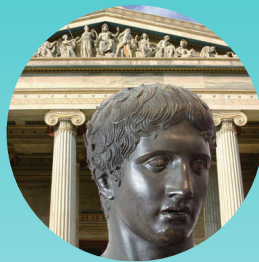


Megaron Athens International Conference Centre

11-13 May 2016 • Athens, Greece



European Symposium on Food Safety



Programme

*In Collaboration with ILSI Europe and with
the Technical Cooperation of the Food and
Agriculture Organization of the United Nations.*



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Organising Committee

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Alejandro Amezcuita, Vice-Chair
Unilever - United Kingdom

Peter Ben Embarek
World Health Organization - Switzerland

Sarah Cahill
Food & Agriculture Organization of the United Nations - Italy

Christina Harzman
BIOTECON Diagnostics - Germany

Lilou van Lieshout
International Life Sciences Institute - Belgium

Akos Jozwiak
National Food Chain Safety - Hungary

Miia Lindstrom
University of Helsinki - Finland

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Cardiff Metropolitan University, Food Industry Centre -
United Kingdom

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CEERAM - France

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Institute of Food Research - United Kingdom

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Agricultural University of Athens - Greece

Panos Skandamis
Agricultural University of Athens - Greece

Daniele Sohler
ADRIA - France

Helmut Steinkamp
German Institute of Food Technologies - Germany

Helen Taylor
Cardiff Metropolitan University, Food Industry Centre -
United Kingdom

Annett Winkler
Mondelez International - Germany

Marcel Zwietering
Wageningen University - Netherlands

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University of California-Davis - United States

David Tharp
International Association for Food Protection - United States

Lisa Hovey
International Association for Food Protection - United States

Tamara Ford
International Association for Food Protection - United States

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Agricultural University of Athens

Panos Skandamis, Co-Chair
Agricultural University of Athens

Ioannis Boziaris
University of Thessaly

Chrysoula Tassou
Senior Scientist, in National Agricultural Research Foundation

Dimitris Iadikos
Yotis Food Industry and Secretary of the Food Industry
Association

John Kourkoutas
Democritus University of Thrace

4 IAFP's European Symposium on Food Safety

Kostas Koutsoumanis
Aristotle University of Thessaloniki

Chrysoula Tassou
Hellenic Agricultural Organisation DEMETER

Rena Tsigarida
National Food Authority (EFET)

John Sofos
Agricultural University of Athens and Colorado State
University

Apostolos Vantarakis
Department of Medicine of the University of Patras

Speakers

Pablo Alvarez

Novolyze, France

Alejandro Amezcuita

Unilever, United Kingdom

Maria Baka

KU Leuven/BioTeC+, Belgium

Roy Betts

Campden BRI, United Kingdom

Sylvain Brisse

Pasteur Institute, France

Michael Brodsky

Brodsky Consultants, Canada

Stanley Brul

Molecular Biology and Microbial Food (SILS), University of Amsterdam, Netherlands

Francesco Capozzi

BioNMR Lab and Università di Bologna, Italy

Vittorio Capozzi

University of Foggia, Italy

Luca Cocolin

University of Turin-DISAFA, Italy

Paul Cook

Food Standards Agency, United Kingdom

René Crevel

Unilever, United Kingdom

Heidy Den Besten

Wageningen University, Netherlands

John Donaghy

Nestlé, Switzerland

Christophe Dufour

Mérieux NutriSciences France, France

Richard Fielder

Elisa Systems, Australia

Anthony Flood

International Food Information Council, USA

Steven Gendel

IEH Laboratories & Consulting Group, USA

Andrea Gianotti

Università di Bologna, Italy

Bruno Gonzalez-Zorn

Complutense University Madrid, Spain

Muriel Guyard-Nicodème

French Agency for Food, Environmental and Occupational Health and Safety, France

Linda J. Harris

University of California, USA

Geert F. Houben

TNO (Netherlands Organisation for Applied Scientific Research), Netherlands

Julie Jean

Université Laval, Canada

Nicholas Johnson

Nestlé, Switzerland

Akos Jozwiak

National Food Chain Safety Office, Hungary

Kostas Konstantinidis

Georgia Institute of Technology, USA

Hilde Kruse

World Health Organisation, Denmark

Roberto Lattanzio

Eurofins Analytik GmbH, Germany

Alvin Lee

Illinois Institute of Technology/IFSH, USA

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University Bordeaux, France

Balkumar Marthi

Unilever, Netherlands

Sandra Martin-Latil

Anses, France

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Buhler AG, Switzerland

Aline Metris

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Universidade Federal de Viçosa, Brazil

Aspasia Nisiotou

ELGO-'DEMETER', Greece

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Cian O'Mahony

Creme Global, Ireland

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Bruno Pot

Pharmabiotic Research Institute, France

Bizhan Pourkomialian

McDonald's Corporation, United Kingdom

Ans Punt

RIKILT, Wageningen University and Research Center, Netherlands

Paul Ross

University College Cork, Ireland

Manuel Jimmy Saint-Cyr

ONIRIS/INRA, LUNAM Université, France

Benoit Schilter

Nestlé Research Center, Switzerland

Oliver Schlüter

Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Germany

Jenny Scott

U.S. Food and Drug Administration-CFSAN, USA

Torstein Skara

NOFIMA, Norway

Daniele Sohler

ADRIA, France

Katherine MJ Swanson

KMJ Swanson Food Safety, Inc., USA

Eirini (Rena) Tsigarida

Hellenic Food Authority, Greece

Eirini Velliou

University of Surrey, United Kingdom

Marjon Wells-Bennik

NIZO Food Research, Netherlands

Pamela Wilger

Cargill, USA

Anett Winkler

Kraft Foods R&D Inc., Germany

Frank Yiannas

Walmart, USA

Sophie Zuber

Nestlé Research Center, Switzerland

IAFP European Symposium on Food Safety Schedule

Room	Skalkotas Hall	MC3	MC2	Kokkali Room	
Wednesday, 11 May 2016					
Wednesday 8.00 - 10.00	Opening Session <i>Skalkotas Hall</i>				
Wednesday 10.00 - 10.30	Networking Coffee Break and Poster Presentations				Poster Session 1 – Antimicrobials, Applied Laboratory Methods, Novel Laboratory Methods, Pathogens and Risk Assessment
Wednesday 10.30 - 12.00	S1 – Challenges and Promises of Systems Biology for Food Safety	S2 – Food Safety: A Professionals Guide to Effective Food Risk Communication	S3 – Probiotics: Myth or Reality?	T1 – Applied Laboratory Methods and Novel Laboratory Methods	
Wednesday 12.00 - 13.30	Lunch				
Wednesday 13.30 - 15.00	S4 – Beyond Whole Genome Sequencing: The Impacts of Omics Technologies on Microbial Food Safety Management	S5 – New Approaches to Food and Chemical Risk Assessment	S6 – How Microbial Interactions are Acting toward Our Safety?	T2 – Communication Outreach and Education and Other Food Commodities	
Wednesday 15.00 - 15.30	Networking Coffee Break and Poster Presentations				
Wednesday 15.30 - 17.00	S7 – Can Whole Genome Sequencing Guide and Inform Intra-Species Virulence Rankings?	S8 – Food (Micro)Structure: Impact on Microbial Dynamics	S9 – Risk-based Sampling; Perspective from Different EU and Non-EU Member States	T3 – Microbial Food Spoilage, Meat and Poultry, and Seafood	
Wednesday 17.00- 18.30	Exhibit Hall Reception				
Thursday, 12 May 2016					
Thursday 8.30 - 10.00	S10 – How to Manage Viruses in the Food Industry	S11 – Metabolomics: A Post-genomic Approach to Study the Effect of Microbial Diversity in Foods	S12 – Risk Assessment or Assessment of the Risk, That's the Question	T4 – Sanitation and Antimicrobials	
Thursday 10.00 - 10.30	Networking Coffee Break and Poster Presentations				Poster Session 2 – Communication, Outreach and Education, Food Toxicology, General Microbiology, Microbial Food Safety, Sanitation, Beverages, Dairy, Seafood, Meat and Poultry, Produce
Thursday 10.30 - 12.00	S13 – Balancing Food Quality and Virus Inactivation for Sensitive Foods	S14 – Sporeformers in Food; Implication of Natural Diversity on Food Safety and Food Quality	S15 – The ISO 16140 Series and the Impact on the Routine Labs' Daily Life	T5 – Risk Assessment	
Thursday 12.00 - 13.30	Lunch				
Thursday 13.30 - 15.00	S16 – Managing Allergens: How Do We Assess the Risk and Protect Allergic Consumers?	S17 – Strategies to Control Foodborne Pathogens: Focus on <i>Campylobacter</i> in Broilers	S18 – Antimicrobial Resistance in the Food Chain	T6 – Pathogens and Produce	
Thursday 15.00 - 15.30	Networking Coffee Break and Poster Presentations				
Thursday 15.30 - 17.00	S19 – Food Allergen Control Under Preventive Food Safety Systems	S20 – FSMA Implications for Suppliers to the USA and Training Implications	S21 – Microbial Inactivation of Dry Foods - Advances in Scientific Knowledge and Industrial Solutions	T7 – General Microbiology and Non-microbial Food Safety	
Friday, 13 May 2016					
Friday 8.30 - 10.00	S22 – Dilemma in Constructive Use of Risk Assessment in a Variable World: All Microbes are Equal but Some Microbes are More Equal Than Others	S23 – Surrogates for Low-moisture Food Validation: What are the Key Steps from Selection to Routine Use?	S24 – Quality, Safety and Spoilage Issues in the Wine Industry	T8 – Modelling, Beverages and Microbial Food Spoilage	
Friday 10.00 - 10.30	Networking Coffee Break				
Friday 10.30 - 12.30	Closing Session <i>Skalkotas Hall</i>				
Friday 12.30 - 13.30	Farewell Refreshments				

DAY 1 – Wednesday, 11 May

7.00 – 17.00 Registration Open

Exhibit Hours 10.00 – 18.30

Posters will be on display 10.00 – 18.30. Poster presentations will take place during coffee breaks.

Opening Session

Skalkotas Hall

Chairs: George-John Nychas and Panagiotis Skandamis

8.00 Introduction to IAFP

DAVID THARP, IAFP Executive Director, Des Moines, IA, USA

8.20 Introduction to the IAFP European Symposium

ALEJANDRO MAZZOTTA, IAFP President, New York, NY, USA

8.30 Food Safety in Athens – On Behalf of Athens Mayor

ELENI MYRIVILLIS, University of the Aegean and City of Athens, Athens, Greece

9.00 Food Safety Management Systems

ALEJANDRO MAZZOTTA, Chobani, LLC, New York, NY, USA

9:30 Risk-based Approaches to Food Safety

EIRINI (RENA) TSIGARIDA, Hellenic Food Authority, Athens, Greece

10.00 Networking Coffee Break in the Exhibit Area

S1 Challenges and Promises of Systems Biology for Food Safety

Skalkotas Hall

*Organizer: Aline Metris
Chair: Jozséf Baranyi*

10.30 From Genomes to Mathematical Models for Systems Biology

ALINE METRIS, Institute of Food Research, Norwich, United Kingdom

11.00 Application of Metabolic Network Models to Develop New Preservation Strategies: An Industrial Perspective

YVAN LE MARC, Unilever, Sharnbrook, United Kingdom – will be presented by ALEJANDRO AMEZQUITA

11.30 The Bacterial Spore Proteome; Identifying Targets for Spore Germination and Outgrowth Inhibition

STANLEY BRUL, Molecular Biology and Microbial Food (SILS), University of Amsterdam, Amsterdam, Netherlands

12.00 Lunch Available in the Exhibit Area

S2 Food Safety: A Professional's Guide to Effective Food Risk Communication

MC3

Organizer: Anthony Flood

Chairs: Anthony Flood and Jack Cooper

10.30 FoodRisC: Perceptions and Communication of Food Risks/Benefits across Europe

NINA MCGRATH, European Food Information Council, Brussels, Belgium

11.00 Building a Practical Framework for Successful Food Safety Risk Communication

ANTHONY FLOOD, International Food Information Council, Washington, D.C., USA

11.30 Effective Food Risk Communication: A Case Study from the Hellenic Food Authority

EIRINI (RENA) TSIGARIDA, Hellenic Food Authority, Athens, Greece

12.00 Lunch Available in the Exhibit Area

S3 Probiotics: Myth or Reality?

MC2

Organizer and Chair: Chrysoula Tassou

10.30 A Lone Voice in the Crowd: Probiotics in the Context of the Microbiome

PAUL ROSS, University College Cork, Cork, Ireland

11.00 Hunting for Probiotic Microorganisms: Is There an Easy Road to Success?

KONSTANTINOS PAPADIMITRIOU, Agricultural University of Athens, Athens, Greece

11.30 What are the Options for the Industry to Promote Probiotic Benefits?

BRUNO POT, Pharmabiotic Research Institute, Aurillac, France

12.00 Lunch Available in the Exhibit Area

T1 Technical Session 1 – Applied Laboratory Methods and Novel Laboratory Methods

Kokkali Room

Chair: Anett Winkler

T1-01 Comparison of Cell-based and PCR-based Assays as Methods for Measuring Infectivity of Tulane Virus

PENG TIAN, Lei Shan, Dapeng Wang, David Yang, U.S. Department of Agriculture-PSMRU-WRRC-ARS, Albany, CA, USA

T1-02 Evaluation of the European Network for Staphylococcal Enterotoxins Detection in Food Matrice
10.45 YACINE NIA, Isabelle Mutel, Adrien Assere, Sabine Messio, Jacques-Antoine Hennekinne, Frédéric Auvray, Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Alfort, France

T1-03 Staphylococcal Enterotoxins Detection in Food Matrices from Various Food Poisoning Outbreaks in Europe
11.00 YACINE NIA, Alexandra Cauquil, Sarah Denayer, Christos Kourtis, Hristo Daskalov, Lucia Decastelli, Bernadette Hickey, Frédéric Auvray, Jacques-Antoine Hennekinne, Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Alfort, France

T1-04 Certified Reference Materials for the Analysis of *Staphylococcus aureus* Enterotoxin A in Cheese
11.15 REINHARD ZELENY, Håkan Emteborg, Jean Charoud-Got, Heinz Schimmel, Isabelle Mutel, Yacine Nia, Frédéric Auvray, Jacques-Antoine Hennekinne, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium

T1-05 A Unique Rapid Detection and Quantification Assay for Total Count of Yeasts and Molds in Dairy Products Based on Multiplex Real-time PCR
11.30 CHRISTINA HARZMAN, Matthias Giese, Cordt Groenewald, Kornelia Berghof-Jaeger, BIOTECON Diagnostics, Potsdam, Germany

T1-06 Detection of Distinct Norovirus Genotypes with a Multiplex Qpcr System in Seafood
11.45 OLAF DEGEN, Arnt Ebinger, BIOTECON Diagnostics, Potsdam, Germany

12.00 Lunch Available in the Exhibit Area

S4 Beyond Whole Genome Sequencing: The Impacts of Omics Technologies on Microbial Food Safety Management

Skalkotas Hall

Organizer and Chair: Cian O'Mahony

13.30 Applications of Metagenomics to Product and Process Design
NICHOLAS JOHNSON, Nestle, Lausanne, Switzerland

14.00 Integrating Microbiomics of the Food Chain into an Effective Food Safety Management System
BALKUMAR MARTHI, Unilever, Vlaardingen, Netherlands

14.30 Molecular-based Surveillance in Food Manufacturing Facilities Using Next Generation Sequencing Techniques and Software
CIAN O'MAHONY, Creme Global, Dublin, Ireland

15.00 Networking Coffee Break in the Exhibit Area

S5 New Approaches to Food and Chemical Risk Assessment

MC3

Sponsored by ILSI Europe

Organizer and Chair: Lilou van Lieshout

13.30 Introduction to Chemicals in Food
BENOIT SCHILTER, Nestlé Research Center, Lausanne, Switzerland

14.00 Current Tools and Approaches in Chemical Risk Assessment
BENOIT SCHILTER, Nestlé Research Center, Lausanne, Switzerland

14.30 New Approaches in Chemical Risk Assessment
ANS PUNT, RIKILT Wageningen University and Research Center, Wageningen, Netherlands

15.00 Networking Coffee Break in the Exhibit Area

S6 How are Microbial Interactions Acting toward Our Safety?

MC2

Organizers and Chairs: Luca Cocolin and Marie-France Pilet

13.30 The Concept of Bioprotection: Microbial Interactions for Safer Foods
LUCA COCOLIN, University of Turin-DISAFA, Turin, Italy

14.00 Protective Bacteria: An Option to Control *Listeria monocytogenes* in Seafood Products
MARIE-FRANCE PILET, UMR SECALIM, INRA, Oniris, Nantes, France

14.30 New Insights on the *LAB-Staphylococcus aureus* Interaction: A Transcriptomic Approach
LUÍS AUGUSTO NERO, Universidade Federal de Viçosa, Viçosa, Brazil

15.00 Networking Coffee Break in the Exhibit Area

T2 Technical Session 2 – Communication Outreach and Education and Other Food Commodities

Kokkali Room

Chair: Michael Brodsky

T2-01 A Food Safety Strategy for Global Logistics Operations: A Global Concept with Local Relevance
13.30 NIKOLAOS BESSAS, METRO Cash & Carry International, Dusseldorf, Germany

T2-02 Consumer Information on the Prevention of Foodborne Microbiological Risks: Improving the Effectiveness of Communication Strategies
13.45 PAULINE KOOH, Thomas Bayeux, Eve Feinblatt, Jean Christophe Augustin, Laure Bonnaud, Olivier Cerf, Michel Gautier, Françoise Gauchard, Laurent Guillier, Nathalie Jourdan-Da-Silva, Thierry Meyer, Lydiane Nabec, Louis-Georges Soler, Isabelle Villena, Moez Sanaa, Sandrine Blanchemanche, ANSES, Maisons-Alfort, France

T2-03 Development of Online Teaching and Learning
14.00 Tools for the Delivery of Poultry Food Safety in the
Veterinary Curriculum
RODRIGO J. NOVA, School of Veterinary Medicine
and Science, Sutton Bonington Campus, University of
Nottingham, Nottingham, United Kingdom

T2-04 Significance of HACCP Implementation on the
14.15 Microbiological Quality of Foods and Environmental
Hygiene in Mass Feeding Facilities in Greece during
the Period 2003 to 2010
Constantin Genigeorgis, Niki Thalassinaki, CHRIS
PANOULIS, University of Crete, Heraklion, Greece

T2-05 Microbiological Quality and Safety in Mass Feeding
14.30 Establishments during the Greece Financial Crisis
Period 2011 to 2015
CHRIS PANOULIS, Constantin Genigeorgis, University
of Crete, Heraklion, Greece

15.00 Networking Coffee Break in the Exhibit Area

S7 Can Whole Genome Sequencing Guide and
Inform Intra-species Virulence Rankings?
Skalkotas Hall
*Organizers and Chairs: Sophia Kathariou and
George-John Nychas*

15.30 Implementation of the Use of Whole Genome
Sequencing Data and Relevant Insights on Virulence
of Bacterial Foodborne Pathogens
To be determined

16.00 An Integrated View on *Listeria* Genomics and Virulence
SYLVAIN BRISSE, Pasteur Institute, Paris, France

16.30 Delineating Virulent and Avirulent Taxa with
Genomics and Metagenomics
KOSTAS KONSTANTINIDIS, Georgia Institute of
Technology, Atlanta, GA, USA

17.00 – 18.30 Reception in Exhibit Area

S8 Food (Micro) Structure: Impact on Microbial
Dynamics
MC3
Organizer and Chair: Jan Van Impe

15.30 Evaluation of (Micro) Structural-related Factors on
Microbial Growth in/on Food-based Model Systems
MARIA BAKA, KU Leuven/BioTeC+, Ghent, Belgium

16.00 Thermal Inactivation of *Listeria* Related to Food
Structure and Processing Technology
TORSTEIN SKARA, NOFIMA, Stavanger, Norway

16.30 Characterisation/Quantification of the Impact of
Food Structure on the Development of Antimicrobial
Resistance of Food-related Pathogens
EIRINI VELLIOU, University of Surrey, Guildford,
United Kingdom

17.00 – 18.30 Reception in Exhibit Area

S9 Risk-based Sampling; Perspective from Different
EU and Non-EU Member States
MC2
*Organizers and Chairs: Akos Jozwiak and
Annemarie Pielaat*

15.30 Risk-based Sampling; Perspective from Hungarian
National Food Chain Safety Office
AKOS JOZWIAK, National Food Chain Safety Office,
Budapest, Hungary

16.00 Risk-based Sampling, Optimal Sampling Design:
Perspective from the Dutch Institute for Public
Health and the Environment
ANNEMARIE PIELAAT, National Institute for Public Health
and the Environment, RIVM, Bilthoven, Netherlands

16.30 Risk-based Sampling; Perspective from CFSAN, USA
JENNY SCOTT, U.S. Food and Drug Administration-
CFSAN, College Park, MD, USA

17.00 – 18.30 Reception in Exhibit Area

T3 Technical Session 3 – Microbial Food Spoilage,
Meat and Poultry, and Seafood
Kokkali Room
Chair: Christina Harzman

T3-01 Metagenomics of Spoiled Meat: Meet the Suspects
15.30 OLAV SLIEKERS, Kyle Brookmeyer, Anira Ruiz Sanchez,
Gaston Bevort, Corbion, Gorinchem, Netherlands

T3-02 Reduction of Broiler Chicken and Turkey *Salmonella*
15.45 Prevalence, Numbers, and Virulence by Diamond V
Original XPC
DOUG SMITH, Steve Carlson, Kristi Anderson, Hilary
Pavlidis, Diamond V, Cedar Rapids, IA, USA

T3-03 Efficacy of Biosecurity Measures for *Campylobacter*
16.00 Control in Spanish Broiler Farms
MARTA CERDÀ-CUÉLLAR, Laura Laureano, Teresa
Ayats, Alfredo Corujo, Birthe Hald, Roser Dolz, IRTA-
CRESA, Bellaterra (Barcelona), Spain

T3-04 High Pressure Inactivation Kinetics for a Better
16.15 Understanding of *Listeria monocytogenes* Behaviour
in RTE Cooked Meat Products Formulated with
Organic Acids
SARA BOVER-CID, Anna Jofré, Cristina Serra, Nicoletta
Belletti, Margarita Garriga, IRTA. Food Safety
Programme, Monells, Spain

T3-05 Distribution of *Salmonella*, ESBL/AmpC-producing
16.30 *Escherichia coli* and Hygiene Indicator Bacteria on
Pig Carcasses after Slaughter
WAUTER BIASINO, Lieven De Zutter, Kurt Houf, Inge
Van Damme, Ghent University, Merelbeke, Belgium

17.00 – 18.30 Reception in Exhibit Area

DAY 2 – Thursday, 12 May

8.00 – 17.00 Registration Open

Exhibit Hours 10.00 – 17.00

Posters will be on display 10.00 – 17.00. Poster presentations will take place during coffee breaks.

S10 How to Manage Viruses in the Food Industry

Skalkotas Hall

Organizer and Chair: Fabienne Loisy-Hamon

8.30 Detection and Assessment of Viral Risk in Food
SANDRA MARTIN-LATIL, Anses, Maisons-Alfort, France

9.00 Viral Risk Management: From Preventive Measures to Sampling Plans
CHRISTOPHE DUFOUR, Mérieux NutriSciences France, Cergy-Pontoise Cedex, France

9.30 Shedding Some Light on Inactivation of Foodborne Viruses
JULIE JEAN, Université Laval, Québec, QC, Canada

10.00 Networking Coffee Break in the Exhibit Area

S11 Metabolomics: A Post-genomic Approach to Study the Effect of Microbial Diversity in Foods

MC3

Organizer and Chair: Andrea Gianotti

8.30 Basic Concepts of Metabolomics and Application to the Food and Nutrition Science
FRANCESCO CAPOZZI, BioNMR Lab - Department of Agricultural and Food Science Alma Mater Studiorum, Università di Bologna - Cesena Campus, Cesena, Italy

9.00 Metabolomics – A Useful Tool to Study the Quality of Fermented Foods
ANDREA GIANOTTI, Department of Agricultural and Food Science Alma Mater Studiorum, Università di Bologna, Bologna, Italy

9.30 Metabolomics Application on Bacterial Safety, Spoilage and Adulteration
GEORGE-JOHN NYCHAS, Agricultural University of Athens, Department of Food Science and Human Nutrition, Athens, Greece

10.00 Networking Coffee Break in the Exhibit Area

S12 Risk Assessment or Assessment of the Risk in Fresh Produce, That's the Question

MC2

Sponsored by ILSI Europe

Organizer and Chair: Lilou van Lieshout

8.30 Developing Practical Risk Assessment for Fresh Produce Industrial Practice: Issues Faced While Putting 'Formal MRA' into Industrial Practice
ROY BETTS, Campden BRI, Gloucestershire, United Kingdom

9.00 Assessment of the Risk for Fresh Produce Primary Producers: Presenting the Example of Fresh Produce Assessment of the Risk
JAMES MONAGHAN, Harper Adams University, Newport, England

9.30 The Fresh Produce Assessment: The Relevance of Risk Assessment for the Food Service
BIZHAN POURKOMAILIAN, McDonald's Corporation, London, United Kingdom

10.00 Networking Coffee Break in the Exhibit Area

T4 Technical Session 4 – Sanitation and Antimicrobials

Kokkali Room

Chair: Sarah Cabill

T4-01 Synergistic Effect of Nitric Oxide Donors in Association with Sanitizers in Dispersing Biofilms of Industrial Interest
8.30 MASSIMILIANO MARVASI, Ian Durie, Raphael Carvalho Prado, Tania Henriquez, Middlesex University, London, United Kingdom

T4-02 Decontamination of Dry and Powdery Food Products by Vaporized Hydrogen Peroxide (VHP)
8.45 Cécile Lacoste, François Zuber, STÉPHANE ANDRÉ, CTCPA, Avignon, FL, France

T4-03 Prevention and Reduction of Bacterial Cross-contamination by Natural Antimicrobials during the Washing of Ready-to-Eat Lettuce
9.00 CRISTINA PABLOS CARRO, Alba Fernández Pulido, Alison Thackeray, Javier Marugán, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain

T4-04 Tolerance to Quaternary Ammonium Compounds May Enhance Growth of *Listeria monocytogenes* in the Food Industry
9.15 TROND MØRETRØ, Bjørn C.T. Schirmer, Even Heir, Annette Fagerlund, Solveig Langsrud, Nofima, Norwegian Institute of Food, Fishery and Aquaculture, Ås, Norway

- T4-05** Impact of Enrofloxacin Treatment on Fecal Populations of *Campylobacter* spp. in Calves
9.30 SOPHIA KATHARIOU, Jeffrey Niedermeyer, Derek Foster, Margaret Kirchner, Hannah Bolinger, William Miller, North Carolina State University, Raleigh, NC, USA
- T4-06** Maleic Acid Enhances Acid Sensitivity of *Listeria monocytogenes* through Inhibition of the Glutamate Decarboxylase Activity
9.45 Ranju Paudyal, ANDREAS KARATZAS, University of Reading, Reading, United Kingdom
- 10.00** **Networking Coffee Break in the Exhibit Area**
- S13** **Balancing Food Quality and Virus Inactivation for Sensitive Foods**
Skalkotas Hall
Organizers and Chairs: Alvin Lee and Sophie Zuber
- 10.30 How Do Viruses Enter the Fruit and Vegetables Food Chain and Estimation of Consumer Risk?
LEENA MAUNULA, University of Helsinki, Helsinki, Finland
- 11.00 Viral Inactivation Using Legacy Thermal Inactivation Technologies and Its Limits
SOPHIE ZUBER, Nestle Research Center, Lausanne, Switzerland
- 11.30 Developments and Optimization of Non-thermal Technologies for Viral Inactivation
ALVIN LEE, Illinois Institute of Technology/IFSH, Bedford Park, IL, USA
- 12.00** **Lunch Available in the Exhibit Area Sponsored by Diamond V**
- S14** **Sporeformers in Food; Implication of Natural Diversity on Food Safety and Food Quality**
MC3
Organizer: Heidy Den Besten
Chairs: Heidy Den Besten and Florence Postollec
- 10.30 Variability in Heat Resistance of Sporeformers; How Diverse is Diversity?
HEIDY DEN BESTEN, Wageningen University, Wageningen, Netherlands
- 11.00 Genetic Biomarkers for High Heat Resistance of *Bacillus* Spores: Relevance for Optimal Design of Heat Treatment
MARJON WELLS-BENNIK, NIZO Food Research, Ede, Netherlands
- 11.30 Combined Approaches to Differentiate the Common Mister *B. licheniformis* and the Super Spoiler
FLORENCE POSTOLLEC, ADRIA UMT14.01 SPORE RISK, Quimper, France
- 12.00** **Lunch Available in the Exhibit Area Sponsored by Diamond V**
- S15** **The ISO 16140 Series and the Impact on the Routine Labs' Daily Life**
MC2
Organizers and Chairs: Patrice Arbault, Paul in't Veld, and Daniele Sobier
- 10.30 The ISO 16140 Series and Their Impact on Routine Laboratories
DANIELE SOHIER, ADRIA, Quimper, France
- 10.50 Testimonials: What Does It Mean to Use Validated and Verified Methods in the Food Industry?
PAMELA WILGER, Cargill, Minneapolis, MN, USA
- 11.10 Roundtable
Panelists:
PATRICE ARBAULT, BioAdvantage Consulting, Orléans, France
FRANÇOIS BOURDICHON, Danone Vitapole, Palaiseau, France
BENJAMIN DIEP, Nestle Research Center, Lausanne, Switzerland
CHRISTOPHE DUFOUR, Mérieux NutriSciences, Cergy-Pontoise Cedex, France
CHRISTINA HARZMAN, BIOTECON Diagnostics, Potsdam, Germany
- 12.00** **Lunch Available in the Exhibit Area Sponsored by Diamond V**
- T5** **Technical Session 5 – Risk Assessment**
Kokkali Room
Chair: Jeanne-Marie Membré
- T5-01** Risk Assessment as a Tool for Evaluating Temperature Requirements in Handling Area of Chill Food Distribution Centers
10.30 Hsin-I Hsiao, YU-TING WENG, National Taiwan Ocean University, Keelung, Taiwan
- T5-02** A Quantitative Microbiological Exposure Assessment Model for *Bacillus cereus* Group IV in Couscous Consumed on a Weekly Basis or during Collective Events
10.45 ZIANE MOHAMMED, Ivan Leguerinel, Jeanne-Marie Membré, Centre universitaire de Ain témouchent, Ain témouchent, Algeria
- T5-03** Phenotypic Behavior of 35 *Salmonella enterica* Serovars Compared to Epidemiological and Genomic Data
11.00 ANNEMARIE PIELAAT, Angelina Kuijpers, Peter Teunis, Ellen Delfgou-van Asch, Wilfrid Van Pelt, Lucas Wijnands, RIVM – Centre for Infectious Disease Control, Bilthoven, Netherlands
- T5-04** Meta-analysis of the Inactivation Effect of High Hydrostatic Pressure on *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*
11:15 SANDRINE GUILLOU, Aude-Marine Makowski, Jeanne-Marie Membré, LUNAM University, Oniris, UMR 1014 Secalim, Nantes, France

T5-05 Risk Ranking of Chemical Hazards in Spices and Herbs

11.30 Esther van Asselt, JENNIFER BANACH, Ine van der Fels-Klerx, RIKILT Wageningen UR, Wageningen, Netherlands

T5-06 Large-scale Molecular Risk Assessment of Shiga Toxin-producing *Escherichia coli* (STEC) by Whole Genome Sequencing

11.45 EELCO FRANZ, Andreas Bauwens, Angela van Hoek, Amanda de Oude, Stefano Morabito, National Institute for Public Health and the Environment, RIVM, Bilthoven, Netherlands

12.00 Lunch Available in the Exhibit Area Sponsored by Diamond V

S16 Managing Allergens: How Do We Assess the Risk and Protect Allergic Consumers?
Skalkotas Hall

Sponsored by ILSI Europe

Organizer and Chair: Estefanía Noriega

13.30 The ILSI-Europe Food Allergy Task Force: Promoting the Safety of Food Allergic Consumers
RENÉ CREVEL, Unilever, Bedford, United Kingdom

14.00 From EuroPrevall to iFAAM – Insights into Food Allergen Management
CLARE MILLS, University of Manchester, Manchester, United Kingdom

14.30 Recent Developments in Risk Assessment of Food Allergens
GEERT F. HOUBEN, TNO (Netherlands Organisation for Applied Scientific Research), Zeist, Netherlands

15.00 Networking Coffee Break in the Exhibit Area

S17 Strategies to Control Foodborne Pathogens: Focus on *Campylobacter* in Broilers
MC3

Organizers and Chairs: Muriel Guyard-Nicodème and Nabila Haddad

13.30 Why is *Campylobacter* the Number One Priority for the Poultry Production Chain?
MURIEL GUYARD-NICODÈME, French Agency for Food, Environmental and Occupational Health and Safety, Ploufragan, France

14.00 An Update about the Different State-of-the-Art Methods to Control *Campylobacter* in Broilers: The European Project CAMPYBRO
PEDRO MEDEL, IMASDE AGROALIMENTARIA, S.L, Calle Nápoles, 3 Pozuelo de Alarcon, Madrid, Spain

14.30 Use of Potential Probiotic Strains to Reduce *Campylobacter jejuni* in Broilers: Recent Developments Using *Lactobacillus salivarius* SMXD51
MANUEL JIMMY SAINT-CYR, ONIRIS/INRA, Luman Université, Nantes, France

15.00 Networking Coffee Break in the Exhibit Area

S18 Antimicrobial Resistance in the Food Chain
MC2

Sponsored by ILSI Europe

Organizer and Chair: Lilou van Lieshout

13.30 Introduction to Antimicrobial Resistance in the Food Chain: The Relevance of Tackling Antimicrobial Resistance from a Global Point of View
HILDE KRUSE, World Health Organisation, Copenhagen, Denmark

14.00 A Global Vision for Antimicrobial Stewardship in Food Animals: Preserving Antimicrobial Effectiveness in the Future through Ethical Practices Today
To be determined

14.30 Biophysical Parameters Affecting Gene Transfer in the Food Chain: First Results from the EFFORT FP7 EU Project
BRUNO GONZALEZ-ZORN, Complutense University Madrid, Madrid, Spain

15.00 Networking Coffee Break in the Exhibit Area

T6 Technical Session 6 – Pathogens and Produce
Kokkali Room

Chair: Panagiotis Skandamis

T6-01 Inactivation of Norovirus in the Presence of Soil Loads Simulating Actual Conditions of Viral Transmission

13.30 JULIE JEAN, Maryline Girard, Ismail Fliss, Université Laval, Québec, QC, Canada

T6-02 Non-protective Role of sigB against Oxidative Stress in *Listeria monocytogenes*

13.45 MARCIA BOURA, Ciara Keating, Conor P. O'Byrne, Andreas Karatzas, University of Reading, Reading, United Kingdom

T6-03 Analysing the Microbial and Sensory Quality of Fresh Produce Following the Application of Ultrasound Decontamination

14.00 Leandra Neto, David Millan-Sango, Jean-Pierre Brincat, Luis Cunha, VASILIS VALDRAMIDIS, University of Malta, Msida, Malta

T6-04 *Salmonella*/Salad Interactions

14.15 GIANNIS KOUKKIDIS, Suzanne Jordan, Primrose Freestone, University of Leicester, Leicester, United Kingdom

T6-05 The Influence of Pre-wash Chopping and Storage Conditions of Parsley on the Efficacy of Disinfection against *Salmonella Typhimurium*

14.30 DIMA FAOUR-KLINGBEIL, Victor Kuri, Ewen Todd, Plymouth University, Plymouth, England

T6-06 14.45 Determination of Fatty Acid Composition of *Pistacia vera* Selected from the Valley of River Platani (Sicily)
GAETANO FELICE CALDARA, Giovanni Lo Cascio, Andrea Macaluso, Antonella Amato, Vincenzo Ferrantelli, Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Università di Palermo, Palermo, Italy

15.00 Networking Coffee Break in the Exhibit Area

S19 Food Allergen Control under Preventive Food Safety Systems

Skalkotas Hall

Organizer: Steven Gendel

Chair: Patrice Arbault

15.30 Food Allergen Controls under FSMA and the FDA Preventive Controls Rule
STEVEN GENDEL, IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA

16.00 Allergen Control, Analysis and Global Food Safety Initiative Schemes
RICHARD FIELDER, Elisa Systems, Windsor, Australia

16.30 Advances in Detection and Measurement Technologies that Support Validation and Verification of Allergen Controls
ROBERTO LATTANZIO, Eurofins Analytik GmbH, Hamburg, Germany

17.00 Adjourn

S20 FSMA Implications for Suppliers to the USA and Training Opportunities

MC3

Sponsored by Food Safety Preventive Controls Alliance

Organizer and Chair: Katherine MJ Swanson

15.30 Preventive Controls for Human Food Regulation Overview
JENNY SCOTT, U.S. Food and Drug Administration, College Park, MD, USA

16.00 FSPCA Preventive Controls for Human Food Curriculum - How is It Different from HACCP Training?
KATHERINE MJ SWANSON, KMJ Swanson Food Safety, Inc., Mendota Heights, MN, USA

16.30 Preventive Controls Implications for Suppliers to the USA
JOHN DONAGHY, Nestle, Vevey, Switzerland

17.00 Adjourn

S21 Microbial Inactivation of Dry Foods – Advances in Scientific Knowledge and Industrial Solutions

MC2

Organizer: Edyta Margas

Chair: Nicolas Meneses

15.30 The Impact of Water and Product Composition on Pathogen Survival and Inactivation
LINDA J. HARRIS, University of California, Davis, CA, USA

16.00 Use of Heat Transfer Properties and Mathematical Modeling for Validation and Monitoring of Industrial Thermal Process for Low-moisture Foods – Case Studies
NICOLAS MENESES, Buhler AG, Uzwil, Switzerland

16.30 Recent Developments in Inactivation Technologies for Low-moisture Foods
OLIVER SCHLUTER, ATB, Leibniz-Institut für Agrartechnik, Potsdam-Bornim e.V., Potsdam, Germany

17.00 Adjourn

T7 Technical Session 7 – General Microbiology and Non-microbial Food Safety

Kokkali Room

Chair: George-John Nychas

T7-01 15.30 Assessment of the Biofilm Formation Interactions between *Cronobacter sakazakii* and *Bacillus subtilis*
Athina Antouva, Eleni Gkana, Alexandra Lianou, Efsthios Panagou, GEORGE-JOHN NYCHAS, Agricultural University of Athens, Department of Food Science and Human Nutrition, Athens, Greece

T7-02 15.45 Electron Beam Processing Improves the Microbiological Safety and Retains the Sensory Qualities of Alfalfa Sprouts
JAMES MCCOY, Suresh D. Pillai, Texas A&M University Department of Nutrition and Food Science, College Station, TX, USA

T7-03 16.00 Behaviour of Psychrotrophic *Bacillus cereus* during the Life Cycle of Food Products for Elderly People
ALIZÉE GUÉRIN, Claire Dargaignaratz, Véronique Broussolle, Thierry Clavel, Christophe Nguyen-the, INRA, Avignon University, Sécurité et Qualité des Produits d'Origine Végétale, Avignon, France

T7-04 16.15 Contribution of the Certified Reference Materials to Food Safety
Alexander Bernreuther, Berit Sejerøe-Olsen, Penka Shegunova, Stefan Harbeck, MARTA DABRIO, European Commission, Geel, Belgium

T7-05 16.30 Mycotoxins Impact Prediction in Food Due to Climate Change Using Big-Data Analysis in Korea
YONG-SOO KIM, Hyun-Ju Lee, Korea Health Industry Development Institute, Chungju, Korea

T7-06 16.45 Efficacy of Aqueous Chlorine Dioxide on *Escherichia coli* Inactivation during Fresh-cut “Lollo Rossa” (*Lactuca sativa*) Washing at the Pilot Scale
JENNIFER BANACH, Leo van Overbeek, Martijntje Vollebregt, Masja Nierop Groot, Patricia van der Zouwen, Kees van Kekem, Lucienne Berendsen, Ine van der Fels-Klerx, RIKILT Wageningen UR, Wageningen, Netherlands

17.00 Adjourn

DAY 3 – Friday, 13 May

8.00 – 11.00 Registration Open

Exhibit Hours 10.00 – 14.00

S22 **Dilemma in Constructive Use of Risk Assessment in a Variable World: All Microbes are Equal But Some Microbes are More Equal Than Others** Skalkotas Hall

Organizers and Chairs: Alejandro Amezcuita and Marcel Zwietering

8.30 Microbiological Sources and Impact of Variability on QMRA (Exposure Assessment and Hazard Characterisation)

HEIDY DEN BESTEN, Wageningen University, Wageningen, Netherlands

9.00 Dealing with Variability in Industry Risk Assessments to Support Safe Product Design

ALEJANDRO AMEZQUITA, Unilever, Sharnbrook, United Kingdom

9.30 Factors to Consider in Decision Making Given Variability and Uncertainty in Microbiological Risk Assessment: A Governmental Perspective

PAUL COOK, Food Standards Agency, London, United Kingdom

10.00 Networking Coffee Break in Exhibit Area

S23 **Surrogates for Low-moisture Food Validation: What are the Key Steps from Selection to Routine Use?**

MC3

Organizer: Pablo Alvarez

Chair: Patrice Arbault

8.30 Validation Studies: An Overview of Currently Used Approaches

ANETT WINKLER, Kraft Foods R&D Inc., München, Germany

9.00 *Enterococcus faecium* as a Surrogate of *Salmonella*: It Works for Almonds, But Does It Work for My Products?

LINDA J. HARRIS, University of California, Davis, CA, USA

9.30 New Surrogates in Low-moisture Food/Petfood Process Validation, Are We Ready to Use Them?

PABLO ALVAREZ, Novolyze, Dijon, France

10.00 Networking Coffee Break in Exhibit Area

S24 **Quality, Safety and Spoilage Issues in the Wine Industry**

MC2

Organizer: Aspasia Nisiotou

Chair: Georgios Banilas

8.30 Shaping Wine Quality by the Use of Native Yeast Microbiota

ASPASIA NISIOTOU, ELGO-'DEMETER', Lycovrissi, Attikis, Greece

9.00 Selecting LAB for Use as Starter Cultures in Winemaking

PATRICK LUCAS, University Bordeaux, Bordeaux, France

9.30 The Importance of Tailored Starter Cultures to Ensure the Quality and the Safety of "Wild", Organic, Biodynamic, and Typical Wines

VITTORIO CAPOZZI, Department of Agriculture, Food and Environment Sciences, University of Foggia, Foggia, Italy

10.00 Networking Coffee Break in Exhibit Area

T8 **Technical Session 8 – Modelling, Beverages and Microbial Food Spoilage**

Kokkali Room

Chair: Helen Taylor

T8-01 Designing a Food Matrix Ontology for Supporting a Predictive Microbiology Database

8.30 Salavador Cubero, FERNANDO PEREZ-RODRIGUEZ, Elena Carrasco, Antonio Valero, Matthias Filter, University of Cordoba, Cordoba, Spain

T8-02 Using Genome-scale Metabolic Models of Foodborne Pathogens to Address Human Disease and Food Safety

8.45 Zachary Metz, Tong Ding, DAVID J. BAUMLER, Department of Food Science and Nutrition and the Biotechnology Institute, University of Minnesota-Twin Cities, St. Paul, MN, USA

T8-03 Validation of a *Vibrio parahaemolyticus* Growth Model for Application in a TTI-based Seafood Safety Management System in Oysters

9.00 Theofania Tsironi, Marianna Giannoglou, PETROS TAOUKIS, Peter Ronnow, National Technical University of Athens, Athens, Greece

T8-04 Fungi in Juices: Survey on the Use of Homogenization and Ultrasound as Efficient Preserving Tools

9.15 ANTONIO BEVILACQUA, Barbara Speranza, Daniela Campaniello, Angela Racioppo, Milena Sinigaglia, Maria Rosaria Corbo, University of Foggia, Foggia, Italy

- T8-05** *Alicyclobacillus acidoterrestris* from Soil and Pear Juice: Do Some Strains Move from Soil to Other Environments?
9.30 ANTONIO BEVILACQUA, Maria Clara Iorio, Milena Sinigaglia, Maria Rosaria Corbo, University of Foggia, Foggia, Italy
- T8-06** Understanding the Fate of Bacterial Transference in a Simulated Wash Process of Fresh-Cut Lettuce
9.45 SOFÍA GONZÁLEZ REBOLLO, Cristina Pablos Carro, Rafael van Grieken, Javier Marugán, Universidad Rey Juan Carlos, Móstoles, Madrid, Spain
- 10.00 Networking Coffee Break in the Exhibit Area**
- Closing Session**
Skalkotas Hall
Chairs: George-John Nychas and Panagiotis Skandamis
- 10.30 **The Fallacious Fecal Coliform**
MICHAEL BRODSKY, Brodsky Consultants, Thornhill, ON, Canada
- 11.00 **Tree Nuts: Food Safety Risk and Intervention Strategies**
LINDA J. HARRIS, University of California, Davis, CA, USA
- 11.30 **Beyond Food Safety Management Systems – Food Safety Culture**
FRANK YIANNAS, Walmart, Bentonville, AR, USA
- 12.00 **Awards Presentation and European Symposium Conclusion**
ALEJANDRO MAZZOTTA, IAFP President, New York, NY, USA
- 12.30 – 13.30 Farewell Refreshments**

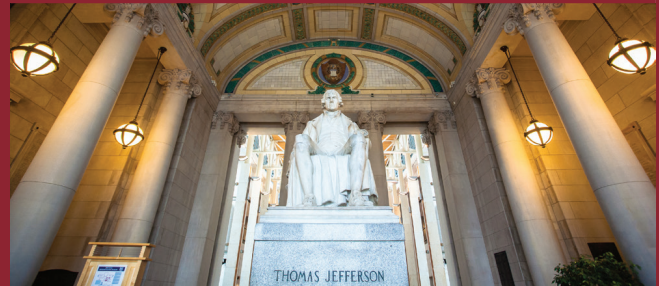
Amanda Demeter
Europe Student Travel Scholarship Award
Eotvos Lorand University, Budapest, Hungary
Institute of Food Research, Norwich, UK



Amanda Demeter is a Ph.D. candidate at Eotvos Lorand University (ELTE) in Budapest, Hungary, and a visiting student at the Institute of Food Research in Norwich, UK. While earning her M.Sc. and throughout her Ph.D. studies, her research has focused on the complex mechanism behind autophagy during infection. Amanda is a member of a Network Biology group in the Department of Genetics at ELTE, where she uses computational techniques to investigate the role of different *Salmonella* Typhimurium proteins in modifying the autophagy.

During her M.Sc. studies, Amanda's research earned second place in the Biology and Bioinformatics section at the Local and the National Scientific Students' Associations' Conferences in Hungary. Amanda is currently receiving laboratory training from the Institute of Food Research, where she regularly spends short-term internships. She recently participated in an international consortium, preparing an EU proposal on the multi-disciplinary training in complexities related to foods.

Amanda considers food as an exciting research area, not only to combine disciplines but also in science, industry and regulation. She is grateful to receive the IAFP European Symposium Student Travel Scholarship, providing a great opportunity for her to learn more about food research and its applications, and to learn from world-leading experts in areas that complement her research field.



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Plenary Speakers

Pablo Alvarez Novolyze, France



Currently working as R&D Manager at Novolyze, Pablo Alvarez possesses a doctorate in microbiology obtained at the University of Oviedo (Spain). During more than 10 years of career in private (Nestlé) and public (Spanish National Research Council, University College Cork and INSA Toulouse) environments, Dr. Alvarez has developed an extensive expertise in food in human microbiology.

Alejandro Amézquita Unilever, United Kingdom



Dr. Alejandro Amézquita is a science leader in microbiological safety at the Safety & Environmental Assurance Centre of Unilever (based in the UK). He has a broad knowledge of risk assessment, predictive microbiology, and food safety management systems at an international level, gained from working for over 15 years in industry and academia. Dr. Amézquita has published numerous research papers in peer-reviewed

scientific journals, and has presented and lectured extensively in his areas of expertise. In addition to his responsibilities at Unilever, he holds an adjunct faculty appointment at the University of Nebraska-Lincoln, and actively contributes to professional societies and scientific organisations, and as a reviewer of key scientific journals. Dr. Amézquita received his B.Sc. in food engineering from La Salle University in his home country (Colombia), and his M.Sc. (food science & technology) and Ph.D. (biological systems engineering) from the University of Nebraska-Lincoln in the U.S.

Maria Baka KU Leuven/BioTeC+, Belgium



Maria Baka was born in Athens, Greece. In 2003, she started her studies in the school of Food Science and Technology of the Agricultural University of Athens, Greece (2003–2009, M.Sc. degree). During her studies, she obtained an Erasmus grant for conducting her Master's thesis in the Laboratory of Microbiology at Ghent University in Belgium. In 2010, she started a Ph.D. in the Chemical and Biochemical Process Technology and

Control section of the Chemical Engineering Department of KU Leuven, Belgium. Currently, she is a post-doctoral researcher in the (Bio)Chemical Systems Technology, Reactor Engineering and Safety Section of KU Leuven Campus Ghent.

Roy Betts Campden BRI, United Kingdom



Roy Betts is Head of Microbiology at Campden BRI, an independent international food research organisation based in the UK. Roy manages a group of 45 food microbiologists, undertaking a range of industry focussed food research and testing projects for a worldwide client base. Roy originally managed a research team at Campden BRI and concentrated on the research, development and validation of microbiological test

methods. After becoming Head of Department, his interests moved to the assessment of the microbiological quality and safety of foods, advising industry on techniques and procedures to produce and market high quality safe foods. Roy has published widely in the area and is a member of the ILSI Europe Microbiological Food Safety Task Force, the UK Food and Drink Federation Food Hygiene Sub Committee and the UK Advisory Committee on the Microbiological Safety of Foods as well as British Standards Institute and ISO committees dealing with microbiological test methods.

Sylvain Brisse Pasteur Institute, France



Dr. Sylvain Brisse is a Research Director in the Microbial Evolutionary Genomics Research Unit at Institut Pasteur, Paris. He obtained a Ph.D. in Evolutionary Genetics at the University of Montpellier, France, where he studied tropical parasites. As a post-doctoral fellow in Utrecht University, Netherlands, he studied the population genetics, antibiotic resistance and molecular epidemiology of hospital-acquired bacterial

pathogens. Dr. Brisse's current research interests cover the microbial population biology, evolution and epidemiology of pathogenic microbial species. The two primary models studied are *Klebsiella pneumoniae* and *Listeria monocytogenes*. Sylvain Brisse has published over 160 peer-reviewed publications and 5 book chapters. He is the manager of Institut Pasteur's microbial genome databases, which provide international nomenclatures of strains of pathogenic bacteria. These reference nomenclatures are widely used globally by microbiologists from research labs, hospitals, epidemiological surveillance networks and public health agencies in order to monitor the spread of pathogenic strains.

Michael Brodsky

Brodsky Consultants, Canada



Michael Brodsky has been an Environmental Microbiologist for more than 44 years. He is a Past President of the Ontario Food Protection Association (OFPA), The International Association for Food Protection (IAFP) and AOAC International. He serves as Chair for the AOAC Expert Review Committee for Microbiology, as a scientific reviewer in Microbiology for the AOAC OMA and the AOAC Research Institute, as a reviewer

for Standard Method for the Examination of Water and Wastewater, as a chapter editor on QA for the Compendium of Methods in Microbiology. He is also a lead auditor/assessor in microbiology for the Canadian Association for Laboratory Accreditation (CALA) and is Vice-chair of the CALA Board of Directors.

Stanley Brul

Molecular Biology and Microbial Food (SILS), University of Amsterdam, Netherlands



Stanley Brul (1964) was trained as Biochemist and graduated "cum laude" in 1986. In 1991 he obtained a Ph.D. with a doctoral thesis entitled "Biochemical and Genetic Aspects of Peroxisome Biogenesis in Mammalian Cells." He then started in 1990 as a post-doctoral fellow at Nijmegen University, did a short-term fellowship supported stay at Rockefeller University and went in 1994 to work for Unilever Research and Development.

From 1994 to 1999, he fulfilled at Unilever several scientific and managerial positions from project to program manager. In addition he received training in teaching, project and program management as well as general management and finance from the London Business School, the Lausanne Business School and INSEAD, France. In 1999, Stanley was appointed professor of Industrial Microbiology on an endowed chair at the Swammerdam Institute for Life Sciences of the University of Amsterdam while staying 4 days, he was appointed to Unilever Research & Development as senior scientist novel (micro)biological technologies in the Science area Food Processing. As of 2002, the University appointed him as full professor of Molecular Biology & Microbial Food Safety (MBMFS) at the Swammerdam Institute for Life Sciences. From 2003 on, he acted as coordinator of the masters program in Medical Biochemistry of the University. Since the end of 2007, Brul has been fully employed by the University. Concomitantly he was appointed director of the bachelor's program in Biomedical Sciences at the University and received training in managing University professionals. Brul teaches courses in molecular microbiology, biochemistry, nutrition and human molecular physiology.

Francesco Capozzi

BioNMR Lab - Department of Agricultural and Food Science Alma Mater Studiorum, Università di Bologna - Cesena Campus, Italy



Associate Professor of chemistry, Francesco Capozzi's research aims at describing the quality of food products and their in vitro digestibility by means of spectroscopic (NMR) molecular profiles. He is the cofounder of Foodomics, a new holistic approach that studies food and human nutrition as a whole, to reach the optimization of health and well-being. Since 2009, he is the organizer of the biennial International

Conference on Foodomics. He was the European coordinator of the KBBE FP7 project "CHANCE - Low-cost technologies and traditional ingredients for the production of affordable, nutritionally correct foods improving health in population groups at risk of poverty."

Vittorio Capozzi

University of Foggia, Italy



Vittorio Capozzi, researcher in Food Microbiology at the University of Foggia, got his Ph.D. in Biotechnology of Food Products. Experiences in Italian Universities and Research Centers: Free University of Bozen, Edmund Mach Foundation (San Michele all'Adige), Cereal Research Centre Council for Agricultural Research and Economics (Council for Agricultural Research and Economics). Experiences in European

Universities and Research Centers: National School of Biology Applied to Nutrition and Food, "Institut Universitaire de la Vigne et du Vin 'Jules Guyot'" - University of Burgundy, "Institut des Sciences de la Vigne et du Vin" (Bordeaux University). ResearcherID: M-4290-2013.

Luca Cocolin

University of Turin-DISAFA, Italy



Luca Cocolin is full professor of food and wine microbiology at the University of Torino, Italy. He is an executive board member of the ICFMH, part of the IUMS, and Editor-in-Chief of the *International Journal of Food Microbiology*. He is also a member of the Editorial Board of *Applied and Environmental Microbiology*, *Frontiers in Microbiology*, *Current Opinion in Food Science* and *Food Analytical Methods*.

Cocolin is co-author of about 250 papers on national and international journals, and an expert in (i) Molecular methods for the detection, quantification and characterization of food-borne pathogens; (ii) Study of the microbial ecology of foods by using culture independent and dependent methods; (iii) Bioprotection; and (iv) Human microbiome.

Paul Cook **Food Standards Agency, United Kingdom**



Following research in environmental and food microbiology, Paul Cook joined the Department of Health in 1993 where he managed the department's research and surveillance programme on microbiological food safety. Since 2000, Paul has worked for the UK Food Standards Agency where he is Head of Microbiological Risk Assessment Branch. The branch is closely involved in managing research on foodborne disease,

the assessment of microbiological food hazards and incidents, antimicrobial resistance and providing the secretariat for the Advisory Committee on the Microbiological Safety of Food (ACMSF). Paul has been an invited expert to various groups including EU Scientific Committees and WHO/FAO consultations.

René Crevel **Unilever, United Kingdom**



Dr. René Crevel works as a Science Leader at Unilever's Safety and Environmental Assurance Centre at Colworth House, Bedfordshire. He qualified initially in mammalian physiology and has postgraduate qualifications in immunology and toxicology. His principal responsibilities include providing scientific advice and guidance on possible adverse effects of materials and their use, arising from their interaction with, or

modulated through the immune system. He is responsible for advice and guidance on food allergy and allergen management to Unilever Companies, and for leading Unilever's food allergy research programme. He participates in major external projects in food allergy, and has regular interactions with other stakeholders in the food allergy field. Other aspects of his work include immunomodulation by different agents, and the effects of diet and other agents on immune responses, including allergy. He has authored papers and book chapters on various aspects of food allergy, including risk assessment and management of food allergens.

Heidy den Besten **Wageningen University, Netherlands**



Heidy den Besten obtained her B.Sc. in Food Technology and Mathematics at Wageningen University. She then obtained an M.Sc. in Food Technology (cum laude) as well as a Ph.D. degree. Her Ph.D. research combined a mathematical modelling approach with single-cell analyses and molecular techniques for elucidating adaptive stress responses in the foodborne human pathogen *B. cereus*. In 2011, she started as

Assistant Professor in the Laboratory of Food Microbiology of Wageningen University. She supervises B.Sc., M.Sc. and Ph.D. projects and teaches in the B.Sc. and M.Sc. Food Microbiology programmes. Her research domain focuses on quantitative pathogen ecology interlinking functional genomics and modelling of microbial behaviour.

John Donaghy **Nestlé, Switzerland**



John Donaghy's current role is Corporate Food Safety Microbiologist at Nestlé, Switzerland, reporting to Global Head of Quality. The role primarily concerns operational aspects of food safety microbiology, including HACCP, PRPs, microbiological specifications and sponsoring R&D projects to underpin microbial safety. He is a member of the International Commission on Microbiological Specifications of Food (ICMSF). A previous

role held by John was as Senior Food Safety Microbiologist in Nestlé R&D. Prior to joining Nestlé, he worked as Project Leader in food safety microbiology at Agri-Food & Biosciences Institute (AFBI), N. Ireland, on projects funded by FSA (UK), FSAI, Government, Industry and European Union.

Christophe Dufour **Mérieux NutriSciences France, France**



After completing veterinarian studies in Maisons-Alfort France in 1986 and UTC Compiègne University DEA degree, Christophe Dufour joined different food testing laboratories. Entering as scientific manager in Silliker in Mérieux NutriSciences France in 1999, Christophe participates in various normalization groups and expert panels in the field of food microbiology, microbiological criteria, food quality, GMO or Allergens

issues. Christophe contributes to many working groups with professional expertise to develop process criteria for food industry.

Richard Fielder **Elisa Systems, Australia**



Over the last 20 years, Richard Fielder's work with food diagnostic producers has involved leading the business growth of a range of immunological and molecular techniques. He is a qualified Microbiologist, Lead Assessor and BRC Third Party Auditor with a keen interest in advising on the provision of objective evidence for Food Safety & Food Quality from supporting analytical testing. Fielder has been an active participant in the Food Allergen analytical community since 1996

providing or assisting with: presentations, publications, codes of practice, guidance documents, factory inspections, training, working groups (e.g., CEN, WGPAT, ILSI, AOAC International).

Anthony O. “Tony” Flood **International Food Information Council, USA**



As Senior Director, Food Safety and Defense with the International Food Information Council (IFIC) and IFIC Foundation, Tony Flood directs the development and continuation of risk/crisis communication programs among academic, government and industry stakeholders on emerging food safety and defense topics. He provides issue management, stakeholder/media and public outreach on a range of food ingredi-

ent and chemical safety issues as well as food safety education for consumers. Tony has worked with IFIC for 20 years.

Tony is a 1986 graduate of James Madison University (JMU), Harrisonburg, VA, where he received a BS degree in Communications. In addition to his communications degree from JMU, Tony has completed risk communication course work at Harvard School of Public Health's Center for Continuing Professional Education.

Tony is an active member of the International Association for Food Protection (IAFP). He served as a collaborator with the Risk Communication project of the National Center for Food Protection and Defense (NCFPD). Recently, Tony has been named to the Advisory Council of the Joint Institute of Food Safety and Applied Nutrition (JIFSAN) at the University of Maryland in College Park, MD, USA.

Steven Gendel **IEH Laboratories & Consulting Group, USA**



Dr. Steven Gendel is the Vice President, Division of Food Allergens for IEH Laboratories and Consulting Group. Previously he was the Food Allergen Coordinator for the U.S. Food and Drug Administration, responsible for activities related to all aspects of food allergen control and regulation. He has 25 years of experience in food safety science and policy with the FDA. He received a B.S. in Chemical Engineering from Case Western

Reserve University and a Ph.D. in Cell and Developmental Biology from the University of California, Irvine. He held postdoctoral positions at Harvard University and the University of Toronto before joining the faculty of the Department of Genetics at Iowa State University.

Andrea Gianotti **Department of Agricultural and Food Science** **Alma Mater Studiorum, Università di Bologna, Italy**



Andrea Gianotti has completed his Ph.D. in Food Biotechnology at the University of Bologna (Italy) where he teaches Food Microbiology. He participated to several national and European research projects. He is Coordinator of an EU funded project on functional bakery foods and participates to other ones on functional foods and recovery of bioactive compounds from by products. He collaborated with different national

and international food and ingredient companies dealing with microbial food safety, food fermentations and bioactive recovery. He has published more than 70 publications including 28 papers and 6 book chapters.

Bruno Gonzalez-Zorn **Complutense University Madrid, Spain**



Professor Bruno Gonzalez-Zorn is Head of the Department of Animal Health at the Veterinary Faculty in the Complutense de University Madrid. He leads a group devoted to the molecular aspects of antimicrobial resistance and resistance transmission.

Muriel Guyard-Nicodème **French Agency for Food, Environmental and Occupational Health and Safety, France**



Muriel Guyard-Nicodème holds a Ph.D. in Microbiology from the University of Nancy, France. Since 2010, she has joined Anses, the French Agency for Food, Environment and Occupational Health and Safety. She works as a researcher in the Hygiene and Quality of Poultry and Pork Products where she contributes mainly to understand host-pathogen interactions and to develop control measures against *Campylobacter*

in poultry. She is currently involved in several national and EU projects aimed at testing non-antibiotics additives in feed to reduce *Campylobacter* colonization and developing a vaccine against this pathogen in poultry.

Linda J. Harris **University of California, USA**



Dr. Linda Harris is a Specialist in Cooperative Extension in Microbial Food Safety in the Department of Food Science and Technology at the University of California Davis campus. She oversees a research program on the microbial food safety of fresh fruits and vegetables and tree nuts and provides expertise on food safety microbiology throughout the food chain. She is currently President-Elect of the International Association for Food Protection.

Geert F. Houben **TNO (Netherlands Organisation for Applied Scientific Research), Netherlands**



Dr. Geert Houben is Principal Scientist with the Netherlands Organisation for Applied Scientific Research TNO, the Netherlands. He also holds a research position as a Visiting Scientist at the University Medical Centre Utrecht (UMCU), Netherlands. Prior to his appointment as TNO Principal Scientist in 2014, he has had various research and management positions at TNO since

1996 and various research positions at the University of Utrecht, Netherlands, prior to 1996. Geert was trained as a Biologist and Toxicologist. His major scientific specializations are food allergy, food and feed toxicology and immunotoxicology, and particularly risk assessment in these areas. He has a strong international involvement in the development and application of probabilistic approaches in food allergy risk assessment and in the elaboration of reference doses and action levels for precautionary labelling of unintended allergen presence in food products.

Julie Jean **Université Laval, Canada**



Dr. Julie Jean is Full Professor at the Department of Food Science, Université Laval, Quebec City, Canada, since 2003. She is a regular member of the Université Laval's Institute of Nutrition and Functional Foods (INAF) and is leading the food virology laboratory. With her research group, they have developed new approaches for the detection, inactivation and control of pathogens including enteric viruses in food and

environmental samples. She advised more than 25 graduate students and post-doctoral fellows and authored close to 50 scientific publications and book chapters. She serves as member or reviewer on international editorial boards of scientific journals related to food safety.

Dr. Jean teaches the undergraduate courses "Food Microbiology," "Recent Progresses in Microbiological Analysis of Foods" and is also involved in different graduate courses. She is the Director of the bachelor curriculum in Food Science and Technology and the Director of the M.Sc. and Ph.D. programs in Agri-Food Microbiology. During 2015–2016, Julie is detached in sabbatical in which she contributed in stays namely in World Health Organization, Geneva, CH, Health Canada, Ottawa, CA and Nestlé, Lausanne, CH.

Since 2010, she has been the President of the Quebec IAFP Affiliate, AQIA (Association Québécoise pour l'innocuité alimentaire). Over the years, the association has organized several symposia and activities for their members from industry, government and academia.

Prof. Jean received her Ph.D. in Food Science and Technology from Université Laval, Quebec City and M.Sc. and B.Sc. in the same fields. She also did a post-doctoral fellow at North Carolina State University, Raleigh, NC, USA.

Nicholas Johnson **Nestlé, Switzerland**



Dr. Nick Johnson is a Food Safety Microbiologist for Nestlé, at the Nestlé Research Centre in Lausanne, Switzerland. In his role since 2010, he is a project manager and technical expert in the fields of product food safety and spoilage management, risk analysis, thermal and preservation processing, and support to Nestlé R&D, businesses and factories in the acceptance of new products. Dr. Johnson received his B.Sc. in

Applied Biology from Coventry University, UK, and his Ph.D. in Environmental Microbiology from Manchester University, UK. He began his food microbiology career in 2001 as a research scientist at Unilever, UK, and latterly as product microbiologist at Unilever, Netherlands, in 2004.

Ákos Józwiak **National Food Chain Safety Office, Hungary**



Ákos Józwiak is a veterinarian, working for the National Food Chain Safety Office in Hungary. He is the head of the unit responsible for risk-based planning of official controls, including strategic, multiannual and annual planning of control and sampling activities. In that wide range of tasks, he leads all the activities along the whole planning process: from risk assessment and risk ranking, through application of IT tools and

new analysis methods in the planning process, to measuring the outcome and impact of control activities. He is a member of the EFSA Advisory Forum and the official delegate for the meeting of the Heads of European Food Safety Agencies. In addition to the work for the competent authority, he is involved in the education of veterinary and food engineer students.

Kostas Konstantinidis **Georgia Institute of Technology, USA**



Dr. Kostas Konstantinidis is an Associate Professor at Georgia Tech, and holds the Carlton S. Wilder Junior Professorship in Environmental Engineering since 2012. He develops bioinformatics approaches and algorithms, and applies them to understand important questions in evolution and ecology of microbial systems and/or provide biotechnological solutions. He has published more than 60 papers in these areas, ten in

PNAS alone, and received several international distinctions and awards for his work, including the 2010 International Skerman Award from the World Federation for Culture Collections, the 2012 Sigma Xi Young Faculty Research Award, and a 2014 Kavli Frontier Fellowship.

Hilde Kruse

World Health Organisation, Denmark



Dr. Hilde Kruse graduated as a DVM from the Norwegian College of Veterinary Sciences in 1990. She holds a Ph.D. in microbiology (1994, thesis “Antimicrobial Resistance - Epidemiological Aspects”) from the same university, and has also done post-graduate training in epidemiology. Dr. Kruse has dedicated all her professional life to the veterinary public health fields having held different positions in the Norwegian

government, working as a guest researcher on antimicrobial resistance at U.S. Centers for Disease Control and Prevention (CDC), being as Fulbright Fellow to the U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) and since 2007, holding the position as Regional Adviser/Programme Manager Food Safety for the WHO Regional Office for Europe. Dr. Kruse is a diplomat of the European College of Veterinary Public Health and is a former member of the European Food Safety Authority’s (EFSA) Scientific Panel on Biological Hazards. Dr. Kruse has supervised four Ph.D. students and 12 master students, several of them in the area of antimicrobial resistance. She is the author of more than 50 scientific articles in peer-reviewed journals, and has published more than 100 abstracts to international scientific conference and more than 50 scientific reports to national and international authorities. Dr. Kruse has been actively involved in communication activities, and has been giving interviews on food safety, antimicrobial resistance and zoonoses to TV, newspapers, and radio - both nationally and internationally.

Roberto Lattanzio

Eurofins Analytik GmbH, Germany



Roberto Lattanzio works as Laboratory Manager at Eurofins Analytik GmbH in Hamburg, which is part of the company’s food division. As a biologist, Roberto’s professional focus lies on the detection of food allergens, animal species determination and irradiation testing. Establishing routine methods and procedures to analyze a broad spectrum of raw and finished products as well as environmental samples has been his

main task after joining the laboratory in 2010. He came across the topic of allergies in the mid-nineties by testing patient sera for specific IgE and IgG. After receiving his diploma in biology from the Karlsruhe Institute of Technology (KIT), having studied molecular processes during embryonic development, he was excited to encounter the subject of allergens again in his subsequent career.

Alvin Lee

Illinois Institute of Technology/IFSH, USA



Dr. Alvin Lee is a microbiologist and virologist with more than 15 years research experience with a Ph.D. from RMIT University. Dr. Lee currently leads IFSH Center for Processing Innovation and co-leads the joint IFSH/FDA Microbiology Research Platform on food safety and defense related projects. He leads the Prevention and Control CORE of NoroCORE, a USDA-NIFA Food Virology Collaborative based at North Carolina

State University. Current research support includes funding from USDA, US FDA, National Center for Food Protection and Defense (NCFPD), Department of Homeland Security (DHS) and various industry contracts. Dr. Lee is an instructor for food microbiology in the IIT’s Master’s of Science program and has mentored more than 25 graduate students and post-doctoral fellows. He is currently an active member of the International Association for Food Protection - serving on the IAFP Scientific Program Committee, American Society for Microbiology and Institute of Food Technologists - serving on the Annual Scientific Meeting Program Advisory Panel.

Patrick Lucas

Bordeaux University, France



Patrick Lucas is Professor of Wine Microbiology at the Institute of Vine and Wine of Sciences of Bordeaux University. His research focuses on wine lactic acid bacteria and malolactic fermentation, with a special emphasis on the species *Oenococcus oeni*. He is using omics-based strategies to investigate its biodiversity, its adaptation to wine(s) and its contribution to wine quality improvement during malolactic fermentation.

He has collaborations with winemakers, professionals of the wine industry and academic partners from projects such as DECARBOXYLATE (FP5), BIAMFOOD (FP7), WILDWINE (FP7) and MICROWINE (H2020). He is author of about 40 per-reviewed publications.

Balkumar Marthi

Unilever, Netherlands



Building on a strong technical and research foundation in Microbiology, Balkumar Marthi developed leading capabilities in Hygiene, Food Safety, Risk Assessment & HACCP, Biotechnology & Sustainability. He is currently Expertise Director and Principal Food Microbiologist at Unilever R & D Vlaardingen, Netherlands.

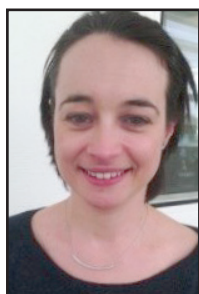
Over the years in Unilever, Dr. Marthi has been successful in building leadership positions in Food Safety in India, where he was one of the pioneers in implementing “Safety by Design” principles in the business. He was also instrumental in influencing adoption of these international principles by the Indian Government – through training, advocacy and participation in high-level Government Committees/Working Groups.

Dr. Marthi has extended this reach considerably after moving to the Netherlands. Within Unilever, he led the Global Microbiology, Biotechnology, Preservation and Risk Assessment Expertise for the Foods Business. Dr. Marthi is Unilever’s Chief Food Microbiologist, thus responsible for Preservation Innovation, Food Safety and Hygiene strategy, implementation and monitoring.

He has also led activities to build bridges with the external scientific world as well as Government and Civil Society to drive strategic R & D and policy agendas in Microbiology & Food Safety. He has been very active in Food Safety-linked technical challenges and represent Unilever on Dutch & EU Programme Committees and initiatives in this area.

Sandra Martin-Latil

Anses, France



Sandra Martin-Latil obtained her Ph.D. in 2004 at the University of Paris XI, France. During the period 2004 to 2009, she performed two post-doctoral stages at the Pasteur Institute (Paris, France). Her research aimed at investigating the interactions of viruses with the human intestinal epithelium for a better understanding of viral diseases.

She is a research scientist at the French Agency for Food, Environmental and Occupational Health & Safety (Anses) since 2009. Her current research interests are varied and focus on food virology: improvement of the existing methods for detecting viruses in complex food matrices, development of methods for measuring infectivity of enteric viruses in food (using cellular systems and/or combined approaches) and understanding of the mechanisms used by enteric viruses for crossing the intestinal epithelial barrier. She has mentored several students and authored or co-authored 23 peer-reviewed publications. She is also a member of the CENTC275/WG6/TAG4 “Viruses in Foods” working group responsible for writing the draft technical specification “Microbiology of food and animal stuffs–Horizontal method for detection of hepatitis A virus and norovirus in food using real-time RT-PCR.”

Leena Maunula

University of Helsinki, Finland



Dr. Leena Maunula is senior scientist and university lecturer working currently at the Department of Food Hygiene and Environmental Health at the University of Helsinki (UH). After graduation as a microbiologist, Dr. Maunula performed her doctoral thesis at the Department of Virology at Haartman Institute (UH). The main research interest of Dr. Maunula and the Ph.D. students under her supervision is in food and environmental

virology, especially tracing transmission routes of enteric viruses between humans, food, environment and animals. Dr. Maunula has joined international expert groups at the European level and participated in several European research projects.

Alejandro Mazzotta

Chobani, LLC, USA



Dr. Alejandro Mazzotta was appointed Vice President of Global Quality, Food Safety and Regulatory Affairs with Chobani in 2013. Prior to joining Chobani, Dr. Mazzotta held various positions with Campbell Soup Company, McDonald’s Corporation, and the Pillsbury Company with General Mills. He joined the National Food Processors Association (currently GMA, Grocery Manufacturers Association) in Washington,

DC in 1998, and conducted research on the heat resistance of *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 in different food products. He became a trainer for the Food Processor Institute and conducted training in Hazard Analysis Critical Control Points (HACCP) and juice pasteurization for numerous member food companies. Between 1986 and 1990, he worked as a Scientist for the Argentine Antarctic Institute. Dr. Mazzotta was posted in the Antarctic Peninsula for two years, where he conducted research on the biochemical composition of Antarctic fish and krill with a focus on the fluoride content in derived meals, as part of a national fishery program. Dr. Mazzotta was elected to serve in the International Association for Food Protection (IAFP) Executive Board for a five-year term of successive positions, and currently serves as IAFP President. Dr. Mazzotta was appointed to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) from 2004 to 2009, where he made significant contributions to several NACMCF reports. He serves on the advisory boards for the Center for Food Safety at the University of Georgia (CFS) and bioMérieux, and is a member of the Institute of Food Technologists (IFT). He has also been published in more than 25 publications in peer reviewed scientific journals in both English and Spanish, and has spoken at numerous international meetings and symposia. He served on the Editorial Boards of Applied and Environmental Microbiology from the American Society for Microbiology and the *Journal of Food Protection* from International Association for Food Protection (IAFP). Dr. Mazzotta is a native of Argentina. He earned a M.S. degree (1985) in Biological Sciences from the University of Buenos Aires. He earned a Doctoral Degree (1997) in Food Science from Rutgers, The State University of New Jersey.

Nina McGrath

European Food Information Council, Belgium



Dr. Nina McGrath joined the European Food Information Council (EUFIC) in May 2015. As Food Safety and Risk Communications Manager, she manages the production of science-based communications on food safety and quality related topics. Prior to this, she worked as Science Counsellor for Euro Chlor, the European chlorine industry federation, where she advised on environmental science issues. She holds a Master's

degree in Chemistry and a Ph.D. in Inorganic Chemistry and Materials Science from the University of Bristol, UK.

Pedro Medel

IMASDE AGROALIMENTARIA, Spain



Pedro Medel is Agricultural Engineer (Universidad Politécnica de Madrid, 1996) and has his Ph.D. in animal nutrition (Universidad Politécnica de Madrid, 2000). He is founder and director of Imasde Agroalimentaria S.L. (2000-today) and Professor of Animal Nutrition at the Veterinary School of the Alfonso X EL Sabio University (2005-2011). Dr. Medel has long experience in Research and Development projects,

founded at Spanish and European level. He is the scientific coordinator of FPVII European project CAMPYBRO. He has been published many times, with 15 peer reviewed articles and more than 50 communications in international congress. Scientific stays in: i) DIAS, Foulum, Denmark; ii) University of Saskatchewan, Canada; iii) Agriculture and Agr-Food Canada, Lennoxville, Canada; iv) University of Iowa, USA; v) University of Maryland, USA.

Nicolas Meneses

Bühler AG, Switzerland



Nicolas Meneses is an Expert on Food & Feed Safety in the Corporate Technology Unit at Bühler AG, Uzwil, Switzerland. He has been working four years in charge of developing technologies for food safety of low water activity food and feed.

Nicolas worked over 3 years in the Department of Food Biotechnology and Food Process Engineering at the Technische Universität Berlin as a Research Associate involved in European and national projects.

He also carried out his Ph.D. studies on non-thermal technologies, has published over 20 articles and presented at over 40 international conferences where he has been awarded with several prizes for the best paper student competition. Nicolas was also Student Representative for Europe at the Non-thermal Processing Division, IFT (2011).

Aline Metris

Institute of Food Research, United Kingdom



Dr. Aline Metris has been working in the field of predictive microbiology for food safety at the Institute of Food Research in the UK for fifteen years. She has been participating in many European projects as well as in the ComBase project. She has developed techniques to study foodborne pathogen kinetics at the single cell level in order to quantify variability in microbiological risk assessments. More recently she

turned to systems biology in order to better understand how molecular interactions allow some cells to survive stresses imposed along the food chain.

Clare Mills

University of Manchester, United Kingdom



After a short spell in London at the Department of Health after she completed her Ph.D., Prof. Clare Mills went to work at the BBSRC Institute of Food Research (IFR) in Norwich where she became a head of the Physical Biochemistry Group in 1999. In 2005, she took over the leadership of the food material science research at IFR and working with four other research leaders developing a new programme of research

relating food structure to health benefits of foods. This also involved promoting a transdisciplinary approach, linking physical scientists with physiologists, clinicians and psychologists to achieve its overall aims and goals. In her capacity as a BBSRC Institute Strategic Programme Grant leader, she was also a member of the IFR Executive Board. In 2011, she moved to the University of Manchester to take up her current position. Based in the Manchester Institute of Biotechnology and working with the Respiratory and Allergy Research team at the University Hospital of South Manchester led by Professor Adnan Custovic, she is now applying molecular science to understand, better diagnose and treat food allergies. This research stems from work she has done through a series of projects funded across several EU Frame Work Programmes. Through these projects she developed a network of researchers that put forward the expression of interest on food allergy which subsequently the consortium applied for, and won, and came on to become the EuroPrevall project. Successfully completed at the end of 2009, the future challenge will be to realise the knowledge currently locked up in the large amounts of data and biological samples (including DNA) collected through the project activities in the coming years to understand the basis of food allergies and deliver more effective management strategies.

James Monaghan

Harper Adams University, United Kingdom



Jim Monaghan has worked in crop production for over 20 years. Following a Biology degree at UCNW Bangor, Jim researched aspects of crop production at Harper Adams and John Innes Centre (Ph.D.), Newcastle University, HRI-Efford and HRI-Wellesbourne. Jim then had a look at the real world for three years at Marks and Spencer as Salads Technologist, where he had responsibility for food safety, pesticide

residue minimisation, and compliance with codes of practice for all salad products and salad ingredients in minimally processed foods, before heading back to Harper Adams to develop teaching and research in the area of fresh produce production in 2005.

Jim leads the Fresh Produce Research Centre at HAU, which is focused on fresh produce production, particularly leafy vegetables and covers three areas: 1) identifying genetic traits that may lead to more sustainable crop production; 2) agronomic manipulation of post-harvest quality and nutritional content in crops; and 3) developing and implementing food safety systems in fresh produce. Jim also chairs the Technical Advisory Committee for Red Tractor Produce.

Eleni Myrivili

University of Aegean and City of Athens, Greece



Eleni Myrivili received her Ph.D. in Social Anthropology from Columbia University, New York. She is an Assistant Professor in the Department of Cultural Technology and Communications, University of the Aegean, Greece. She has been active in projects that go beyond the walls of the academy: designing multimedia exhibitions/applications, curating events/festivals, participating in civil society organizations, and hosting public

television documentary series on sustainability. She has been a member of EastboardNet (COST ESF), a Deputy Coordinator for EUNAMUS (FP7) and a researcher for the Centre National d'Art et de Culture George Pompidou, France. Currently, she is an elected Council member in the City of Athens (urban-sustainability portfolio) and the Chief Resilience Officer for Athens (100ResilientCities).

Luís Augusto Nero

Universidade Federal de Viçosa, Brazil

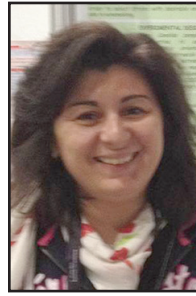


Prof. Luís Augusto Nero has a degree in Veterinary Medicine and Doctorate in Food Sciences. He is a professor in Universidade Federal de Viçosa, Brazil, since 2005, teaching Food Inspection and Microbiology for Veterinary Medicine students. His research area is food microbiology, with many scientific projects and articles in foodborne pathogens and lactic acid bacteria. Considering this area, his main interests are:

epidemiological studies and identification of key contamination points of foodborne pathogens in the food chains, bacteriocins characterization and interactions among foodborne pathogens and lactic acid bacteria in food matrices.

Aspasia Nisiotou

ELGO-'DEMETER', Greece



Aspasia Nisiotou, a wine microbiologist, received her M.Sc. in food biotechnology from the University of Reading and a Ph.D. in food microbiology from the Agricultural University of Athens. She is currently a researcher at ITAP of the National Agriculture Research Foundation of Greece. Her work has been focused on food and wine microbiology with special interest in molecular microbial ecology and in spoilage and safety aspects of foods. She has participated as a researcher or coordinator in national and European research programs and has published several papers and book chapters.

George-John Nychas

Agricultural University of Athens, Department of Food Science and Human Nutrition, Greece



George Nychas is Professor of Food Microbiology at the Agricultural University of Athens. In the last 25 years, he has coordinated 4 and participated in more than 30 EU projects (budget >6M€), while his research interests focus on: a) the assessment of food safety and spoilage through microbiological and physicochemical analysis (metabolomics) in combination with advanced statistical methods, b) responses of stress

adapted pathogens grown either planktonically or as biofilms, and c) modelling the behaviour of pathogenic bacteria for the assessment of food safety. He has published 182 papers in SCI journals & 30 book chapters as invited author (ca. 6600 citations, h = 45).

Cian O'Mahony

Creme Global, Ireland



Cian O'Mahony is head of Expert Modelling and Statistics at Creme Global. He has a BSc in Mathematical Sciences and an M.Sc. in Applied Mathematics and Pharmacy. He currently leads a team of analysts developing exposure and risk assessment models in a number of areas including food and cosmetics, working with both industry and regulatory bodies. Many of the models developed at Creme Global are now built

into a range of software applications used by regulators and industry. He is also involved in a number of expert panels and groups including at ILSI, ECETOC, and the JRC.

Konstantinos Papadimitriou

Agricultural University of Athens, Greece



Konstantinos Papadimitriou was awarded his Ph.D. degree on the stress physiology of lactic acid bacteria in 2006 with distinction. Since then, Dr. Papadimitriou has worked in different projects funded by EU or national funds as a research associate in the group of Prof. Tsakalidou. His main research interests include the microbiology of milk and milk products, the physiology, the genetics and the genomics of lactic acid bacteria, the

metagenomics of food ecosystems, single cell microbiology, plasmid biology and applied bioinformatics. Dr. Papadimitriou has published twenty-one original research articles and has received more than 200 citations. He and Prof. Tsakalidou are the editors of the book entitled *The Stress Physiology of Lactic Acid Bacteria* published by Springer (2011). Furthermore, Dr. Papadimitriou is a member of the editorial board of Applied and Environmental Microbiology (ASM) and he has served as an ad-hoc reviewer in more than twenty research journals.

Annemarie Pielat

National Institute for Public Health and the Environment, RIVM, Netherlands



Annemarie Pielat has a Master's in Mathematical Biology in the field of ecotoxicology at the Free University of Amsterdam. Her Ph.D. graduation was on 'Splash Dispersal of Plant Pathogens' at the Wageningen University, after which she had a two year post-doc position at the University of Alberta, where she did research on 'Long-distance Seed Dispersal'. Currently, she works at the Dutch Institute for Public Health and the

Environment (RIVM). Her main interest is in setting up biologically relevant experiments, sampling plans and subsequent statistical data analysis as input for microbiological risk assessment. The last few years she has been working on methodology development for the implementation of molecular data in Hazard Characterisation.

Marie-France Pilet

INRA-Oniris, UMR1014 SECALIM, France



Marie-France Pilet is Assistant Professor in Food Hygiene and Microbiology at National College of Veterinary Medicine, Food Science and Engineering (Oniris) at Nantes, France, and Head of the Joint Research Unit Food Safety and Microbiology INRA - Oniris (UMR1014). Her research activities are focused on microbial ecosystems of seafood products and bacterial interactions. She has worked on selection and characterization of

protective lactic acid bacteria (LAB) showing inhibition activities against *Listeria monocytogenes* or spoilage flora, investigation of their inhibition mechanisms including bacteriocin production, and protective LAB application for the biopreservation of seafood.

Florence Postollec

ADRIA UMT14.01 SPORE RISK, France



Florence Postollec is project manager in ADRIA food technology institute, Quimper (Fr). Within the frame of a competitive technological cluster ACTIA UMT 14.01 SPORE RISK, she is collaborating with the Mafart Team on risks associated to spore-former contaminants. She was trained as biochemist and obtained a Ph.D. degree on bacterial interactions at the faculty of medical sciences in Groningen (NI). She gained

experience on molecular microbiology when she integrated ADRIA in 2005 as a post doc working on the detection and identification of sporeformers involved in food spoilage.

Bruno Pot

Pharmabiotic Research Institute, France



Bruno Pot started an academic career as microbial taxonomist at the Laboratory of Microbiology (University Ghent), where he obtained a Ph.D. in Biology. His research interest in lactic acid bacteria started in 1986 and continuous today. Bruno is currently Director of Business Development at bioinformatics company Applied Maths and Research Director of the lab 'Bactéries Lactiques et Immunité des Muqueuses' of

the Institut Pasteur de Lille, with research topics on probiotics and microbiota. In parallel, Bruno is a guest professor at the Vrije Universiteit Brussel for food microbiology. Since 2014 he is president of the Pharmabiotics Research Institute.

Bizhan Pourkomialian

McDonald's Corporation, United Kingdom



Bizhan Pourkomialian holds an Hons. Degree in Biochemistry, Ph.D. in Microbial Physiology from Aberdeen University and MBA from Warwick University. He holds the Chair for the Microbiology Members Interest Group at Campden BRI, Vice Chair for Microbiological Food Safety Task Force of ILSI Europe, and is a member of GFSI technical committees.

After graduating in 1994, Dr. Pourkomialian took the position of research scientist at Unilever Research, where he was involved in sauce development and botulinum laboratory management. In 1997, he moved to Leatherhead Food International to become Principal Scientist. The role required developing, training and auditing food safety systems.

Dr. Pourkomialian joined McDonald's Europe in January 2000 and is now the Global Restaurant Food Safety and Consumer Product Safety Director. He is responsible for providing direction and developing management systems for the company on matters relating to food and consumer product safety at restaurants. In this role advice is also given to the supply chain, farm to across the counter, in 120 countries across the world. The world includes over 36000 restaurants and over 3500 supplier facilities.

Ans Punt **RIKILT Wageningen University and Research Center, Netherlands**



Dr. Ans Punt earned her Ph.D. degree (Cum Laude) in Toxicology from Wageningen University in 2009 in collaboration with Nestlé Research Centre. Between 2010 and 2015 she worked as an assistant professor at the division of Toxicology at Wageningen University. Within her research, she successfully developed various in physiologically based kinetic and dynamic (PBK/D) models to simulate the fate and effects of naturally

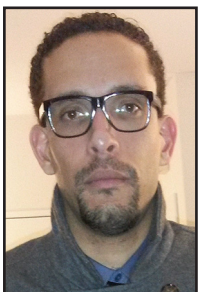
dietary ingredients in intact organisms and has over 35 publications in this field. Dr. Punt is both a Dutch and European Registered toxicologist and currently works as Food Toxicologist at RIKILT Wageningen University and Research Center. Dr. Punt chairs the ILSI Europe Expert Group on “Exploitation of ToxCast Data on Food Chemicals for Risk Assessment” of the “New Approaches to Chemical Risk Assessment Task Force.”

Paul Ross **University College Cork, Ireland**



Paul Ross graduated from University College Cork with a B.Sc. in Biochemistry and Microbiology, and a Ph.D. in Microbiology. Following postdoctoral research and an Assistant Professorship at Wake Forest University, NC, he moved to Teagasc's Moorepark Food Research Centre to lead the Food Biotechnology programme, which encompasses aspects ranging from gut flora to novel antimicrobials, including bacteriocins and bacteriophage. Until recently he was Head of Food Research at Teagasc and Adjunct Professor at UCC. Paul is now Head of College of Science Engineering & Food Science at UCC and is a Principal Investigator in the APC's Microbes to Molecules Research Spoke and the Food Health Ireland research centre. He was awarded a D.Sc. in 2009 based on published work and received the William C. Haines Dairy Science in 2007 and the Enterprise Ireland Commercialization award in 2008.

Manuel Jimmy Saint-Cyr **ONIRIS/INRA, France**



Dr. Saint-Cyr's background is in human health and food safety. He obtained his Ph.D. from University of Rennes I in December 2013. His thesis was done at ANSES (French Agency for Food, Environmental and Occupational Health & Safety) in the Antibiotics, Biocides, Residues and Resistance Unit. He investigated the impact of mycotoxins on the human gut microbiota. He is working as a postdoctoral research fellow at ONIRIS (Nantes Atlantic National College of Veterinary Medicine, Food Science and Engineering) in the Secalim unit, since October 2014. His research focuses on *Campylobacter* and probiotics use to reduce this foodborne pathogen at the farm level.

Benoit Schilter **Nestlé Research Center, Switzerland**



Benoit Schilter earned his a Ph.D. in biology at the University of Lausanne, Switzerland. After a postdoctoral fellowship in neurotoxicology, he was visiting scientist at the University of Washington. He then joined the Nestlé Research Center, Switzerland, as head of molecular toxicology, and currently as head of the Chemical Food Safety Group. Dr. Schilter has more than 25 years of experience in toxicology/risk assessment. He has

established over 200 safety/risk evaluations and is the author of more than 100 peer-reviewed publications/reviews/book chapters. Dr. Schilter has contributed, as member or chair, to over 35 international scientific expert groups and task forces.

Oliver Schlüter **ATB, Leibniz-Institut für Agricultural Engineering, Potsdam-Bornim e.V., Germany**



Oliver Schlüter is the coordinator of the research program on “Quality and Safety of Food and Feed” at the Leibniz Institute for Agricultural Engineering Potsdam e.V. (ATB). The ATB is a European Center of Agricultural Engineering Research at the nexus between biological and technical systems. The research targets a knowledge-based bioeconomy by developing highly innovative and efficient technologies for the use of natural resources in agricultural production systems – from

basic research to application.

Dr. Schlüter is also Head of the ATB working group on food safety and vice-head of the Department of Horticultural Engineering. His research work focuses on emerging technologies in primary food production (fruits, vegetables, milk, meat, edible insects) and fresh food processing (high pressure, ozone, plasma, etc.), optimization and innovation of processing steps along the food chain of perishables including quality and safety monitoring.

Among others, Dr. Schlüter is member-at-large of the IFT Food Engineering Division, member of the Executive Committee of EFFoST, Secretary of the Technical Board of CIGR Section VI: Bioprocesses, and German representative of IFA (ISEKI Food Association) and GHI (Global Harmonization Initiative). He is member of the editorial board of *Innovative Food Science and Emerging Technologies*, *Journal of Insects as Food and Feed*, and *International Journal of Food Science*. Click here for his website: <http://www.atb-potsdam.de/en/institute/about-us/team/portrait/portrait/oliver-schluter.html>.

Jenny Scott **U.S. Food and Drug Administration-CFSAN, USA**



Jenny Scott is a Senior Advisor in the Office of Food Safety with the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition, where she leads the FDA teams on the Preventive Controls for Human Food rule and guidance. She is a past president of the International Association for Food Protection and a fellow of both IAFP and the Institute of Food Technologists. In addition, she serves as the U.S. Delegate to the Codex Committee on Food Hygiene.

Torstein Skara **NOFIMA, Norway**



Torstein Skara graduated from the Norwegian Institute of Technology, at the department of Organic Chemistry. He continued his studies at Cornell University, where he obtained his M.Sc. in Food Science. Having worked for a number of years with research in fish processing, he was awarded his Ph.D. at the KU Leuven (Department of Chemical Engineering), for research on modelling of inactivation of *Listeria* on fish products, by steam surface pasteurization. Throughout his career, Torstein Skara has been working mainly with applied research in processing and preservation. In recent years, however, with an increasing interest in modelling and model systems.

Danièle Sohier **ADRIA, France**



Danièle Sohier is heading the Food Safety & Quality unit in ADRIA (www.adria.fr), a Food Technology Institute qualified by the French Ministry of Agriculture. The team is involved in food microbiology R&D activities and expertises, and coordinates national and European innovation programs in close collaboration with food and diagnosis industrial partners. In relation with the oral communications planned at the IAFP-Eu meeting, ADRIA is one of the most important expert lab, which provides validation studies of alternative methods. Danièle participates to the AFNOR and MicroVal Certification committees. She is also involved in the ISO working group dealing with the ISO 16140 standard revision, as well as in the AOAC International Stakeholder Panel on Alternative Methods. ADRIA is as well managing the SPORE-RISK Joined Technology Unit, which deals with food safety and spoilage risks linked to spore-forming bacteria. Indeed, Danièle is the convenor of the ISO working group on "Guidelines for conducting challenge-tests."

Katherine M.J. Swanson **KMJ Swanson Food Safety, Inc., USA**



With over 30 years of experience, Katherine M.J. Swanson provides food safety consulting and is Curriculum Development Program Manager for the Food Safety Preventive Control Alliance. She led food safety efforts at The Pillsbury Company, General Mills and Ecolab, and is an IAFP past president and fellow, an IFT fellow, and secretary of the International Commission on Microbiological Specifications for Foods. She contributed to numerous scientific committee reports, books and papers, and delivered over 100 presentations around the globe. She earned her B.S. from the University of Delaware, and M.S. and Ph.D. from the University of Minnesota, and numerous awards and recognitions.

Erini Tsigarida **Hellenic Food Authority, Greece**



Dr. Eirini Tsidarida, Hellenic Food Authority, Head of Nutrition and Research Policy Directorate. Eirini Tsigarida graduated from the Department of Food Science and Technology of the Agricultural University of Athens, where she also received her doctorate in Food Microbiology.

In 2002, as scientific officer of the Hellenic Food Authority (EFET), she gained experience as a national expert on the European legislation on microbiological criteria of foodstuffs, as a scientific member of the working group of EFET on the preparation of guidelines of Good Hygiene and Manufacturing practices of various food sectors, and as a coordinator of scientific panel of EFET on specific food safety risks.

In 2005, she joined the Unit of Biological Hazards (BIOHAZ) of European Food Safety Authority where she was the scientific co-ordinator of the working groups related to food microbiology and hygiene. Her working experience in the European Food Safety Authority has contributed to expertise on working in a multicultural environment, coordinating working groups in the area of microbial risk assessment and supporting close liaison with Member States and with the Commission services responsible for the development and implementation of European legislation in the field of food safety.

Eirini Velliou **University of Surrey, United Kingdom**



Dr. Eirini Velliou is a Lecturer (Assistant Professor) at the Department of Chemical and Process Engineering of the University of Surrey, UK. Her research interests fall within the domain of bioprocess optimization under stress. More specifically, she is focusing on understanding the impact of environmental stress on the response and potential development of resistance in biological systems, i.e., microbial cells, healthy/diseased human cells and, micro-algae cells. Within the domain of predictive (food) microbiology she has experimentally studied and mathematically quantified, through parameter estimation, the effect of a variety of factors, i.e., acids, proteins, chemicals etc., on the heat stress adaptation of food pathogens in liquid and solid(like) systems. Lately, she is focusing on understanding, quantifying and further predicting the antimicrobial resistance development of food pathogens when exposed to natural antimicrobials in food(model) systems.

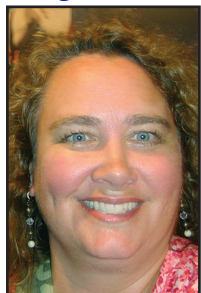
Marjon Wells-Bennik NIZO Food Research, Netherlands



Dr. Marjon Wells-Bennik is Principle Scientist Food Safety at NIZO food research. She obtained her Ph.D. in Food Microbiology at Wageningen University in the Netherlands and was a Postdoctoral Fellow (molecular microbiology) at Harvard University (Boston). She subsequently worked as a Scientist at the Agrotechnological Research Institute (Wageningen) supervising Ph.D. projects on

Listeria monocytogenes and *Bacillus cereus*, and continued research on sporeformers, namely *Clostridium botulinum*, at the Institute of Food Research (Norwich UK) from 2001 to 2004. Since she started at NIZO in 2004, she has managed diverse projects relating to contaminants in foods, and over the past four years, this included a program on heat-resistant bacterial spores which was funded by the Top Institute Food and Nutrition TIFN, a public-private centre of excellence in the Netherlands.

Pamela Wilger Cargill, USA



Pamela Wilger received her B.S. and M.S. degrees in Bacteriology from the University of Wisconsin at Madison where she also worked at the Food Research Institute. After graduating, she worked for Quest International for just over 11 years in two different locations as the facility microbiologist and lab supervisor and then a researcher in product development of fermented Hydrocolloids. In January of 2001, Pamela joined

Cargill's Corporate Food Safety, Quality & Regulatory Affairs Department as a Global Food Safety and Microbiology Specialist. As of September 1st, 2015 she is now a Senior Applied Food Safety and Quality Microbiologist working more closely with the Cargill businesses' manufacturing facilities and laboratories. Pamela is a very active member of the International Association for Food Protection (IAFP), AOAC International, and represents the U.S. through ANSI on the ISO Technical Committee 34/Sub-Committee 9 Microbiology including Working Group 3 for Method Validation.

Anett Winkler Kraft Foods R&D Inc., Germany



Anett Winkler joined Kraft Jacobs Suchard in December 1998 to head up the research microbiology laboratory in Munich. She developed the internal Microbiological Methods Manual before moving towards manufacturing and Research & Development microbiological support in all Kraft Foods categories (Cheese, Coffee, Chocolate, Beverages). Later on, Anett concentrated on chocolate, biscuits and other low-moisture

foods including supplier developments and approvals. She also consolidated the scientific basis for microbiological process controls in low-moisture foods by performing validation studies for nut and cocoa processing. Following a regional role for Microbiology in the Eastern European, Middle East and African Region she is now globally designing food safety programs,

rolling out training modules related to food safety and further supporting supplier development. She is also the global expert for thermal processing within the company. Before moving to industry, Anett had worked in Basic Research on different projects in Germany and the USA looking at molecular and biochemical changes during drug withdrawal and studying relapse mechanisms. She has studied Microbiology in Germany, but completed her Master's degree at the Lomonosov State University in Moscow about mutations in bacteriophages. After that she returned to Germany where she obtained her Ph.D. on work about stress responses in *Bacillus subtilis*.

Frank Yiannas Walmart, USA



As Vice President of Food Safety and Health, Frank Yiannas oversees all food safety, as well as other public health functions, for the world's largest food retailer, Walmart. Training and education of thousands of associates, hundreds of food suppliers, and a number of critical regulatory compliance issues also come under his purview. Prior to joining Walmart in 2008, Frank spent 19

years as Director of Safety & Health for the Walt Disney World Company. In 2001, under his tenure, Walt Disney World received the prestigious Black Pearl Award for corporate excellence in food safety by the International Association for Food Protection. He is the 2007 recipient of the NSF International Lifetime Achievement Award for Leadership in Food Safety. Frank is also a Past President of the International Association for Food Protection (IAFP) and the Past Chair of the Food Allergy and Anaphylaxis Network's Board of Directors. He is the author of the book, *Food Safety Culture, Creating a Behaviour-based Food Safety Management System*, by Springer Scientific. Frank holds memberships with several professional associations, including the National Environmental Health Association, the American Society of Microbiology, and the Institute of Food Technologists.

Sophie Zuber Nestlé Research Center, Switzerland



Sophie Zuber works as Food Safety Microbiologist at the Nestlé Research Centre, based in Lausanne, Switzerland. She received her Ph.D. in Microbiology from the Department of Genetics, University of Melbourne, Australia. In her current position, her principal responsibilities include providing scientific advice and guidance on possible risks of viruses in the food chain and developing risk management strategies in this field. In

this context, Sophie Zuber has published peer-reviewed publications focusing on the effects of treatments used in food processing on viruses.

11-13 May 2016 • Athens, Greece

Symposium Abstracts



IAFP'S EUROPEAN
SYMPOSIUM
ON FOOD SAFETY
11-13 MAY 2016 • ATHENS, GREECE

Symposium Abstracts

Opening Session

Food Safety Management Systems

ALEJANDRO MAZZOTTA, Chobani, LLC, New York, NY, USA

If we were to predict the future, I have no doubt that soon we will be surprised to find an “unexpected” outbreak related to a food that has been always considered innocuous, and has been out of the scope of food safety topics, symposia and research activities. That was the case of surprising events from botulism in yogurt in the UK (1989), *Salmonella* in peanut butter (2007), *Listeria monocytogenes* in cantaloupe (2011) and in caramel apples (2011) in the U.S. The food safety community jumps on full investigations, research, and implementation of programs in processing plants after the devastating news. But, how can we be more proactive and establish stronger food safety management systems that rely on a realistic hazard analysis; a hazard analysis that is constantly revised to incorporate changes in practices, ingredients, operation efficiencies, distribution and consumer behavior. We have become experts in studying the past, but we need to continue to educate and promote appropriate food safety practices to prevent incidents that affect public health.

Risk-based Approaches to Food Safety

EIRINI (RENA) TSIGARIDA, Hellenic Food Authority, Athens, Greece

The current food safety legislation requires the following principles (i) a comprehensive and integrated approach to food safety throughout the whole food chain, (ii) the precautionary principle, (iii) the responsibilities of food business operators, (iv) the establishment of traceability at all stages of production, processing and distribution, (v) the transparency through public consultation and information, and (vi) the requirement of food law and any subsequent measures to be based on comprehensive and integrated scientific advice and a shift from hazard analysis approach-to-risk analysis approach." The hazard-based approach represents an operational system to select and implement effective control measures to ensure the safety of food product. The approach is that the simple presence of a potentially harmful agent at a detectable level in food is a basis for legislation and/or risk management action and potential reason for considering a food unsafe. Risk-based approaches, on the other hand, consider the likelihood and the nature of consequences of an exposure to decide if a food is unsafe. The “risk analysis” approach is a structured process consisting of three separate but interconnected elements: risk assessment,

risk management and risk communication. It is now a fact that food safety risk analysis has been repeatedly developed and integrated into the food safety policy at national, international and European level as a tool to encourage science-based decision-making processes, to improve food control systems, to identify gaps in scientific knowledge, to set priorities among different food safety problems and to contribute to smooth domestic and international food trade. There is no golden rule of which approach to follow when there is a need to take a risk management measure. Each approach has its advantages and its disadvantages and the use of each one depends on various factors such as the context of the food safety problem, the availability of data and time, the uncertainties and the technical capacity.

S1 Challenges and Promises of Systems Biology for Food Safety

As the cost of sequencing continues to fall and the use of omics technologies such as RNA-sequencing is rapidly progressing, massive amount of data continues to be generated for lab-strains as well as many real food isolates. This ‘data deluge’ provides new opportunities to design novel strategies for antimicrobial intervention in the food chain. In particular, in-silico approaches could potentially be applied to identify new target sites to prevent either spore germination or growth of vegetative pathogens and promote die-off.

However, these means are seldom exploited in the food industry because appropriate methods to model them have yet to be established. We propose a symposium that illustrates both the promises and the challenges of using Systems Biology approaches for food safety in practical cases.

The symposium will begin with a presentation on how genome sequences can be converted into genome-scale metabolic or regulatory models for foodborne pathogens such as *Campylobacter* and *Salmonella*. Then we will explore how the metabolic networks can be analysed to unravel potential antimicrobial targets or select optimized combinations of antimicrobials which efficiently inhibit organisms and prevent resistance. Finally, we will illustrate how proteomics can advance the understanding of spore germination and maturation and hence the possibility of finding targets for intervention.

This symposium will be of interest to students and professionals of all knowledge levels and expertise in the food industry, academia, as well as regulatory agencies who have an interest in exploiting molecular biology data.

From Genomes to Mathematical Models for Systems Biology

ALINE METRIS, Institute of Food Research, Norwich, United Kingdom

Systems biology is a discipline which has mainly been developed in biotechnology for enhancing yields of production of bacterial by-products or synthetic biology. As a consequence, models have been developed with organisms used in biotechnology, such as *Escherichia coli* and *Saccharomyces cerevisiae*, and in environmental conditions that are not necessarily relevant to the food industry. We propose that existing modelling methods could be used in the food industry to design novel strategies for antimicrobial intervention in the food chain. However, the models have to be extrapolated to foodborne pathogens and genomic sequences provide insufficient information; condition specific data are also essential to build meaningful models.

In the first part, we will illustrate how a metabolic model of *Campylobacter jejuni* was derived from an existing model of *Helicobacter pylori*. Metabolic networks can be analysed mathematically with constrained-based analysis. How this method may be exploited in the food industry will be illustrated in the subsequent talk, so the method will not be detailed but the steps involved in the conversion of the genome annotation into a mathematical model will be explained.

In the second part, we will introduce a qualitative method to model the response of bacteria to osmotic stress and show the challenges of inferring a model for *Salmonella* from *E. coli*. We will also prove that potential regulatory interactions deduced from bioinformatics analyses are not sufficient to explain regulation as they are condition specific.

Application of Metabolic Network Models to Develop New Preservation Strategies: An Industrial Perspective

Yvan Le Marc, **ALEJANDRO AMEZQUITA**, Unilever, Sharnbrook, United Kingdom

The development of omics technologies and systems biology provides new opportunities to design novel strategies for antimicrobial intervention in the food chain. Among the systems approaches of interest, genome-scale metabolic models (GSMMs) provide a biologically meaningful mechanistic basis for elucidating the genotype-phenotype relationship. GSMMs contain curated and systematized information about known metabolites and metabolic reactions of a given bacterial cell. GSMMs have been used in a number of applications, including the discovery of drug targets. The method consists in simulating gene knockouts to identifying genes that could be targeted to prevent bacterial growth. However, little or no application of GSMMs to support the design of preservation strategies to control bacterial growth in consumer products has been reported. It is expected that incorporating specific information on the metabolism of the bacterial cell exposed to

preservatives in the GSMMs would facilitate this application.

We present two case studies that demonstrate the potential of GSMMs to design novel antimicrobial strategies in an industrial context. The first study involves using light-emitting luciferase biosensor mutants in combination with GSMM modelling as a novel approach to identify key genes/pathways (either cellular targets of the preservatives or resistance pathways). Light-emitting mutants corresponding to the selected genes can be assembled in biosensor panels and be used to identify optimized combinations of preservatives. The second study focuses on developing metabolic models that are specific to the cell metabolism under the action of the selected preservatives. This can be achieved by mapping expression data and metabolomics profiles of bacteria exposed to selected preservatives onto the GSMMs. Gene(s) deletion analysis will then allow identifying targets that can specially potentiate the efficacy of the antibacterial treatment. Note that the benefit that this second study can bring to the industry will also depend on our ability to find compounds that can inhibit the identified cellular targets.

The Bacterial Spore Proteome; Identifying Targets for Spore Germination and Outgrowth Inhibition

STANLEY BRUL, Wishwas Abhyankar, Sacha Stelder, Leo J. de Koning, Chris G. de Koster, Molecular Biology and Microbial Food (SILS), University of Amsterdam, Amsterdam, Netherlands

Bacterial spores are ubiquitous in nature. They are stress-resistant entities that can withstand high environmental temperatures, chemical insults and physical stress such as radiation or increased pressure. Spores are a concern to microbiological food stability due to these characteristics as upon survival of a preservation process they may start to germinate and grow out in food leading to vegetative cells that may cause food spoilage. In addition germinating and outgrowing spores at undesired times and places pose a significant health burden. The challenge is amplified due to the heterogeneous germination and outgrowth behaviour of an isogenic spore population. We discuss the role of different Omics techniques with the aid of state-of-the-art technology to study spore biology in detail. With examples, the use of quantitative proteomics approaches in understanding the spore physiology is demonstrated. Also the need of single cell analyses and analyses of cellular physiology, in particular intracellular pH, is discussed briefly. Certainly the accurate data obtained from these Omics methods will lead to development of robust molecular models of bacterial spore germination and outgrowth and thus assist in the identification of novel systems biology based antimicrobial targets to inhibit spore germination and outgrowth.

S2 Food Safety: A Professionals Guide to Effective Food Risk Communication

Safe food for today's growing global population is a universal goal and while food producers, processors, manufacturers, retailers, regulatory and consumers globally strive to keep food safe, not all food is safe all of the time. Food safety professionals and others who are tasked with explaining why this is and when and how it affects the food chain is imperative to maintain consumer confidence and build trust and to minimize and/or prevent a food crisis from occurring. The proposed interactive session will bring together global partner organizations from Europe and the United States to discuss the plan and execution of food safety risk communication through a series of regional case study examples that demonstrate a local and national cultural context in which the communication occurs. While these case studies are examples, they will be presented as a roadmap on what types of information should be communicated to the public; which channels and communication and social media networks should be used as templates for attendees to better understand the communication needs of target audiences. Communication tools and resources will be shared from the International Food Information Council Foundation's newly developed "Food Safety: A Communicator's Guide to Improving Understanding" as well as The European Food Information Centre (EUFIC) "FoodRisc" Program.

FoodRisC: Perceptions and Communication of Food Risks/Benefits across Europe

NINA MCGRATH, European Food Information Council, Brussels, Belgium

What is the role played by traditional and social media in food risk communication? What prompts journalists to communicate on food risks? What are the main barriers to effective risk communication? These are among the issues investigated by the European Commission funded FoodRisC project which ran for 3 years from 2010 to 2013. This presentation will provide an overview of some of the key findings of the FoodRisc project, which addressed challenges in food risk and food benefit communication. Research conducted in this project included media analysis (traditional and social media) of three food crises, i.e., 2008 Irish dioxin crisis, the 2010 to 2011 German dioxin crisis and the 2011 German *Escherichia coli* crisis. This analysis provided information on media coverage, communication channels (e.g., newspapers, online news, twitter, blogs), inter-relationship between communication channels, story content and tone, and primary source of information (e.g., official source, food industry). Interviews conducted with professional journalists and bloggers in four European countries, who had reported on the 2010-2011 German dioxin crisis and/or the 2011 German *Escherichia coli* crisis provided qualitative data on the factors influencing their choice of story topic and source(s) and the barriers they experience to effective risk

communication. In addition, an online deliberation tool, VIZZATA was employed to gain insights on the impact of the discovery of horsemeat in beef products in Europe in 2013 on consumer confidence and how to improve communication strategies during future food adulteration incidents. Based on this research, an innovative e-resource centre was developed which offers practical guidance to enable key communicators across Europe to produce coherent, evidence-based communication about food risk/benefit issues.

Building a Practical Framework for Successful Food Safety Risk Communication

ANTHONY FLOOD, International Food Information Council, Washington, D.C., USA

Safe food for today's growing global population is a universal goal and while food producers, processors, manufacturers, retailers, regulatory and consumers globally strive to keep food safe, not all food is safe all of the time. Food safety professionals and others who are tasked with explaining why this is and when and how it affects the food chain is imperative to maintain consumer confidence and build trust and to minimize and/or prevent a food crisis from occurring.

To achieve food safety from farm to fork, whether domestically or globally, and to reduce the frequency, impact and severity of outbreaks of foodborne illness, we have to partner. We must cooperate up and down the entire food chain to ensure that consumers, stakeholders, regulators and policy makers have the right information on which to make informed decisions and food choices. In addition and as warranted, we must consider the global impact of food decisions in any food risk scenario.

In the 21st century, the food chain is, more often than not, a global chain that relies on growers and ingredients suppliers, food processors, food distributors and food retailers in many different world regions. If we fail to cooperate effectively, to share knowledge, insight and response initiatives along the entire food supply chain, I ask you all, "How effective is a very long chain with a link missing in the middle?"

Or to put it another way, you can have near perfect food science, near perfect agricultural practice and near perfect regulation but if the system doesn't function as a whole you can still have a near perfect disaster!

In bringing together unique, individual and regionally diverse case studies, we seek to identify best practices to improve public understanding about the safety to the food supply at home and abroad.

Effective Food Risk Communication: A Case Study from the Hellenic Food Authority

EIRINI (RENA) TSIGARIDA, Hellenic Food Authority, Athens, Greece

Risk communication is an essential part of the risk analysis paradigm. Risk communication is an interactive process of exchange of information

and sharing of knowledge as regards hazards and risks when conducting the risk assessment and/or when willing to communicate to the consumers. Depending on the scope of the risk communication, the players in risk communication shall involve all interested parties such as risk assessors, data providers, food businesses, risk managers and consumers. Public information is a prerequisite for national authorities with competence on the control of food safety. In particular, where there are reasonable grounds to suspect that a food or feed may present a risk for human or animal health. Depending on the nature, seriousness, and extent of that risk, public authorities shall take appropriate steps to inform the general public of the nature of the risk to health. Public authorities must identify, to the fullest extent possible, the food or feed, or type of food or feed, the risk that it may present, and the measures which are taken or about to be taken to prevent, reduce or eliminate that risk. Several factors can influence the effectiveness of a risk communication strategy. The main goal is to bridge risk assessment and the science into clear and understandable messages in a manner that helps consumers make well-informed decisions.

S3 Probiotics: Myth or Reality?

Probiotics are microorganisms, mainly bacteria and yeasts, which when ingested in sufficient amounts have a positive effect on human health. Human intestinal strains play an important role in ensuring gastrointestinal microbial balance. Thus, the manipulation of gut microbiota is considered able to enhance protective and beneficial role regarding the alleviation or remedy of GI tract disorders. However, nowadays the benefits of probiotics are considered to go far beyond this, including stimulation of immune system, anti-cancer properties, low-cholesterol levels as well as brain behavior.

The incorporation of probiotic bacteria into foods and their use as starter cultures in fermented foods, presents many technical challenges. The food matrix contains a variety of ingredients that impact the ability of live bacteria to survive through processing and storage that precede consumption. In this sense, techniques, such as encapsulation, can protect probiotic bacteria in food applications.

Probiotics must be assessed for health benefits and safety before they can be introduced into the food products. Many probiotics have a long history of use in fermented foods that bestowed them GRAS status, but when novel microbes are introduced, their efficacy, risk-to-benefit ratio and safety aspects, particularly in an at-risk population need to be assessed. LAB in food and the GI tract could act as a potential reservoir of antibiotic-resistance genes and may participate in the exchange of genes with strains present in the same environment to produce multidrug resistant strains.

This symposium considers the concept of probiotics as a whole, as well as ranging from their selection criteria mode of actions up to their food applications. Last but not least, regulations and

guidance available in the EU to substantiate a health claim and the qualified perception of safety (QPS) concept will be also considered.

A Lone Voice in the Crowd - Probiotics in the Context of the Microbiome

R. PAUL ROSS, University College Cork, Cork, Ireland

The study of the human microbiome is transforming much of our understanding of human diet and its relation to health and disease. Moreover, many of these studies are now making associations between particular microorganisms and groups of organisms and their impact on human disease states ranging from diabetes and obesity to cancer. Related to this, research on probiotic microorganisms such as many *Lactobacillus* species have demonstrated compelling health benefits associated with their ingestion and therein lies a longstanding conundrum. How could an organism representing such a small fraction of the trillions of bacteria in human faeces have such an influence on human health? In this talk a rationale for the efficacy of probiotics is given in the context of their complex microbiota niche. Moreover, examples of mechanisms of actions of probiotic cultures will be discussed which range from pathogen inhibition to the reduction of serum cholesterol. These studies demonstrate the potential for manipulation of the human gut microbiota including the use of probiotics, antimicrobials and bacteriophage but highlights the complexity of the microbiome/host relationship.

Hunting for Probiotic Microorganisms: Is There an Easy Road to Success?

KONSTANTINOS PAPADIMITRIOU, Agricultural University of Athens, Athens, Greece

By definition probiotic foods must contain live microorganisms in adequate amounts so as to be beneficial for the consumer's health. There are numerous probiotic products marketed today and many probiotic strains are commercially available. However, the question that arises is how to determine the real probiotic potential of microorganisms. This is becoming increasingly important, as even a superficial search of the relevant literature reveals that the number of proclaimed probiotics is growing fast. Potential probiotics are selected after in vitro or in vivo assays by evaluating simple traits such as resistance to the acidic conditions of the stomach or bile resistance, or by assessing their impact on complicated host functions such as immune development, metabolic function or gut-brain interaction. Human clinical trials are considered mandatory for communicating health benefits but only few strains with positive studies have been able to convince legal authorities with these health claims. Here an overview of the most common assays employed in screening for probiotics will be presented, highlighting the potential strengths and limitations of these approaches.

What are the Options for the Industry to Promote Probiotic Benefits?

BRUNO POT, Magali Cordaillat-Simmons,
Pharmabiotic Research Institute, Aurillac, France

Probiotics have been considered a healthy food for more than a century, especially in Asian countries. Research on their health benefits was mainly structured as food research. In the nineties probiotics gained interest in Europe and the USA, mainly because of their extensive commercialization. This evolution increased the pressure to supply scientific support for the claimed health benefits. In parallel there was an evolution in the USA and Europe to better regulate health claims for food, in order to protect the consumer. In Europe this resulted in the nutrition and health claims Regulation 1924/2006, which today has approved only one health claim in the probiotics area. While there might be several reasons for this, it is clear that current and future research efforts to sustain health benefits will need to be of very high quality, approaching what is generally requested for drugs. Current technological developments involving -omics technologies will largely assist in improving the quality and profoundness of this health claim supporting research. Depending on the target population, the nature (species designation) of the strains used, the quality of the supporting science and the expected commercial margins, probiotic products can be put on the market as foods or drugs. If the food was not used for human consumption to a significant degree within the EU before 15 May 1997, it could be considered as a novel food. This will be especially the case for the 'new type' of probiotics that are the result of extensive metagenomics research, resulting in non-QPS probiotic candidates. Alternative categories for probiotics might be foods for special medical purpose, medical devices, or medicinal products. According to the European Directorate for the Quality of Medicines and Healthcare, the latter is considered as live biotherapeutic products, Biological medicinal products containing living microorganisms (bacteria or yeast).

S4 Beyond Whole Genome Sequencing: The Impacts of Omics Technologies on Microbial Food Safety Management

Genomics and molecular biology are revolutionising microbiology, particularly with the rapid advances in Whole Genome Sequencing (WGS) technologies and the insights these technologies bring. It is now possible to sequence an entire bacterial genome in a matter of days. By coupling WGS with high-throughput capabilities, it is estimated that it is now possible to bring the cost of sequencing an entire microbial genome to around \$40.00. The US FDA for example, has been very active in sequencing genomes of food microorganisms, adding about 1000 new genomes per month into their GenomeTrakr database. As practitioners in the field of food safety microbiology, it is imperative that we fully understand

the impacts of these new technologies on how we manage and improve food safety.

It should be noted that WGS is but one element of the molecular revolution. As we combine WGS with transcriptomics, metabolomics, metagenomics and ultimately linking this to phenotypes and community behaviour, we have the unique opportunity to study both food and manufacturing microbial communities at an unprecedented level of detail and accuracy.

How do we harness the potential of this technology? Is it enough that we sequence microbial genome to completeness? What is the link between the microbiome and specific bacterial isolates? In this symposium, we propose to explore how 'Omics technologies will help us build better risk assessment tools and consequently, better and more effective food safety management systems. Speakers will cover the whole breadth of these issues; from rapidly and unambiguously identifying contaminants to understanding the community behaviour and ecology of spoilage microbes or pathogens. The Symposium will also examine the legal and regulatory ramifications of the application of these technologies.

Applications of Metagenomics to Product and Process Design

NICHOLAS JOHNSON, Nestle, Lausanne,
Switzerland

Recent technology advances in the fields of culture-independent genomics and 'Big Data' computation are providing opportunities for unprecedented insight into food microbial ecology and physiology. Improvements in analytics and informatics ("Omics technologies") have got faster, cheaper, and more accurate, and are now delivering more extensive and detailed data with each passing year. However, as these technologies have progressed, the scientific knowledge and understanding to interpret and manipulate the information for applications of risk analysis and product design has largely been attempting 'catch up', particularly when compared to some other environmental microbiology or even other food disciplines (e.g., fermentation). The use of community profiling and microbiomics using culture-independent methods, such as metagenomics, are now beginning to be published, and are demonstrating potential benefits for improved product or process design. Most are focused on food fermentation and spoilage studies, but also reveal opportunities for their use in risk analysis applications. The ability to better identify and quantify the connections between members of microbial communities and environmental factors has the potential for a profound impact upon the future way food companies conduct a large number of activities; for example, raw material sourcing and specifications setting, plant hygiene management, adulterant risk assessment, manufacturing process design, and root-cause analysis, etc.

A particular food industry perspective will be

presented as an overview of some of the current and possible future applications of metagenomics technologies as applied to safer and better food design.

Integrating Microbiomics of the Food Chain into an Effective Food Safety Management System

BALKUMAR MARTHI, Unilever, Vlaardingen, Netherlands

The term “Microbiomics” encompasses the use of omic technologies to develop insights into the behaviour of communities of microorganisms. The power of omics approaches (Whole Genome Sequencing, metagenomics, metatranscriptomics, etc.) resides in the fact that we can now start profiling microbial communities in far greater detail than previously possible.

These advances in science open up several opportunities to truly apply a ‘systems’ approach to microbial contaminants in the food chain and map their behaviour across the chain. From predominantly research applications involving the identification of stress survival mechanisms, we can now extend the application to predicting the behaviour and fate of contaminants (pathogens as well as spoilage organisms) in the food chain. This allows us to develop an integrated approach to safe and stable design of foods and processes, leading to enhanced trust in the foods that we produce.

In this talk, I will present an industry perspective on how Microbiomics approaches can become an essential part of a holistic approach to Food Safety and Stability, which at the same time is sustainable and has a positive impact on product quality.

Molecular-based Surveillance in Food Manufacturing Facilities Using Next Generation Sequencing Techniques and Software

CIAN O'MAHONY, Seamus Fanning, Creme Global, Dublin, Ireland

Next generation sequencing (NGS) techniques can provide significant insights for safety and quality programmes in food manufacturing facilities, by characterising specific bacterial isolates using Whole Genome Sequencing (WGS) or by examining the complete microbiome of a facility using metagenomics. The Origin Green Micro Surveillance project, commencing in 2016 for a period of three years, is a multi-disciplinary, multi-partner initiative with the aim of gathering and integrating WGS and metagenomics data from manufacturing environments into a harmonised risk and quality assessment system and software application. Industry partners in the project include a global leader in paediatric nutrition, three international infant formula grade ingredient suppliers, an international cooked and fermented meat supplier and an SME nutritional ingredient supplier. The project will identify a sampling plan that will be used to capture

the dynamics of bacterial adaptation and microbial population changes over time and space, using WGS and metagenomic analysis. Specific isolates, identified from monitoring, will be sequenced in parallel with a microbiome characterisation of production environments. The molecular epidemiology will be described and predictive models based on both bacterial isolates and their connection with the microbiome will be developed. All gathered data and models will be housed in a risk and quality assessment software application built on a cloud computing architecture, for use by industry to analyse, monitor and improve food safety and quality in specific manufacturing environments.

S5 New Approaches to Food and Chemical Risk Assessment

Food is composed of a wide range of chemicals and includes those which are naturally occurring, those which are intentionally added to food (e.g., food additives and nutrients) and those which are unintentionally added to food (e.g., contaminants). Scientific risk assessments are evidence based and consider both hazard and consumer exposure in assessing whether there may be unacceptable risks to health. Safety factors are typically applied to extrapolate the hazards to humans once they are identified.

For nutritional constituents of the diet, additional risk assessment approaches such as substantial equivalence, weighing risk-benefit and history of safe use can be considered.

The diversity of questions now being asked in food safety (e.g., combined exposures, process intermediates, environmental degradates), together with rapid advances in scientific knowledge and challenges to the reliability of animal testing, is resulting in a paradigm shift in toxicology where new approaches involving MIE (molecular initiating events) and AOPs (adverse outcome pathways) are being developed. The challenge is for these new approaches to be robust enough to assure product safety and for them to be accepted by regulatory agencies.

Introduction to Chemicals in Food and Current Tools and Approaches in Chemical Risk Assessment

BENOIT SCHILTER, Nestlé Research Center, Lausanne, Switzerland

Chemical analysis of food has revealed the presence of a multitude of substances occurring over a large range of concentrations, from picog to g levels. Some are intrinsic components of food, including macro- and micro- nutrients, anti-nutrients and inherent toxicants, while others are extrinsic, either added or resulting from natural or industrial sources. In addition, process-related chemicals are formed during processing. So from scientific point of view, food can be assumed as a highly complex mixture of chemicals. To understand the health

significance of food chemicals is complex. Many provide but others reduce nutritional value. Some may be associated with beneficial effects. A number have been shown to be harmful, producing toxicity. This is why chemicals must be managed to ensure that food is safe. This is not straightforward. Toxicity depends on the properties of each chemical and toxic potencies spans over 6 orders of magnitude. In this context it appears important to establish priorities. Risk assessment, combining exposure and toxicological information, has been valuable to identify chemicals of concern on which management efforts should focus. Toxicological characterization is a resources intensive process based on feeding experimental animal with high doses of the chemical investigated. Only a limited number of chemicals to which human is exposed have actually been characterized toxicologically. Currently the application of the standard toxicology approach to the untested chemicals is getting increasingly questioned from scientific, feasibility and ethical perspectives. New approaches using *in vitro* and computational methods are now being developed to tackle these challenges.

New Approaches in Chemical Risk Assessment

ANS PUNT, RIKILT, Wageningen University and Research Center, Wageningen, Netherlands

Rapid advances in scientific knowledge and challenges with respect to the reliability of animal testing have resulted in a paradigm shift in toxicology where new approaches are being developed. The core of these new approaches to chemical risk assessment includes (high-throughput) *in vitro* toxicity screening that forms an efficient way to identify potential biological targets of chemicals and adverse outcome pathways. Yet, relying on *in vitro* results only may misrepresent the *in vivo* situation as *in vivo* kinetics (i.e., absorption, distribution, metabolism and excretion) of the chemicals are not accounted for in the *in vitro* assays. Key to new approaches in chemical risk assessment is therefore to also integrate the *in vitro* data with kinetic information to extrapolate the results to the *in vivo* situation. Several approaches exist, varying from comparing the results with human plasma data, or applying reverse dosimetry by combining *in vitro* toxicity results with simple (steady-state) kinetic models or more complex physiologically based pharmacokinetic (PBPK) models, to obtain *in vivo* equivalent oral dose levels. Within this lecture the work of the ILSI expert group on the exploitation of ToxCast data will be presented. This expert group explores opportunities to understand better how these new data and methods may be used in the safety risk assessment for foods.

S6 How are Microbial Interactions Acting toward Our Safety?

Biopreservation is considered as one of the emerging non-thermal and sustainable strategy to increase the safety of fermented and non-fermented food products. In most cases, this technology involves microbial interactions between foodborne pathogens and protective bacteria selected for their inhibiting capacities. Lactic acid bacteria (LAB) have been widely used as protective bacteria to improve food safety due to: their wide distribution in the microbial ecosystems of ready-to-eat food products (such as dairy, meat and seafood products); their capacities to produce organic acids and bacteriocins; and because they are able to compete with other bacteria. The objective of this symposium is to discuss how microbial interactions can be enhanced in food products using selected protective bacteria showing inhibition properties towards unwanted microorganisms. Case studies concerning inhibition of pathogenic microorganisms, namely *Listeria monocytogenes* and *Staphylococcus aureus*, in seafood and dairy products, respectively, by LAB will be illustrated. Moreover regulatory aspects regarding the use of bacteriocins in foods will be taken into account.

The Concept of Bioprotection: Microbial Interactions for Safer Foods

LUCA COCOLIN, Valentina Alessandria, Paola Dolci, Kalliopi Rantsiou, University of Turin-DISAFA, Turin, Italy

Bioprotection is defined as the improvement of safety and/or the extension of the shelf life of foodstuffs using microorganisms and their metabolites which interfere with the growth of pathogenic and spoilage microbiota. Bioprotection has gained interest from food producing companies since it can be considered a “natural” preservation strategy.

Lactic acid bacteria (LAB) are the most frequently used agents for bioprotection because of their capability to produce bactericidal organic acids (e.g., acetic and lactic and acids) and bacteriocins, defined as peptides able to hamper microorganism growth by interfering with the structure of the cell membrane and/or cell wall.

While the literature on the use of bacteriocins and LAB to inhibit the growth of foodborne pathogens is large, only in the last years scientists have put efforts in order to exploit this approach also to limit the growth of spoilage microorganisms, thereby increasing food shelf life, mainly fresh produce.

The use of starter cultures able to produce antimicrobial substances and the direct employment of the purified bacteriocins are both considered as bioprotection interventions. While in the first case it is essential to understand if the bacteriocin is produced also when the strain faces the food environment, which for ecological and technological

reasons often results to be stressful, in the second option regulatory aspects have to be taken into account. Lastly, the functionalization of packaging films with bacteriocins has recently been proposed as a way to extend the shelf life, especially of meat and meat products.

In this contribution, an overview of bacteriocins in food production and the challenges inherent with their use will be presented, taking into account also the limitation of their use as food ingredients.

Protective Bacteria: An Option to Control *Listeria monocytogenes* in Seafood Products

MARIE-FRANCE PILET, UMR SECALIM, INRA, Oniris, France

Listeria monocytogenes is responsible of human listeriosis, a severe foodborne disease (1,763 human cases in Europe in 2013, 15.6% fatality rate (1)). Among ready to-eat-food products, seafood and fishery products usually show the highest level of non-compliance (presence in 25g) according to the European microbiological criteria (1). A wide range of seafood products such as sushi, fish carpaccio, smoked or marinated fish, cooked shrimp are considered as low preserved since they can be consumed raw or after salting, cold smoking or mild cooking. During cold storage of these foodstuffs, usual preservative methods are vacuum or modified atmosphere packing or/and addition of preservatives such as organic acids or sulphites, that are in some cases inefficient to limit the growth of *L. monocytogenes* if a contamination has occurred. The use of protective bacteria is one of the alternative or additional methods that have been proposed for several years. Lactic acid bacteria that have been selected for their ability to limit the growth of *L. monocytogenes* in seafood belong to the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*. Some of them have been tested successfully against the pathogenic bacteria added in real products by challenge test during the shelf life of the product and their relative efficiency will be compared. In some cases, the effect of the selected lactic acid bacteria on the organoleptic characteristics of seafood has been tested by sensorial and/or physicochemical analysis. Finally, the mode of action of these bacteria involving bacteriocins, nutritional competitions or contact dependent inhibition and the way it has been demonstrated will be investigated. (1)EFSA Journal 2015;13(1):3991

New Insights on the LAB-*Staphylococcus aureus* Interaction: A Transcriptomic Approach

LUÍS AUGUSTO NERO, Gabriela Nogueira Vicosa, Luca Cocolin, Universidade Federal de Viçosa, Viçosa, Brazil

The impact of lactic acid bacteria (LAB) on *Staphylococcus aureus* growth and enterotoxin production is the focus of several studies targeting food safety. LAB-mediated inhibition in foods often involves rapid pH decrease, nutrient competition and

the production of several antagonistic substances, including bacteriocins. The challenges in studying bacterial interactions in complex food systems, such as raw milk and cheese, can be overcome by using Next Generation Sequencing based methodologies, once these techniques can provide a broader perspective on this specific issue. Therefore, we considered a RNA sequencing approach to study the occurrence of interactions between an enterotoxigenic *S. aureus* (ATCC 29213) and a bacteriocin-producing *Enterococcus faecalis* strain, previously isolated from raw milk cheese. These strains were inoculated alone or in combination in skim milk and the kinetics of bacterial growth, bacteriocin and enterotoxin production were monitored for 48 h at 30°C. Phenotypic analysis revealed a lack of enterotoxin production in mixed culture with minor impact on *S. aureus* final population. Preliminary metatranscriptomic analysis revealed a negative impact of *E. faecalis* on the expression of global regulators of *S. aureus* virulence, such as *agr* and *sar*. Also, the expression of oxidative and acidic stress related genes in *S. aureus* was altered. Considering these data, transcriptomic-based techniques constitute reliable tools for the study of bacterial behavior in food systems and may eventually help us to develop new ways of approaching food safety.

S7 Can Whole Genome Sequencing Guide and Inform Intra-species Virulence Rankings?

Whole Genome Sequencing (WGS) is emerging as the molecular epidemiology platform of choice for bacterial agents implicated in human foodborne disease. In the United States, coordinated efforts between the U.S. FDA, USDA and the Centers for Disease Control and Prevention now include WGS-based data of *Listeria*, *Salmonella* and other pathogens during outbreak investigations as well as in routine surveillance. Similar initiatives are being developed and implemented in other nations. These developments are unprecedented not only in the sheer amount of WGS information that is being collected and the real-time nature of the collection and use of WGS data (e.g., while an outbreak is being investigated) but also in the fact that the archived sequenced data are open-access, free of charge, to a diverse community of stakeholders that includes academia, industry, and regulatory agencies. The objective of this symposium is to present cutting-edge developments in WGS-based molecular epidemiology and to address the capacity of WGS and omics platforms stemming from it, e.g., epigenetic analysis and global transcriptome profiling, to help address the following long-standing query, of key interest to the entire food safety community: are there intra-specific differences in human virulence among different strains of foodborne pathogens, and how can such differences be best identified and interpreted? The symposium will focus on uses and capacity of WGS and related omics-derived data to

complement epidemiological trend analysis, targeted molecular biologic studies and lab-based virulence assessments, in order to guide and inform detection and elucidation of strain-specific differences in the ability of major pathogens to cause human foodborne disease.

Implementation of the Use of Whole Genome Sequencing Data and Relevant Insights on Virulence of Bacterial Foodborne Pathogens

To be determined

An Integrated View on *Listeria* Genomics and Virulence

SYLVAIN BRISSE, Pasteur Institute, Paris, France

Listeria monocytogenes is genetically highly diverse. I will present the latest research on whether and how strains differ in their pathogenic or ecological characteristics. I will show how high-throughput sequencing has revolutionized our abilities to analyse the diversity and phylogenetic structure of *L. monocytogenes* and to define the association of genotypes with phenotypes. I will also present current approaches to translate population genomics research into standardized molecular typing strategies and unified strain nomenclatures that enable internationally coordinated epidemiological surveillance of *L. monocytogenes* at the global scale.

Delineating Virulent and Avirulent Taxa with Genomics and Metagenomics

KOSTAS KONSTANTINIDIS, Georgia Institute of Technology, Atlanta, GA, USA

Culture-independent analysis (aka metagenomics) has recently revealed tremendous diversity in the microbial communities inhabiting the human body and revolutionized our understanding of these communities primarily because the majority of microbial species cannot be cultured in the laboratory and thus, they remain poorly understood. The ability to characterize in detail the microbial constituents in human clinical specimens without culture may prove invaluable in public health as all surveillance and outbreak detection methods to date rely on culture, frequently failing to identify the key causative agent of a disease. In this talk, I will summarize our recently developed bioinformatics algorithms and approaches to deal with several challenges associated with metagenome-based analysis of clinical samples such as how to detect and quantify target species (e.g., pathogens) and genes (e.g., toxins) in complex, short-read metagenomes, and how to identify and genotype organisms with no previously sequenced representatives. Application of these tools to samples from foodborne diarrheal outbreaks showed that in many cases – but not all – the disease and healthy states of the gut microbial community can be distinguished from each other, opening new possibilities for diagnostics. I will also present our approaches to delineate between highly virulent and a-virulent populations recovered

in metagenomes from these samples, as well as examples from *Clostridium botulinum* and *Bacillus anthracis* on how to distinguish pathogenic strains from their innocuous close relatives based on comparative analysis of the genomes of isolates. The tools are available for online analysis or download for standalone application through <http://enve-omics.gatech.edu/>

S8 Food (Micro)Structure: Impact on Microbial Dynamics

The microbiological safety of food products is affected by many factors, both intrinsic and extrinsic. Most of the extrinsic factors (e.g., atmosphere, temperature) are easy to control and measure, therefore they have extensively been studied. Food products are systems with complex intrinsic characteristics ((micro)structure, composition and physicochemical properties). All these factors influence the microbial dynamics of potential microbiological contaminants. The influence of most of the physicochemical properties of foods, e.g., pH and water activity (a_w) and compositional aspects has also been widely studied. However, the influence of the food (micro)structure has not yet been assessed in depth.

Food (micro)structure is a property extremely difficult to objectively quantify. (Micro)structures have been classified qualitatively in five categories (liquids, emulsions, aqueous gels, gelled emulsions and food surfaces). Emerging studies are being conducted in this field. The target of the studies is oriented to the identification of quantifiable characteristics related to the food (micro)structure. These characteristics could further be used to update the already existing predictive models, by incorporating the relevant food (micro)structural parameters. Most predictive models have been developed on the basis of experimental data conducted in liquid microbiological media or with real foods. In liquid microbiological media, food (micro)structural characteristics are not present while real food products exhibit a high batch to batch variability.

This session aims at discussing recent studies, where (micro)structural aspects of foods have been identified and quantified and their influence on microbial dynamics is being documented. Overall, the future research directions for the scientific community in this domain will emerge from the Workshop. In the end, this influencing factor will be better characterised and incorporated in already existing predictive model structures; as a result, the microbiological food safety of the relevant food products will be assured to a higher extent.

Evaluation of (Micro)Structural-related Factors on Microbial Growth in/on Food-based Model Systems

MARIA BAKA, KU Leuven/BioTeC+, Ghent, Belgium

Microbial growth is influenced by variations in intrinsic complexity of foods ((micro)structure, composition and physicochemical characteristics). So far, the effect of food (micro)structure has been assessed mainly by comparing planktonic growth in liquid (microbiological) media with colonial growth in/on solid-like systems or on real food surfaces. However, many foods are emulsions or gelled emulsions with complex intrinsic characteristics as compared to liquids or solids.

In this study, the effect of food (micro)structure on the dynamics of *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus*, is investigated via food (model) systems with variable (micro)structural complexity, composition and physicochemical characteristics. The (micro) structures studied were: liquids, aqueous gels, emulsions and gelled emulsions. The composition and physicochemical characteristics of gelled emulsions targeted two different types of foods: (i) Frankfurter sausages and (ii) fish patés. All model systems were incubated at 4, 8 and 12°C and analysed for their pH and water activity values. The model systems approaching Frankfurters in composition were vacuum packed and analysed for their resistance to penetration.

This study illustrated the significance influence of food (micro)structure on microbial dynamics. Also, the maximum specific growth rate estimated on the collected experimental data was compared to the predictions of ComBase at the specific storage temperature and pH and aw values of the different model systems. Further studies are required to fully quantify the interaction of food (micro)structure with other food intrinsic and extrinsic factors.

Thermal Inactivation of Listeria Related to Food Structure and Processing Technology

TORSTEIN SKARA, Mehmet Baris Ates, NOFIMA, Stavanger, Norway

Food structure plays an important role in the dynamics of growth and inactivation of microorganisms. In this presentation, structural aspects will be outlined, and correlations between real foods and models will be drawn. Also, important aspects of experimental conditions and limitations will be discussed; inoculation levels and techniques as well as model target organisms, with special reference to *Listeria*. For quantifying the structural effects during inactivation, thermal characterization is required, but foods are often complex solids or structured liquids whose thermal behavior can be challenging to measure or model. The often low thermal conductivity of foods limits heat transfer, and increasing the heating and cooling rates is important in order to maximize the product quality. But there are various processing technologies available, and

the challenge lies in finding the optimal process for each product. It is important to understand the criteria for choosing thermal process technology. The presentation will give examples of inactivation of *Listeria* in fish based model products using shaka retorting surface pasteurization and microwave heating, and discuss relevant aspects related to each technology.

Characterisation/Quantification of the Impact of Food Structure on the Development of Antimicrobial Resistance of Food-related Pathogens

EIRINI VELLIYOU, University of Surrey, Guildford, United Kingdom

Nowadays, emerging industrial processing techniques are increasing rapidly and are progressively replacing the classical decontamination processes of food systems. One emerging alternative is the use of natural antimicrobials, produced by microorganisms such as lactic acid bacteria (LAB), as there is evidence that those components may act against food pathogens. However, the efficiency of such antimicrobial components is still unclear. Moreover, most of the available studies describe the action of antimicrobials against pathogens in liquid systems, although the majority of food products are solids. In a solid system immobilization of microorganisms leads to their evolution as colonies and due to diffusional limitations of oxygen and nutrients as well as the accumulation of acidic metabolic products around the colony microorganisms may experience a self-induced (acid) stress that could affect their overall response and tolerance to the antimicrobial component. Indeed, our recent findings point interesting differences in the development of antimicrobial resistance of *Listeria* in liquid vs solid state when exposed to natural antimicrobials. Development of antimicrobial resistance could lead to higher microbial tolerance on industrial processing conditions due to the action of the so-called cross-protection mechanism which enables cells adapted to a specific stress to resist other types of stress stimuli as well. Therefore, understanding and precisely quantifying the response of food related pathogens on the one hand to natural antimicrobials and on the other hands to natural antimicrobials in combination with an industrial(like) treatment in a food(like) in vitro models is of significant importance for the design of safe industrial processes and consequently for the achievement of food safety.

S9 Risk-based Sampling; Perspective from Different EU and Non-EU Member States

Theme: Supply chain management is the basis to govern food safety and to prevent foodborne outbreaks in the ever increasing complexity of the (inter)national food chain. An appropriate test system is required to support managers in their decision-making regarding safety measures in the food supply

chain. A "risk-based sampling plan" is a crucial component of this test system with the ultimate goal to take the right measures at the right time and the right place in the food supply chain in order to prevent food borne disease. Yet, the term "risk based" can be, and has been, interpreted in many different ways depending on, for example, the manager and scientists involved, data and time availability and public health status of the population concerned. This has resulted in different "risk-based sampling plan" approaches in different EU and non-EU member states.

Purpose: To identify the current status on "risk-based sampling approaches" in different EU and non-EU member states and how we can learn from each others' approaches taking into account the ever increasing complexity of international food trade and accompanying microbiological dynamics.

Methods: Present three different approaches from different EU member states and one approach from a non-EU member state.

Results: Document a discussion session on the pro's and con's of the different methods. With that, this symposium will be the basis for a network of stakeholders (scientists, supply chain managers from government and industry) with a view towards future risk-based sampling approaches.

Significance: A risk-based sampling plan will help stakeholders to monitor the occurrence of the most important pathogen/product combinations from a public health risk perspective in a consistent manner (e.g., by trend analysis) and, with that, give input for targeted intervention programs.

Risk-based Sampling: Perspective from Hungarian National Food Chain Safety Office
AKOS JOZWIAK, National Food Chain Safety Office, Budapest, Hungary

One of the most important tasks of the central competent authorities in Europe is to plan an effective, risk-based control plan. As an effect of different institutional, political, economic and cultural background, there are different interpretations of being risk-based and different solutions and approaches co-exist in the EU Member States.

The risk-based concept offers the most effective planning methodology, however, different definitions exist for risk. Should only health risks be taken into consideration or other risks as well? How should the planning process be built to serve those different needs? What input data could be used and how the information needed could be collected? How risk assessment is connected to the operative sampling plan (as a part of risk management)? What is the outcome of an effective planning and how it could be measured?

All the experts dealing with planning exercises face those questions from time to time. The presentation shows the interpretation of those questions and examples from the perspective of Hungarian central competent authority.

Risk-based Sampling, Optimal Sampling Design: Perspective from the Dutch Institute for Public Health and the Environment

ANNEMARIE PIELAAT, Jurgen Chardon, Lucas Wijnands, Eric Evers, National Institute for Public Health and the Environment, RIVM, Bilthoven, Netherlands

Introduction: We propose a sampling plan optimized to produce public health risk estimates related to microbiological contamination in food according to the following definition:

"Distribute the sampling capacity of the food safety authority over products in retail according to their relative contribution to the microbiological public health risk within the available budget."

Purpose: Develop a monitoring program including those pathogen/product combinations that will capture as many Disability Adjusted Life Years (DALYs) as possible for the least amount of money.

Methods: Estimating food related microbiological public health risks involves knowledge about different variables. Beside prevalence and concentration data of specific pathogens in specific food products and consumption data, food handling by consumers and human exposure are important aspects for the ultimate risk.

Exposure assessment was combined with DALYs and a monitoring budget which resulted in the following optimizing criteria for risk based sampling:

A. Exposure to a certain pathogen depending on the consumed amount of its associated food product, pathogen concentration and food preparation,

B. Based on exposure estimates, attribute contributions of pathogen/product combinations (from A.) to disease burden expressed in DALYs for pathogen-product groups,

C. A predefined uncertainty in the risk estimate (expressed in the prevalence) and subsequent amount of samples needed of products in A,

D. Costs per sample.

The optimizing criterion is $(C * D)/B$ and has the units costs per DALY.

Results: The method is explored using five case studies: *Campylobacter* in pork and poultry, *Salmonella* and *Toxoplasma* in pork, Shiga-toxin producing *E. coli* in beef.

Significance: A risk based sampling plan will help authorities to monitor the prevalence of the most important pathogen/product combinations from a public health risk perspective in a consistent manner (e.g., by trend analysis) and, with that, allowing them to advise on targeted intervention programs.

Risk-based Sampling: Perspective from CFSAN, USA

JENNY SCOTT, Yuhuan Chen, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

The safety of food is principally ensured by the effective implementation of scientifically valid preventive control measures throughout the food chain. Nevertheless, risk-based sampling can play an important role in food safety systems.

Microbiological sampling and testing can be used to verify whether food operations are under control, to determine the acceptability of individual lots of food, and for other purposes, such as to gather baseline data, to inform traceback in epidemiological investigations, and to monitor environmental pathogens or indicators. This presentation will describe criteria and data to consider in risk ranking to inform risk-based sampling. As part of the FDA's risk-based and preventive approach to food safety, which is at the core of the FDA Food Safety Modernization Act, the agency has developed a new, more robust, surveillance sampling approach in the last several years. This presentation will highlight components of this new approach, including the types of sampling (e.g., product sampling, environmental sampling, and combinations thereof), the pathogens targeted, the number of samples, commodities sampled, recent sampling activities, and how data will be evaluated and shared to engage stakeholders throughout the process. Risk-based sampling is part of FDA's efforts to keep contaminated products from reaching consumers and to facilitate a greater understanding of hazards to minimize risks.

S10 How to Manage Viruses in the Food Industry

Although human viruses are a major cause of foodborne illness worldwide, the way of managing this foodborne issue is still not well understood. In this symposium proposal, three speakers with expertise in foodborne virus risks will provide the audience with information and practical approaches about the challenges faced in the management of foodborne viruses.

Attendees will learn about the risk of viruses all along the food chain and will gain insights into the best practices for their control. An overview of the situation in Europe will be addressed, incorporating new methodologies for their detection and control. In addition, the way of implementing virus testing in food will be discussed, highlighting the challenges when embarking on a routine testing regime. Regulatory considerations and successes from implementing virus testing in the food industry will be also detailed. Finally, the way of inactivation of viruses in food and the impact of various technologies will be also covered.

It is anticipated that attendees will be better equipped to address the viral risk in their products after attending the symposium. They will have a better understanding of how susceptible their food is to viral contamination, how foodborne viruses issues can be part of a food safety plan, how reliable methods are in preventing viral contamination, and how efficient food processes are on virus elimination.

Detection and Assessment of Viral Risk in Food

SANDRA MARTIN-LATIL, Anses, Maisons-Alfort, France

The number of foodborne disease outbreaks caused by viruses has more than doubled since 2011 and reached the highest level yet reported in 2014 (20.4% of all outbreaks) (*EFSA Journal 2015;13(12):4329*). Enteric viruses involved in foodborne diseases belong to various viral families and can cause a wide variety of diseases in humans. Human norovirus (NoV) and hepatitis A virus (HAV) are the most frequent causes of viral foodborne outbreaks worldwide.

Detection of HAV and NoV in foodstuffs is complicated by the absence of a reliable cell culture method and the low contamination levels of food. Then, the standardized method (CEN/ISO/TS 15216 published in 2013) for detection and quantification of NoV and HAV in foodstuffs is based on a final detection of viral genome using real-time reverse transcriptase PCR (RT-qPCR). Despite its sensitivity, specificity and rapidity, RT-qPCR is sensitive to substances present in tested samples that can inhibit PCR amplification of viral genomes and may lead to false-negative results. This drawback may be overcome by digital RT-PCR that is more robust for detecting viral genomes in presence of PCR inhibitors.

Another challenge in food virology is the assessment of infectious viral risk. The current RT-qPCR detects viral genomes of both infectious and non-infectious viruses. Two different approaches were evaluated in our lab for measuring the infectivity of enteric viruses: a combined method using intercalating dyes with RT-qPCR and a real-time cell analysis method (xCELLigence, ACEA). This last system measures in real time the electrical impedance of cell monolayers which allows the continuous monitoring of cell behavior following viral infection.

In conclusion, the improvement of the existing methods and the development of new tools for detecting the infectivity of enteric viruses are future challenges needed to be addressed in food virology.

Viral Risk Management: From Preventive Measures to Sampling Plans

CHRISTOPHE DUFOUR, Scientific Director Microbiology Europe, Mérieux NutriSciences

Food Industry has a growing awareness of the specific risks in relationship with viral contamination of food including norovirus and hepatitis A. Industry progressively develops HACCP based approach to implement adapted measures to prevent contamination of food as many process can't rely on robust CCP to limit this risk. Testing is necessary to verify the efficiency of the preventive measures and evaluate supplies.

Preventive measures include quality of water, personal health, good hygienic practices, cleaning and disinfection, selection of ingredients and raw materials based on good agricultural practices.

Examples will be given of practical actions to implement the preventive measures in a field to fork approach.

Control plan is constructed to verify the efficiency of the preventive measures and the surveillance of critical supplies. Traditional microbiological indicators such as *Escherichia coli* are useful but need to be completed by targeted sampling of water, raw ingredients, finished products and surfaces for virus detections. Limits of sampling plans in relationship with contamination heterogeneity within product lots will be discussed.

Shedding Some Light on Inactivation of Foodborne Viruses

JULIE JEAN, Université Laval, Québec, QC, Canada

The majority of non-bacterial gastroenteritis is caused by norovirus, which is transmitted primarily through surfaces, food and water. Most household disinfectants are ineffective against these viruses with the exception of the bleach which, however, loses partially its activity and generates toxic residues in the presence of organic matter. Alternative, effective and safer methods of disinfection are necessary to hamper the spread of norovirus. This presentation will focus on the work of our research team on two promising approaches, namely peroxy-carboxylic acids and pulsed light.

First, four peroxy-carboxylic acids were evaluated based on their concentration and contact time. Monoperoxy-carboxylic acids, namely peracetic and perpropionic acids, were the most effective by reducing the viral load of about $4 \log_{10}$ after a treatment with 50 mg L^{-1} for 5 minutes. These molecules maintained their activity against noroviruses attached on stainless steel and PVC, clean or dirty, and entrapped in an artificial biofilm. In the case of peracetic acid, we showed that this inactivation was mainly due to damages on the RNA, probably through free radicals. At high concentrations, the capsid was also altered.

On the other hand, noroviruses were treated with pulsed light (200-1000 nm; 0.69 J cm^{-2} per pulse). Three pulses (1.6 seconds) decreased viral load of approximately $4 \log_{10}$ in all media tested (buffered saline, hard water, mineral water and sewage treatment effluent) with the exception of turbid water. At the maximum turbidity (1000 NTU), the reduction was $2.4 \log_{10}$. This technology is also effective to disinfect clean surfaces even in the presence of an artificial biofilm ($4 \log_{10}$ after 7 pulses), but is affected by the presence of proteinaceous material. We demonstrated that the pulsed light inactivated norovirus by inducing damage to both the RNA and the integrity of the particles.

S11 Metabolomics: A Post-genomic Approach to Study the Effect of Microbial Diversity in Foods

Foodomics was defined as a discipline that studies the food and nutrition domains through the application and integration of advanced omics technologies to improve consumers' well-being, health, and confidence. In particular is an emerging field within omics sciences dealing with the simultaneous determination and quantitative analysis of intracellular metabolites, which have been defined as low-molecular-mass compounds that are not genetically encoded and produced modified by the metabolism of living organisms including microbes those compounds small molecules such as peptides amino acids nucleic carbohydrates organic vitamins polyphenols alkaloids minerals. Although metabolic footprinting gives important information about only a small part of the entire bacterial metabolome, it provides key information that may contribute to the understanding of cell communication mechanisms. In fact, metabolomics has recently been applied for monitoring the quality, processing, chemical and microbiological safety of both raw materials and final products to improve the consumers' health and confidence. Moreover metabolomics may be applied in fermented foods to observe metabolite modifications during fermentation and the possibility to predict, among others, the sensory and nutritional quality of the fermented final product. Since the crucial roles in the success and safety of food industry, microbial functions in food are increasingly attracted researchers' interest. The emerged foodomics approaches that integrated advanced high-throughput analytical techniques and bioinformatics tools have been used in modern food microbiology. In fact, two analytical platforms are currently used for metabolomic analyses: nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). They have achieved success in revealing many fundamental processes driven by both pathogens and commensals in food. The suggested session focuses on the application of foodomics in food microbiology in order to clarify basic concepts and their application to food safety, quality and food fermentation.

Basic Concepts of Metabolomics and Application to the Food and Nutrition Science

FRANCESCO CAPOZZI, BioNMR Lab - Department of Agricultural and Food Science Alma Mater Studiorum, Università di Bologna - Cesena Campus, Cesena, Italy

NMR spectroscopy is widely used to investigate living systems mainly for two reasons: i) it is usually regarded as the universal detector for practically all organic substances of natural origin, because it quantitatively records signals for all substances containing hydrogen atoms above the micromolar

concentration limit, and ii) the spectrum obtained under controlled experimental conditions (temperature and pH) is highly reproducible so that it is suitable, as it is, for direct multivariate data analysis. For this reason, the NMR spectrum of the cell extract is generally regarded as the metabolic profile of an organism, and from these premises metabolomics has been developed based on the chemometrics analysis of NMR spectra of biological samples, including tissues and cell extracts. The approach allows a measure, against a control population, of the perturbations induced by a treatment, through a holistic view of the cellular composition. Subsequently, the identification of the metabolites contributing to the description of the perturbation is also possible. NMR spectroscopy, thus, is increasingly used in the investigation on the metabolic effects of natural molecules with bacteriostatic and/or bactericidal actions on bacterial growth. The presentation will provide an overview on the methodology, discussing limits and advantages of NMR in the metabolomics approach, and will present possible applications for the evaluation of food transformations during processing, storage and digestion.

Metabolomics - A Useful Tool to Study the Quality of Fermented Foods

ANDREA GIANOTTI, Department of Agricultural and Food Science Alma Mater Studiorum, Università di Bologna, Bologna, Italy

Metabolomics was recently applied in food science for monitoring the quality, processing, safety, and microbiology of both raw materials and final products to improve the consumer's health and confidence. Specifically, the study of metabolite profile in fermented foods was applied to record metabolite modifications during fermentation and the possibility to predict, among others, the sensory and nutritional quality of the fermented final product. The metabolomics application to fermented products will be focused: i) to characterize volatile metabolites by gas chromatography (GC-MS) and electronic nose in real sourdough; ii) to discriminate chemically acidified fermented dough mimicking sourdough fermentation; iii) to exploit different *L. plantarum* strains to improve sensorial and functional properties of cereal-fermented foods. GC-MS discriminated the fermentation type and cereal source due to its ability to detect specific patterns of alcohols, ketones, aldehydes and carboxylic acids. The chemical acidification induced changes allowing GC-MS to discriminate the cereal source basing on 1-octen-3-ol, 2-methyl-propanol and 1-hexanol. On the other hand e-nose sensors revealed the different formulations and sourdough process. Finally, the effect of *L. plantarum* fermentation on sensorial and healthy compounds was significant in wheat flours. However KAMUT® khorasan wheat represents itself a highly specific source of volatile and phenolic compounds. Finally it was used metabolomics to correlate, in fermented cereals, specific groups of volatile molecules to antioxidant activity and

polyphenols assays. Metabolomics may represent an important information tool suitable to support a rapid selection or prediction of strain/substrate combination to simultaneously increase sensorial and healthy characteristics of cereal-fermented foods.

Metabolomics Application on Bacterial Safety, Spoilage and Adulteration

GEORGE-JOHN NYCHAS, Agricultural University of Athens, Department of Food Science and Human Nutrition, Athens, Greece

In food science, metabolomics has been proven a valuable tool to investigate quality changes during processing, distribution and storage of raw materials and final products. In the area of quality and safety of processed foods metabolomics can contribute in (a) food classification, food adulteration and authenticity assessment, (b) quality control in food industry, (c) food spoilage, (d) association between sensory characteristics and chemical composition, and (e) impact of processing on food composition. Metabolomics have been proved very efficient in the detection of microbial metabolites that can be used as biomarkers in food spoilage under different packaging conditions (air, vacuum, modified atmosphere packaging) using HPLC, gas chromatography (GC) and/or mass spectrometry (GC-MS). In the context of the current presentation selected examples will be presented on the implementation of metabolomics in table olive fermentation, pasteurized vanilla cream spoilage, beef and pork meat spoilage and marinade poultry as well as meat (beef, pork and horse) adulteration stored under aerobic and modified atmosphere packaging, through the evolution of volatile compounds detected by headspace solid phase microextraction gas chromatography/mass spectroscopy (Headspace SPME-GC/MS) and HPLC analysis. Moreover, pasteurized vanilla cream spoilage will be presented using SPME-GC/MS analysis.

S12 Risk Assessment or Assessment of the Risk in Fresh Produce, That's the Question

The information that currently exists in the public domain is highly theoretical and very generic; application to each industry sector can be difficult.

The purpose of this session is to a public-private view with speakers from the food industry, and academics to present on the current findings of an ILSI Europe expert group on industrial MRA in fresh produce. There is a lack of practical and applicable data and guidelines to perform Microbiological Risk Assessment (MRA). This expert group aims to provide easy-to-follow and practical MRA recommendations specific to fresh produce and aims to provide guidance on implementation of risk assessment strategies.

Developing Practical Risk Assessment for Fresh Produce Industrial Practice: Issues Faced While Putting 'Formal MRA' into Industrial Practice

ROY BETTS, Campden BRI, Gloucestershire, United Kingdom

Assessment and management of microbiological risks is of critical importance within any organisation producing foods. Risk assessment is a key part of food safety assurance and there are numerous published examples of large risk assessments done using Codex approaches, by governmental agencies and research bodies that tackle risks associated with particular products in great detail. But how does that leave smaller food producers that do not have the time or the access to expertise and information available to those involved in these Codex style assessments? Does this mean they are unable to make use of "Risk Assessment" to help in producing safe products? Or perhaps an alternative route is available to help such producers make use of a "Risk Assessment" approach. An ILSI sub group has considered how such an approach of "An assessment of Risk" could be used by producers of fresh produce to help make key decisions and reduce risks. Fresh produce is an interesting product from a microbiological view, in most cases it is grown in an open environment. Control over environmental contamination may be considered to be difficult to achieve, yet this product is essentially ready to eat and true HACCP CCPs are virtually impossible to apply. Many of the microbiological hazards that apply to fresh produce will originate at the growing stage. Once those hazards are in place, they can be very difficult to effectively control. The "Assessment of Risk" approach suggested by the ILSI group, gives fresh produce growers a simple route forward by which they can look at the practices they employ in field growing and harvesting, can make well considered judgements as to the relative risks of various alternative ways of growing/harvesting product and finally employ a transparent and recorded system of "approaches" that can reduce risks during growth and harvest.

Assessment of the Risk for Fresh Produce Primary Producers: Presenting the Example of Fresh Produce Assessment of the Risk

JAMES MONAGHAN, Harper Adams University, Newport, England

Growers are required to complete risk assessments for the production of leafy crops supplied either to retail or for further processing. There is an assumption that this will reduce the risk of supplying contaminated produce to the end consumer. In contrast to quantitative microbial risk assessments commonly used in international hygiene criteria setting, grower risk assessments (RA) are based primarily on qualitative judgements of hazard and risks at a number of stages in the RA process. This approach leads to a RA based on subjective opinion allowing different businesses to view the same hazard as posing a different level of risk and having differing

levels of intervention and hence risk reduction. This presentation looks at the reality of leafy vegetable primary production and highlights the areas of potential risk to contamination of fresh produce that require RA, namely water used in production, manure and soil, pest incursion, equipment and worker hygiene. The second part of the presentation discusses a number of examples/scenarios using a structured qualitative assessment which requires all decisions to be based on evidence and outlines a framework for describing the decision process that can be challenged and defended within the supply chain.

The Fresh Produce Assessment: The Relevance of Risk Assessment for the Food Service

BIZHAN POURKOMAILIAN, McDonald's Corporation, London, United Kingdom

Fresh produce at the end of the supply chain, the restaurant, does not often receive any intervention for microbial load reduction. The restaurant relies on the partners upstream to have taken the necessary steps to assure the safety of the product, as at this point, the product is considered ready-to-eat. The question arises, how confident can the restaurant be of this assurance? End product testing? Is that the answer? By the time the test results come through, the product has been consumed. If positive release is employed, then half the shelf life, at best, has perished. The provision of sound, simple and practical assessment of risk in the supply chain is essential in elevating security of product safety and further safeguarding consumer safety. Risk assessments have traditionally been directed towards the processor. This practice must change and risk assessment must be extended to cover the complete supply chain, from farm to fork. Risk-based food safety tools and risk assessment are being employed more at the end and middle of the supply chain, but not often found at the beginning, the field growing stage. Rarely have we seen simple, focused and practical approaches to assessment of risk at the field level. This approach would strengthen the supply chain and give more confidence to the consumer through reduction in food safety incidences, arising from pathogen contamination of fresh produce.

S13 Balancing Food Quality and Virus Inactivation for Sensitive Foods

Enteric viruses, particularly human noroviruses (NoV) are the most common cause of foodborne disease, responsible for up to 50% of all outbreaks and cases per year in the U.S. and internationally. Viruses enter the food supply across the farm-to-fork chain by exposure to contaminated waters, surfaces, food and/or human hands and could persist in the environment and food contact surfaces for long periods of time. Unlike bacterial pathogens, for which there are widely used validated inactivation methods, such methods may not be effective or

applicable to viruses. Foods attributed to virus contamination are usually minimally processed or handled incorrectly. Therefore, any developed methods for the inactivation of viruses in these sensitive foods should balance between the need to inactivate viruses and bacterial pathogens and optimized food quality. There have been a number of new developments in the area of legacy thermal and non-thermal processing technologies where tweaking of the processes can enhance the inactivation of viral pathogens. The session will explore the use of models to predict viral loads, help to define the risk and criteria for viral inactivation and where are the gaps or limitations of inactivation strategies.

How Do Viruses Enter the Fruit and Vegetables Food Chain and Estimation of Consumer Risk

LEENA MAUNULA, University of Helsinki, Helsinki, Finland

In developed countries, a substantial number of foodborne disease outbreaks is caused by enteric pathogenic viruses. Contamination by viruses like noroviruses (NoV), hepatitis A virus (HAV) and hepatitis E virus (HEV) in foods does not correlate with indicator bacterial loads that are used for assessment of microbiological quality of food products. Typically also backtracking of foods such as shellfish and frozen berries is challenging; one batch includes berries picked at numerous farms and oysters from different cultivation areas can be mixed, even across borders. The viral contamination of food is closely linked to human population and sewage, except for the zoonotic HEV that is linked to animal products. The viruses enter the soft fruit chain in production mainly via contaminated irrigation water and/or harvesters' hands. During processing, the critical points are washing and chlorination for leafy greens, freezing and packaging for soft fruit. Manual handling of food produce at point-of-sale has also to be taken into account. Recent epidemiological investigations of outbreaks have shown evidence about the role of asymptomatic food handlers in contaminating food. However, virus transmission by food handlers leading to outbreaks occurs often at food serving facilities rather than at food industry. With data obtained by harmonized quantitative viral detection methods, it is possible to build more accurate risk models and compare scenarios, although due to numerous data gaps, many assumptions are still made. Here we describe our long-term experiences of analyzing NoV from foods, mainly soft fruits.

Viral Inactivation Using Legacy Thermal Inactivation Technologies and Its Limits

SOPHIE ZUBER, Nestle Research Center, Lausanne, Switzerland

The characteristics of foodborne viruses present new challenges for the food industry. Human enteric viruses such as Norovirus (NoV), hepatitis A and E viruses (HAV, HEV) are an emerging food safety

concern and represent a major cause of outbreaks of gastroenteritis and hepatitis. Animal viruses are also of concern to the food industry, as the risk of importing economically important viral pathogens such as foot and mouth disease virus (FMDV) restricts trade in livestock and their products. Heat represents the most efficient treatment to inactivate foodborne viruses and as a general rule, the higher the temperature, the higher and the faster the reduction in viral infectivity. Thermal inactivation kinetics data for foodborne enteric viruses and their surrogates in cell culture media are available. However, the impact of certain industrially applied thermal processes on viruses in complex matrices is not well described. This is owing to the difficulties in designing relevant laboratory scale experiments, achieving recovery of spiked virus in subsequent tissue culture and natural microbial contamination problems linked to the matrix. This presentation will focus on the work done in the frame of a collaborative project looking at the inactivation of virus surrogate and pathogen in extrusion cooking of petfood. Other processes will also be discussed, especially the effect of heat on viruses in low-water activity food matrices and the limitations of thermal processing for certain sensitive foods where non-thermal technologies need to be further explored to ensure the inactivation of viruses.

Developments and Optimization of Non-thermal Technologies for Viral Inactivation

ALVIN LEE, Illinois Institute of Technology/IFSH, Bedford Park, IL, USA

Enteric viruses, particularly human noroviruses (NoV) are the most common cause of foodborne disease, responsible for up to 50% of all outbreaks and cases per year in the U.S. and internationally. Enteric viruses including NoV, hepatitis A and E viruses can enter the food supply through contaminated environmental factors or by contamination during handling and processing, resulting in outbreaks ranging from small isolated ones to epidemic. Foods attributed to viral contamination are usually minimally processed, sensitive to quality changes and often without any thermal treatments prior to consumption. A number of innovative non-thermal food processing technologies such as high pressure processing, high intensity pulsed light and traditional ultra-violet light and high powered ultrasound can be optimized for viral inactivation in/on these sensitive foods often without adverse effects to visual appearance. Levels of viral inactivation can vary depending on technologies, surrogates used in the study and its analytical methods. Inactivation levels usually range between 1 to 5 log reduction of human NoV surrogates. The session will introduce audience to these technologies and steps taken to optimize these technologies for viral inactivation. The session will also help to define the risk and criteria for viral inactivation and where are the gaps or limitations are for inactivation strategies.

S14 Sporeformers in Food; Implication of Natural Diversity on Food Safety and Food Quality

Bacterial spores are extremely resistant and therefore widespread in food ingredients and foods. Efficacy of control of sporeformers by the food processing industry is challenging due to diversity between strains and species with respect to their spore robustness and ability to rapidly germinate. Food processing and food characteristics can impose significant selective pressure on sporeformers, whereby most robust sporeformers and/or the best growers predominate. This symposium will focus on diversity of relevant food sporeformers (both pathogens and spoilage organisms) and the implication for controlling food safety and quality. We will address these issues with leading experts in the spore research field, discussing quantification of natural diversity between strains and between species, genetic biomarkers for high spore heat resistance and diversity in growth performance of germinated spores, thereby highlighting the relevance for these aspects in the actual control of sporeformers in food.

Variability in Heat Resistance of Sporeformers; How Diverse is Diversity?

HEIDY DEN BESTEN, Wageningen University, Wageningen, Netherlands

Realistic prediction of microbial inactivation in food requires quantitative information on variability introduced by the microorganisms. *Bacillus* species form heat-resistant spores and variability in spore heat resistance varies among species. Therefore, the spore heat resistance of the spoilage organisms *Bacillus subtilis* and *Geobacillus stearothermophilis*, and the foodborne pathogen *Bacillus cereus* were characterized in detail using twenty strains per species. This allowed comparison of variability in spore heat resistance between and within *Bacillus* species. In addition, reproduction variability was determined using two biologically independent spore crops for each strain which were heat treated on different days. Reproduction variability was significantly lower than strain variability for all three species. A meta-analysis on spore heat resistance of *Bacillus* species demonstrated that strain variability explained at least 50% of all variability in heat resistance of sporeformers. This indicates that integration of microbiological variability in prediction makes predictions not more accurate, yet more realistic.

Genetic Biomarkers for High Heat Resistance of *Bacillus* Spores: Relevance for Optimal Design of Heat Treatment

MARJON WELLS-BENNIK, NIZO Food Research, Ede, Netherlands

Spores of various *Bacillus* species can display very high levels of heat resistance, allowing for survival of even sterilization processes that are commonly applied in the food industry. This includes spores of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* and various other species. However, high level heat resistance of spores is seen only for certain strains of these species. This makes it hard to predict if their presence will cause problems in final heat-treated products. So far, it was not possible to establish whether spores of *Bacillus* isolates could survive high heat treatments (i.e., at least 30 min 100°C) unless their survival was assessed in an experimental setting. We recently identified a mobile genetic element that is responsible for high level heat resistance of *Bacillus subtilis* spores. It encodes proteins that are specifically expressed during spore formation. The presence of this element in the genomes of wide array of *Bacillus* species was investigated and could be linked directly with high-level heat resistance of spores of certain *B. subtilis* strains. Moreover, this was also demonstrated for high-level heat resistance of spores of certain *B. amyloliquefaciens* and *B. licheniformis* strains. In addition, we established that the copy number of the element correlated positively with further increased heat resistance of spores. The presence or absence of this element can now be established by PCR, and based on the established heat inactivation kinetics of the spores of a large range of individual strains, optimal heat treatments can be designed to inactivate such spores in foods.

Combined Approaches to Differentiate the Common Mister *B. licheniformis* and the Super Spoiler

FLORENCE POSTOLLEC, ADRIA UMT14.01 SPORE RISK, Quimper, France

Sporeforming bacteria are ubiquitous in the environment and exhibit a wide range of diversity leading to their natural prevalence in foodstuff. In most cases, *Bacillus* and related genus enter the industrial plants via spore contaminations of raw material or dehydrated ingredients and may persist in the environment. While spore is the resistant but dormant state of these bacteria, food spoilage and food poisoning is only due to the vegetative cells multiplication of specific strains. Better knowledge on biodiversity, in particular regarding spore resistance, germination, growth and spoilage abilities may explain why specific strains, called here super spoiler, are able to survive process and develop in food during shelf life. Based on combined screening approaches, either common *Bacillus* contaminant or super spoiler may be identified. Deep characterization of these contaminants provides an added value when conducting challenge tests in food

as it ensures the evaluation of different scenario of relevance for food business operators to better adjust process and shelf life.

S15 The ISO 16140 Series and the Impact on the Routine Labs' Daily Life

The ISO 16140 standard (validation of alternative microbiological method) has now been split into 6 different parts, addressing various needs in the validation of alternative and reference microbiological methods. The first two parts will be published in 2016. Part 1 describes the terminology, while Part 2 is dedicated to the validation of proprietary microbiological methods, i.e., detection and enumeration methods. Both parts have already been implemented in ongoing validation studies in Europe by different certification bodies. Additionally, Part 3 will provide protocols for the implementation and verification of reference and alternative methods in routine labs, Parts 4 and 5 will be a new opening for internal method development and Part 6 will target the validation of confirmation methods. What are the links between the different ISO 16140 parts, particularly Part 3 which deals with method implementation and verification in routine laboratories? How will that impact on routine testing, on quality management and accreditation requirements, on the relationship between labs and their clients? How will the kit manufacturing industry deal between Parts 2 and 3? The session is fully dedicated to FBO, particularly to analysts and quality managers from contract labs and food industries. The roundtable will start with two oral presentations, introducing the ISO 16140 series, with a special focus on the needs for method verification. Then the roundtable will gather key parties. - Patrice Arbault, BioAdvantage Consulting, CEO, France - François Bourdichon, Danone Vitapole, Food Safety Analytical Governance Director, France - Benjamin Diep, Nestle Research Center, Switzerland - Christophe Dufour, Scientific Director of Mérieux NutriSciences (Fr) - Christina Harzman, BioTecon Diagnostics GmbH, Key Account Manager, Germany

The ISO 16140 Series and Their Impact on Routine Laboratories

DANIELE SOHIER, ADRIA, Quimper, France

After a short introduction of all the ISO 16140 series, the presentation will focus mainly on the Part 3, which is fully dedicated to routine laboratories. The Part 3 will provide the technical protocols for the implementation and verification of reference and alternative methods. Many accreditation bodies and food business operators (FBO) are waiting for that Part 3 of the ISO 16140 standard. The study designs are now available for both qualitative and quantitative methods, but intensive discussions are still going on at the international level regarding the selection and the number of the matrices to be tested, the required inoculation levels, the data interpretations.

The session will be a wonderful opportunity to

get the opinion of the FBO regarding the technical and economic aspects of the developed approach.

Testimonials: What Does It Mean to Use Validated and Verified Methods in the Food Industry?

PAMELA WILGER, Cargill, Minneapolis, MN, USA

The food and feed industry spend millions of U.S. dollars every year analyzing their products to make sure the product is the correct functionality, quality and safe. The industry also monitors their environment to be more proactive in discovering and reacting to preventing issues before they show up in the product. The reason we do all this testing is to obtain data to use to make decisions on the products being produced and our environment. Thus, it is critical to get the most accurate data science can provide. The use of scientifically approved and validated methods is essential to be successful at making the correct decisions. The problem is many people do not understand what it means to be an approved and validated method. I will discuss the importance to the food and feed industry to use validated and verified methods and the challenges of why this is so hard today.

S16 Managing Allergens: How Do We Assess the Risk and Protect Allergic Consumers?

Food allergy is an issue of high and growing importance to public health, affecting consumers' quality of life and resulting in increasing calls on health service resources. There is growing evidence that this impact is increasing at a global level as developing countries adopt a "Western" lifestyle. Therefore, minimising the risk from allergenic foods is a shared responsibility of all the stakeholders involved (e.g., patients, clinicians, food manufacturers, regulators). ILSI Europe's Food Allergy Task Force aims to foster an international evidence-based consensus on how to assess the risk and develop the tools which will help to manage this risk. The task force and its expert groups consist of a strong network of international experts and include among their partners world-class experts and leading organisations such as the Food Allergy Research & Resource Program and the European Academy of Allergy and Clinical Immunology. Moreover, ILSI Europe plays an important role in the FP7-funded project iFAAM (Integrated Approaches to Food Allergen and Allergy Risk Management; 38 partners), coordinating effective dissemination of project results to relevant stakeholder groups to ensure impact in terms of improved quality of life for allergic consumers, improved food safety and increased competitiveness of the European food industry. The overall objective of this project, which was built to address some of the questions and data gaps that emerged from the prior EuroPrevall project, is to develop evidence-based approaches and tools, based on e-Health concepts, for management of allergens in

food and to integrate knowledge derived from their application and new knowledge from intervention studies into food allergy management plans and dietary advice. Within this session, the task force activities and main outcomes will be outlined and the objectives and achievements of the iFAAM project will be presented, together with valuable insight into recent developments in risk assessment of food allergenicity.

The ILSI-Europe Food Allergy Task Force: Promoting the Safety of Food Allergic Consumers

RENÉ CREVEL, Unilever, Bedford, United Kingdom

ILSI Europe's Food Allergy Task Force was established in the late 1990s when food allergy had just been recognised as a food safety issue rather than a medical curiosity. From the very start, the Task Force formulated its approach around a risk assessment framework, aiming to demystify food allergy as a toxicological phenomenon, while mindful of its unique characteristics. Thus, the work which the Task Force undertook can be mapped chronologically within a risk analysis framework encompassing hazard identification and characterisation, exposure and risk assessment. In that context, the Task Force has and continues to aim to foster an international evidence-based consensus on how to assess the risk and develop the tools which will help to manage this risk. It also recognised early that minimising the risk from allergenic foods is a responsibility shared by all the stakeholders involved, including patients, clinicians, food manufacturers, regulators. On that basis, it developed collaborations with a strong network of international experts, representing all those stakeholders. This presentation will illustrate the work of the Task Force and the role it has played in the development of the current approach to assessing the risk from food allergens and thereby protecting allergic consumers.

From EuroPrevall to iFAAM - Insights into Food Allergen Management

CLARE MILLS, University of Manchester, Manchester, United Kingdom

It has been estimated that up to 20 million European citizens suffer from food allergy. However management of both food allergy (by patients and health practitioners) and allergens (by industry) is thwarted by lack of evidence to either prevent food allergy developing or protect adequately those who are already allergic. The EU-funded EuroPrevall project collected data on the patterns and prevalence of food allergies across Europe and beyond, to enable effective, evidence-based approaches to managing food allergies. These complex data are now being utilised in the iFAAM project to:

(1) Extend and integrate existing cohorts from observation and intervention studies to provide evidence as to how maternal diet and infant feeding practices (including weaning) modulate the patterns and prevalence of allergies across Europe;

(2) Establish risk factors for the development of severe reactions to food and identify associated biomarkers;

(3) Develop a clinically validated tiered risk assessment and evidence-based risk management approach for food allergens for allergens in the food chain;

(4) Develop clinically relevant multi-analyte methods of analysis suited to allergen management across the food chain.

The new data generated in iFAAM will broaden the evidence base to support efforts to revise and update the list of allergenic ingredients in Annex II of the EU Food Information Regulation. The data on threshold doses, risk models and their clinical validation will also support the development of evidence-based action levels for allergens in foods. In parallel, clinically relevant analytical tools are being developed, utilising ingredients and foods that have a defined allergenic activity, thus helping to provide analysts with much needed quality control samples with potential to be developed into properly validated reference materials in the future. The iFAAM project will also deliver a knowledge base that is needed by public health authorities to develop new guidance with respect to dietary advice to pregnant and breast feeding mothers and with regards infant feeding practices (including weaning).

Recent Developments in Risk Assessment of Food Allergens

GEERT F. HOUBEN, TNO (Netherlands Organisation for Applied Scientific Research), Zeist, Netherlands

Risk assessment of food allergens has made an enormous development during the last decade. Depending on the risk management question and goal, different approaches can be followed in risk assessment, which is also the case in food allergen risk assessment. Particular for population risk management purposes, probabilistic quantitative risk assessment is nowadays considered the most appropriate approach. Probabilistic quantitative risk assessment was first proposed and developed for food allergen risk assessment by TNO, The Netherlands, and has been used in the development of the current Voluntary Incidental Trace Allergen Labeling (VITAL[®] 2.0) guidance of the Australian-New Zealand Allergen Bureau and for the quantification of risks of (possible) unintended allergen presence in food products. The approach provides many advantages over other approaches and many different applications are possible, like for instance to assess and compare the efficacy of various management options in reducing the risks of allergens in food chains. However, the approach also depends on stakeholder's ability to deal with risks. Some background on the need for and principles of probabilistic risk assessment in food allergy as well as some examples of its application and interpretation will be presented and discussed.

S17 Strategies to Control Foodborne Pathogens: Focus on *Campylobacter* in Broilers

As reported by the European Food Safety Authority (EFSA), *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) since 2005. The disease is characterized by watery or bloody diarrhea, abdominal cramps and nausea (Blaser et al., 2008). Post-infection complications include peripheral neuropathies, Guillain-Barré syndrome, and functional bowel diseases (Moore et al., 2005). Numerous studies have already emphasized the importance of poultry as a reservoir and source of *Campylobacter* (Hermans et al., 2012; Sasaki et al., 2013) and broiler meat is considered the main foodborne source of *Campylobacter* human infection (EFSA 2015; Nadeau et al., 2003; Nielsen et al., 2006; Silva et al., 2011).

In Europe, the mean prevalence of *Campylobacter* in primary poultry production is very high, up to 70% of broiler batches being contaminated (EFSA 2010). Moreover, the prevalence of *Campylobacter* on broiler carcasses is much higher at the slaughterhouse due to cross-contamination between infected and non-infected birds, standing at about 75%. A quantitative microbiological risk assessment of campylobacteriosis in Europe demonstrated that controlling *Campylobacter* in broiler flocks could be highly beneficial to public health because of its impact all along the broiler food chain (slaughter, retail sales and consumption) (Romero-Barrios et al., 2013). Treatments of meat on an industrial scale could even eliminate human campylobacteriosis. However, several of these processes could impact meat quality (Meunier et al., 2015). The current proposal will point out the epidemiology of *Campylobacter* in the broiler chain and highlight few strategies experimented to reduce *Campylobacter* prevalence in primary poultry production, and so prevalence of human campylobacteriosis. Three talks are proposed and will be presented by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), a private research center (IMASDE AGROALIMENTARIA), and a public research unit (INRA, UMR 1014 Secalim).

Why is *Campylobacter* the Number One Priority for the Poultry Production Chain?

MURIEL GUYARD-NICODÈME, Ségolène Quesne, Typhaine Poezevara, Sandra Rouxel, Emmanuelle Houard, Valérie Rose, Katell Rivoal, Marianne Chemaly, French Agency for Food, Environmental and Occupational Health and Safety, Ploufragan, France

Campylobacteriosis is the most frequently reported zoonotic disease in humans in the European Union with 236,851 cases in 2014. Unfortunately, this number is largely under-reported. The species most commonly associated with human infections are the

thermotolerant *C. jejuni* and *C. coli* that can colonize poultry, cattle, pigs and sheep in addition to wild birds and mammals. However, it is recognized that contaminated broiler meat could account for 20% to 30% of human campylobacteriosis, while the chicken reservoir as a whole could be responsible for 50% to 80% of cases. The prevalence of *Campylobacter* at the flock level is very high and it is the same along the food chain. The mean count of *Campylobacter* in the intestinal tract of birds is about 8 log₁₀ CFU/g. In France, the prevalence of *Campylobacter* spp. was around 77% at the flock level, 87% at the slaughtering level and 76% at the retail level. The mean count of *Campylobacter* in the intestinal tract of birds was about 8 log₁₀ CFU/g and on meat products the higher counts reached more than 4 log₁₀ CFU/g. The main risk factors identified were hot season, age of birds, thinned flocks, slaughtering practices and products packed under plastic wrap film. The molecular characterization of *C. jejuni* poultry isolates showed great diversity and the majority could be potentially virulent for humans harboring sialylated lipooligosaccharides. As a consequence, the contaminated broiler meat products are a source for cross-contamination to other foodstuffs and surfaces during meal preparation in the consumer's kitchen. Both, *C. jejuni* and *C. coli* showed abilities to transfer during handling in the kitchen. Risk reduction of human campylobacteriosis requires therefore implementation of *Campylobacter* control measures at the primary production level and also at the following levels of the poultry production chain.

An Update about the Different State-of-the-Art Methods to Control *Campylobacter* in Broilers: The European Project CAMPYBRO

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There is no a simple effective strategy to decrease *Campylobacter* infection in chicken carcasses. Starting from the premise that there is no vertical transmission, reduction strategies fall into two groups: pre-slaughter and post sacrifice. Within the post sacrifice strategies; there are various measures that can be applied in the slaughterhouse: logistic slaughtering, strict cleaning and disinfection procedures, specific-designed physical steps to decrease the *Campylobacter* counts (crust freezing or ultrasound-steam systems) and the controversial use of chemicals. Another post sacrifice measures are the heat processing or freezing the contaminated batches. Within the pre-slaughter strategies, there are different alternatives: increase biosafety measures, insect control, decrease slaughtering age, avoid thinning and maybe in the future, vaccination. Finally it is possible try to control the infection by feeding through two strategies: the feed composition and structure, and the use of additives. Unfortunately, the feed composition and structure has limited effect on *Campylobacter* infection. However, a combination of additives (a probiotic based on *B. subtilis* and a mixture of organic acids with monoglycerides of medium chain fatty acids) has demonstrated an

effective control of infection in challenge trials. This effectiveness has to be proven in field trials with natural strains of *Campylobacter jejuni* and *Campylobacter coli*. The project CAMPYBRO "Control of *Campylobacter* infection in broiler flocks through two-steps strategy: nutrition and vaccination" <http://campybro.eu> funded by the 7th Framework Program of the European Union, try to develop strategies to reduce levels of *Campylobacter* in poultry production through i) nutritional dietary interventions ii) development a vaccine by reverse vaccinology.

Use of Potential Probiotic Strains to Reduce *Campylobacter jejuni* in Broilers: Recent Developments Using *Lactobacillus salivarius* SMXD51

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Campylobacteriosis is the most frequently reported zoonotic disease in humans in the European Union, and poultry meat the main source of human infection. Several strategies to reduce the prevalence and colonization of *Campylobacter* in broiler chickens are being developed in the primary production, including the use of probiotics. Recent advances will be presented with a focus on *Lactobacillus salivarius* SMXD51. SMXD51, a potential probiotic strain shows an *in vitro* and *in vivo* anti-*Campylobacter* activity. But, the delivery route and the administration form are inconsistent for an industrial application. Freeze-dried SMXD51 were produced from a laboratory (LFD-SMXD51) and an industrial (IFD-SMXD51) scale. The aim of the study was to assess i) the competitive exclusion of *Campylobacter jejuni* in broiler chickens by freeze-dried SMXD51, and ii) the influence of the freeze-drying processes on physiological parameters and probiotic abilities of SMXD51 through *in vitro* assays.

Administration of freeze-dried SMXD51 showed variable impact on *Campylobacter* colonization. Per os treatment with LFD-SMXD51 led to a significant reduction of cecal *C. jejuni* loads by 1.5 log ($P < 0.001$) in broilers of 35 days of age while no effect was observed in another trial. IFD-SMXD51 treatments (per os or in feed) showed no effect on *Campylobacter* colonization. *In vitro* comparison of the freeze-dried forms with the native strain showed differences in membrane cell properties and probiotics abilities. Literature highlights interesting but variable results on reduction of *Campylobacter* levels in broiler chickens. Our results suggest that freeze-drying processes and differences of avian gut microbiota are two factors that could influence the *in vivo* anti-*Campylobacter* activity of SMXD51.

S18 Antimicrobial Resistance in the Food Chain

The purpose of this session is to give an overview of the problem of antimicrobial resistance in the food chain. The session will feature one speaker from the food industry, one from WHO and updates from two academic scientists that are involved in EFFORT (Ecology from Farm to Fork Of microbial drug Resistance and Transmission), the FP7 EC project that was launched in 2013.

Introduction to Antimicrobial Resistance in the Food Chain: The Relevance of Tackling Antimicrobial Resistance from a Global Point of View

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Antimicrobial Resistance (AMR) is a significant public health problem resulting from use and misuse of antimicrobial agents. Any kind of antimicrobial use in people, animals or plants can promote the development and spread of AMR. Also, AMR does not respect geographical or biological borders. Thus, the use of antimicrobial in one sector, setting or country affects the spread of resistance in others. AMR is also a food safety concern considering the use of antimicrobials in food animals, for treatment, disease prevention or growth promotion, thus allowing resistant bacteria and resistance genes to pass through the food chain from food animals to humans. Resistance in the foodborne zoonotic bacteria *Salmonella* and *Campylobacter* is clearly linked to antimicrobial use in food animals, and foodborne diseases caused by such resistant bacteria are well documented in people.

Tackling AMR requires a multi-faceted holistic approach, which includes collaboration, cooperation and information-sharing between the public health and the veterinary sectors. Addressing use of antimicrobial agents in food chain and the occurrence and spread of AMR in the food chain is an important aspect of the efforts to combat AMR.

At the Sixty-eight World Health Assembly in May 2015, the World Health Assembly endorsed a global action plan to tackle AMR. The World Health Assembly also urged all Member States to develop and have in place by 2017, national action plans on AMR that are aligned with the objectives of the global action plan. A manual has been developed by WHO, in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE), to assist countries in preparing or refining their national action plans. It aims to facilitate the participation of all relevant sectors, and outlines an incremental approach that can be adapted by countries to their specific needs, circumstances and available resources.

A Global Vision for Antimicrobial Stewardship in Food Animals: Preserving Antimicrobial Effectiveness in the Future through Ethical Practices Today

To be determined

Biophysical Parameters Affecting Gene Transfer in the Food Chain: First Results from the EFFORT FP7 EU Project

BRUNO GONZALEZ-ZORN, Complutense University Madrid, Madrid, Spain

Microbial antibiotic resistance (AMR) is widely accepted as a growing concern. It is well recognized that the application of antibiotics in human clinical therapy, agriculture and aquaculture, all contribute to the emergence and persistence of antibiotic resistance due to selective pressure. Throughout the food chain, from live animals to retail, bacteria are subjected to harsh conditions. These conditions often stress the microorganisms, and may enhance horizontal gene transfer between bacteria and thus spread antimicrobial resistance determinants.

Therefore, the main aim of this presentation is to present an in depth analysis of the conditions to which the bacteria are subjected throughout the food chain. The conditions have been analysed and classified for the demonstrated or potential role in enhancing dissemination of antimicrobial resistance determinants. Further, we'll show how knowledge gaps are being filled using experimental approaches in order to establish their potential effect on gene transfer and selection pressure.

S19 Food Allergen Control under Preventive Food Safety Systems

The widespread adoption of HACCP, Preventive Controls, and GFSI food safety schemes requires that producers, auditors, and regulators all understand how to design and implement effective food allergen controls within these frameworks. This includes considering regulatory requirements, label declarations, and process controls as well as the fact that allergens may be present either as ingredients or through cross-contact. Risk management for food allergens differs significantly from that for microbial and other chemical hazards through reliance on label declarations, both mandatory and voluntary, as a primary risk mitigation tool. Differences between various regulatory and audit schemes introduces an additional level of complexity, particularly those rules (such as the U.S. Foreign Supplier Verification Programs rule) that affect international trade. Further, the allergen detection and measurement technologies needed to support validation and verification of allergen controls is evolving rapidly. This symposium will provide updates on how allergen control is integrated into the new GMP and preventive controls rule in the U.S. and into GFSI recognized audit schemes, and on new detection and quantitation techniques that are being used to support food allergen controls. This information will make it

possible for food producers to assess the adequacy of their current food allergen controls and to plan for any needed updates.

Food Allergen Controls under FSMA and the FDA Preventive Controls Rule

STEVEN GENDEL, IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA

The new FDA Preventive Control (PC) rule (Current Good Manufacturing Practice, Hazard Analysis, and Risk-based Preventive Controls for Human Food) requires each food manufacturing facility to implement Good Manufacturing Practices (GMP) to control allergen cross-contact, to assess food allergen hazards during development of the Food Safety Plan, and to develop allergen control plans as needed to prevent or minimize both cross-contact and mislabeling. This includes the need to consider the potential for allergen cross-contact and mislabeling when developing supply chain controls. These requirements apply to facilities in the U.S. and to those that export foods and ingredients to the U.S. This combination of GMPs, PCs, and supply chain controls represents a significant increase in the level of concern for food allergen safety and the documentation needed to demonstrate control. Careful planning will be needed to adapt existing HACCP-based allergen control systems to meet the requirements of the PC rule; and extended efforts will be needed by facilities that have previously considered allergen control as a prerequisite program.

Allergen Control, Analysis and Global Food Safety Initiative Schemes

RICHARD FIELDER, Elisa Systems, Windsor, Australia

Providing safe food to an increasing number of adults and children susceptible to food allergies, intolerances and sensitivities is challenging enough. However, more often it needs to be offered in different social settings with near family and friends. The food industry has embraced both the challenge and the opportunity. Allergen-free-from foods have moved from occupying a few shelves in a retailers store to the main aisles, and from a sub-section of a restaurant menu to the main provision. Today, there's a broader array of food manufacturers and food service operators from around the globe providing greater choice together with a broader range of consumers seeking more information on healthier choices. Safeguarding the health of those at risk from allergenic ingredients and foods is the industry's responsibility and minimum legal obligation. In a new era of globally recognised food safety frameworks and evidence-based regulatory systems; providing reliable evidence to support effective Allergen Management systems can't be that difficult surely? We have evolved our systems, implemented new technology, empowered our staff through training, reinforced a supportive culture and introduced new testing. Yet, as we work through our risk assessments and validation exercises it is often the simple questions which still

prove the most difficult to answer: how much is too much? How clean is clean? Testing should provide the objective evidence that management and auditors need to assess the effectiveness of their Allergen Control plans. Whilst it is apparent that new tests have enabled us to take better control, recent events have revealed that interpreting the data from testing is often problematic.

Advances in Detection and Measurement Technologies that Support Validation and Verification of Allergen Controls

ROBERTO LATTANZIO, Eurofins Analytik GmbH, Hamburg, Germany

The great relevance of the allergen topic motivates many food analysts to develop sensitive and reliable detection and determination methods. Until today a variety of different analytical approaches are being published. Driven by the effort of the food industry to address risk management for food allergens and the consequent need for allergen tests, some of these techniques are commercially available as so called “kits” and widely used for routine testing (mainly ELISA and PCR). During the recent years this established methods were improved with some success in terms of their analytical properties (sensitivity, accuracy and precision). Since the diversity of food products and raw material being tested is constantly increasing, extended matrix validations of the applied method become more and more essential. Therefore the question what might be the “best analytical strategy” is quite context depending. What is the given sample matrix? What is the possible contaminating product? Which test is the most robust in terms of the applied production conditions (heat, pressure, acidity etc.)? Approaches like LC-MS/MS bypass some of the weak points of the established methods by design but also here the development of a robust routine method is difficult and tedious. On the other hand, a consequent risk assessment and transparency along the complete production can make all this choices much easier and improve the value of allergen tests significantly. Given that even complex issues can be resolved with astonishing imperfect techniques.

S20 FSMA Implications for Suppliers to the USA and Training Opportunities

The Food Safety Modernization Act (FSMA) represents sweeping reforms of the USA's food safety regulations. FDA's Current Good Manufacturing Practice, Hazard Analysis and Risk-based Preventive Controls for Human Food regulation and Foreign Supplier Verification regulation were published in late 2015 and go into effect over the next few years, depending on facility size and type. The Food Safety Preventive Alliance (FSPCA) was established in 2011 to help small and midsize food companies to comply with the Preventive Controls regulations through development of training and technical assistance. This session provides an overview of the Preventive

Controls for Human Food regulation. It also reviews FSPCA training, how individuals can access the training, and how qualified persons can become Lead Instructors. The session also addresses implications for food companies sending food to the USA.

Preventive Controls for Human Food Regulation Overview

JENNY SCOTT, U.S. Food and Drug Administration, College Park, MD, USA

The Preventive Controls for Human Food rule promulgated in accordance with the FDA Food Safety Modernization Act (FSMA) requires that domestic and foreign facilities that must register with FDA because they manufacture, process, pack or hold food for consumption in the U.S. develop a food safety plan. The food safety plan contains a hazard analysis, preventive controls for identified hazards, and procedures for monitoring, verification, and corrective actions. When a hazard in a raw material or other ingredient has been controlled by a supplier, a supply-chain program must be implemented and control of the hazard verified. Certain activities related to the food safety plan must be conducted by a “preventive controls qualified individual,” who is qualified by training or job experience to conduct those activities. In addition, U.S. importers must verify that food imported into the United States has been produced in a manner that meets the same level of public health protection as U.S. safety standards. Thus, it is important for companies exporting to the U.S. to understand how to comply with the new FSMA regulations.

FSPCA Preventive Controls for Human Food Curriculum - How is It Different from HACCP Training?

KATHERINE MJ SWANSON, KMJ Swanson Food Safety, Inc., Mendota Heights, MN, USA

The Food Safety Preventive Controls Alliance (FSPCA) was established as part of a grant from FDA to the Illinois Institute of Technology's Institute of Food Safety and Health. FSPCA is a public/private partnership among U.S. federal and state regulatory officials, academic food safety researchers and educators, and food industry representatives. The FSPCA *Preventive Controls for Human Food* course is the standardized curriculum recognized by FDA. The FSPCA training materials are designed to meet the requirements for training preventive controls qualified individuals who conduct certain Food Safety Plan activities. Attending an FSPCA course is not mandatory, but it provides assurance that the course content is consistent with regulatory expectations. The FSPCA curriculum is built on established food safety principles, thus there are many similarities with HACCP. However, consistent with the preventive controls regulations, the hazard analysis process is used to identify other preventive controls that must be implemented, and may include process controls, food allergen controls, sanitation controls,

supply-chain-applied controls, and other controls as appropriate to the food, the facility, and the nature of the hazard. The FSPCA course manual and information on training for FSPCA Lead Instructors can be found on the FSPCA website.

Preventive Controls Implications for Suppliers to the USA

JOHN DONAGHY, Nestle, Vevey, Switzerland

From 17 September 2016, suppliers based outside of the USA whose food products are imported for the USA market, potentially must comply with the “FSMA Final Rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals.” In essence, this rule requires that foreign suppliers produce food in a manner that provides the same level of public health protection as the preventive controls required under FSMA for relevant local manufacturers. To that end, FDA requires local food business operators and importers alike to ensure due compliance with the Preventive Controls approach launched under FSMA. The onus is on the importer to verify compliance of its supplier to the Foreign Suppliers rule and to ensure that the foreign supplier’s food is not adulterated or misbranded with respect to allergen labeling. The various FSMA rules have been rolled out in concrete terms since second half of 2015 and a major effort is being made in the USA to provide the necessary capability building within the regulatory bodies and the food industry to allow for effective and timely implementation. While this already poses quite a challenge to food business operators based in the USA, it is potentially posing an even larger challenge to FBOs located outside of the USA that provide food products to importers based in the USA. In this presentation, some of the challenges for relevant non-USA suppliers to implement preventive controls are discussed in addition to some international efforts to address these challenges.

S21 Microbial Inactivation of Dry Foods – Advances in Scientific Knowledge and Industrial Solutions

The concern about microbiological safety of low-moisture foods is relatively new comparing to the high-moisture products. Only in recent decade, the legal requirements for such foods became more stringent, some industrial guidelines were developed and large amount of research projects related to this topic were undertaken. As the understanding of factors influencing microbial survival in dry conditions is advancing, it is still challenging to transfer this knowledge to the industrial applications. The seminar will focus on recent developments in inactivation technologies for low-moisture foods, as well as their main limitations and solutions for industrialization. Additionally, current advances in kill step validation for dry foods will be discussed, with the main focus on the use of mathematical models as a validation tool. In this symposium, it will be also

explained how product types and environmental factors affect the survival and inactivation of pathogens in low-water activity products, and how these issues can be considered in an industrial process.

The Impact of Water and Product Composition on Pathogen Survival and Inactivation

LINDA J. HARRIS, Bradley Marks, University of California, Davis, Davis, CA, USA

Salmonella is known to persist for long periods in desiccated environments and food products. Additionally, its enhanced resistance to lethal treatments in low-moisture foods is a well-known challenge in designing, validating, and operating pathogen reduction processes for these types of products. In general, *Salmonella* thermal resistance is known to increase with decreasing water activity; however, the interactions between water content, other product components, product structure, and dynamic process conditions are complex and heretofore rarely reported. The purpose of this presentation is to summarize the state-of-knowledge related to pathogen survival and inactivation in low water activity food products, and the resulting impacts on process validation. Field survey data will illustrate the differential persistence of *Salmonella* in various components of real-world processing systems (e.g., tree nuts). Laboratory-scale data will demonstrate the significant impact of product characteristics (e.g., water activity, composition, structure) on resulting inactivation kinetics of multiple low-moisture products (e.g., nut, cereal, and fruit products). Lastly, pilot-scale data will show how processing conditions (e.g., humidity and air velocity) can significantly reduce the impact of product water activity on the inactivation of *Salmonella* during thermal processes.

Use of Heat Transfer Properties and Mathematical Modeling for Validation and Monitoring of Industrial Thermal Process for Low-moisture Foods - Case Studies

NICOLAS MENESES, Buhler AG, Uzwil, Switzerland

See online programme for abstract

Recent Developments in Inactivation Technologies for Low-moisture Foods

Oliver Schlüter, Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Germany

Cold atmospheric pressure plasma (CAPP) has been applied in diverse fields of research to achieve several purposes, e.g., to produce specific functional groups at the surface, increase surface energy and hydrophobicity, introduce surface cross-linking and remove weak boundary layers or contaminants. The application of CAPP is an alternative process for the

inactivation of microorganisms on dry and also heat sensitive surfaces. The inactivation of microorganisms on the surface of dry products, like herbs, spices, and/or almonds is difficult, because of the higher resistance especially of sporulated microorganisms when compared to a medium with a lower water activity. The main challenges in plasma processing of food materials are: i) proper selection of the plasma source, ii) characterization of product-process interactions including quality and safety attributes, and iii) optimized process design and up-scaling for industrial application.

In this presentation different plasma sources were discussed regarding their inactivation of selected microorganisms, e.g., *Bacillus subtilis* spores (PS832). A radio-frequency (RF) plasma jet was used for the direct treatment, working with argon as a process gas with the admixture of O₂ and N₂. Furthermore, a DBD (dielectric barrier discharge) system in a static atmosphere was used with different process gases (air, N₂, O₂). For an indirect treatment plasma processed air (PPA) was used, which was generated by a microwave driven plasma torch. The different generated plasmas were characterized using optical emission spectroscopy, gas analysis tubes and the quantification of ozone. Furthermore, the temperature inside the different plasmas was measured. A quantitative PCR assay was used to detect the effect of the plasma treatment on the spore DNA, by monitoring the destruction of *dnaK* fragments. Additionally, selected isogenic *B. subtilis* mutant strain spores were plasma treated to evaluate the main inactivation effects of different plasma sources. Photons emitted by the generated plasma ((V)UV) take a key role in the inactivation process, as shown for direct treatment using DBD and plasma jet systems, but for PPA the inactivation process is dominated by diffusion of reactive species.

S22 Dilemma in Constructive Use of Risk Assessment in a Variable World: All Microbes are Equal But Some Microbes are More Equal Than Others

Risk assessment often deals with variability and uncertainty, while food safety management often needs to make discrete decisions. The objective of this proposed symposium is to facilitate connecting probabilistic variability (and uncertainty) in Quantitative Risk Assessment (QRA) on the one side and management need for “discrete” decisions on the other side, for a better understanding of how to manage food safety risks in a variable world.

Microbiological criteria, processing targets and limits for CCPs, are examples of “lines in the sand.” Decisions from legislation or in standard settings are often discrete. But we live in a variable world: microbiology, food processes, raw materials, humans all are inherently variable. All these aspects are treated in QRAs, but sometimes this variability, as well as explicit communication of uncertainty, undermines the understanding and the confidence in these analyses and their applications.

Making models more “accurate” than reality is simply not possible in a variable world, making them more realistic is possible. Understanding the magnitude and sources of variability and uncertainty can aid in decision making, including selecting the most efficient control measures.

The symposium brings speakers together from academia, industry and government to share the latest developments in QRA, lessons learned and experiences in constructive use of QRA to inform decision making under variability and uncertainty. The symposium intends to make connections between risk assessments and decision makers in government, industry and beyond.

Microbiological Sources and Impact of Variability on QMRA (Exposure Assessment and Hazard Characterisation)

HEIDY DEN BESTEN, Wageningen University, Wageningen, Netherlands

Quantitative microbiology is used in risk assessment studies, microbial shelf-life studies, product development, and experimental design. Realistic risk estimation is, however, complicated by different sources of variability. The variability in hazard characterization is fairly unexplored, though highly relevant. The final concentration of microorganisms at the moment of consumption (exposure assessment) depends, amongst others, on the variability in the storage times and temperatures, variability in product characteristics, variability in process characteristics, variability in the initial contamination of the raw materials, and last but not least, microbiological variability. This presentation compares different sources of microbiological variability in growth and inactivation kinetics of a pathogen, namely experimental variability, reproduction variability (within strain variability), strain variability (between strain variability) and variability between individual cells within a population (population heterogeneity), and prioritizes their importance. Also, the microbiological variability is compared to other variability factors encountered in a model food chain to evaluate the impact of different variability factors on the variability in microbial levels encountered in the final product.

Dealing with Variability in Industry Risk Assessments to Support Safe Product Design

ALEJANDRO AMEZQUITA, Unilever, Sharnbrook, United Kingdom

Making decisions on the safety of food products under uncertainty and based on variable data/information may undermine confidence in the decision. This has traditionally led to the application of deterministic calculations with conservative assumptions (to account for uncertainty and variability) in the food industry when establishing the basis for safety of new products. Performance criteria and related metrics based on such conservative

approach, have been in existence for a long time and provided a good safety record. In some circumstances, though, probabilistic approaches offer advantages for innovative product design, as they enable the quantification of the relative likelihood of alternative outcomes, thus better informing decision making under uncertainty and variability. Sources of uncertainty and variability may come from product/process parameters, microbial responses to control measures, and prevalence/level of microbial contaminants, among others. Methodologies used for articulation of uncertainty and variability in safety assessments are well established (e.g., Monte Carlo simulation, sensitivity and scenario analyses techniques). This presentation illustrates how a combination of deterministic and probabilistic approaches can be used to ensure products are safe by design in a fit-for-purpose manner. A case study related to the validation of a continuous thermal sterilisation processing line for low-acid liquid foods will be used to illustrate application in industry. It describes how process and microbiological data can be integrated iteratively with microbial inactivation and thermal process models in a Monte Carlo simulation framework to define a safe operating space. The suitable application and interpretation of these methods requires specialist knowledge and training, and therefore are unlikely to be the 'norm' for industry-based risk assessors. However, they are extremely valuable in increasing confidence in decision making for complex product designs where the decision would otherwise be hindered if such methods were not available to industry.

Factors to Consider in Decision Making Given Variability and Uncertainty in Microbiological Risk Assessment: A Governmental Perspective

PAUL COOK, Food Standards Agency, UK, London, United Kingdom

Dealing with hazards and risks is an important part of the Food Standards Agency's work and the approach taken to microbiological risk assessment will depend on the scale, complexity and urgency of the situation. Variability and uncertainty are key factors in microbiological risk assessment and understanding their nature, magnitude and impact are important for both risk assessors and risk managers. Risk assessment outputs can be particularly challenging for risk managers when faced with difficult or uncertain risk management options. Interaction and clarity between risk assessors and risk managers is important in both framing the risk assessment question and beginning preparation for dealing with the risk assessment outputs. Even during the early stages it is important to recognise whether there are any limitations for example, where preliminary scoping activities are undertaken (e.g., data gaps, data quality, spatial or temporal alignment of information) as these could have implications for the assessment including variability and uncertainty. The emergence of new microbiological hazards,

methodology, more complex and diverse foods as well as consumer behaviour will continue to challenge our ability to assess and manage the attendant variability and uncertainty associated with risk assessments and how we compare and communicate risks to consumers.

S23 Surrogates for Low-moisture Food Validation: What are the Key Steps from Selection to Routine Use?

Nowadays, multinational food companies as well as medium and small food producers, face a real issue regarding the variety and complexity of processes to be validated before final adoption. Furthermore, they also face the diversity of the type and origin of food matrixes and the various raw materials to be used. Therefore, It is necessary to pay particular attention to the verification programs for foreign suppliers and the compliance with U.S. food safety rules. The compliance requirements with FSMA rules have put in evidence the limits of the current process validation strategies and thereby the need to develop new specific and well-characterized surrogates. Surrogates dedicated to the validation of food process have been in use for many years, such as *Enterococcus faecium* NRRL B-2354 for the validation of the almond roasting steps. Nevertheless, if surrogates need to be applied to other process validation and other food types, it is important to better define the requirements. A good surrogate can be only defined after having completed a qualification program taking into account a number of variables: the process to be validated, the food matrix, thermal resistance of the surrogate in comparison with a specific pathogen, stability and its capacity to be implanted in the matrix.

Validation Studies: An Overview of Currently Used Approaches

ANETT WINKLER, Kraft Foods R&D Inc., München, Germany

To provide a safe food supply many factors (materials, processes, people, environment) have to be taken into account and need to be adequately controlled. Systematically, that is addressed by applying HACCP principles throughout the food chain. In that context specific attention has been given to processes in food production controlling hazards. Such processes need to be controllable, reliable and validated. "Validated thereby referring to Codex Alimentarius: "Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specific outcome." That purpose can be achieved by different means, where the two main approaches focus on either validation using known scientific, technical, observational information or measuring performance against a desired food safety outcome/target. With respect to process controls for biological hazards and their validation both approaches are used, depending on available

knowledge. However, each of them has advantages and disadvantages, which will be discussed during the presentation. Also, elements to be included in a credible validation study, as well as pitfalls to be avoided will be pointed out. Overall, the presentation is aiming to provide the audience with an insight into applicability and uncertainties of validation data and their interpretation.

Enterococcus faecium as a Surrogate of Salmonella: It Works for Almonds, But Does It Work for My Products?

LINDA J. HARRIS, University of California, Davis, Davis, CA, USA

Validation of food processing equipment often requires the in-facility use of a nonpathogenic surrogate organism. The organism should have similar or more robust survival capabilities under the conditions being studied and, ideally, have been characterized with respect to pathogenicity or lack thereof. Historically, *Enterococcus faecium* NRRL B-2354 has been used as a surrogate for foodborne pathogens in thermal processes used for dairy products, juice, and meat. Under a wide range of laboratory and pilot-scale conditions, the thermal tolerance of this organism on almonds has been shown to be similar to that of *Salmonella*. As a result *E. faecium* NRRL B-2354 is recommended and widely used as a surrogate for *Salmonella* in the validation of commercial thermal processes that are used for almonds. The organism has also been adopted as a surrogate for *Salmonella* and other enteric pathogens in a wide range of low-moisture foods and a wide range of thermal and non-thermal processes. The assumptions supporting this adoption and the data needs will be discussed.

New Surrogates in Low-moisture Food/ Petfood Process Validation, Are We Ready to Use Them?

PABLO ALVAREZ, Novolyze, Dijon, France

The publication of Food Safety Modernization Act final rules has forced the multinational food companies as well as medium and small food producers to move forward and to start, or continue, the work to validate a variety of different processes for numerous products. Many of these processes have never been validated in the past and no validation approach or strategy has been established for this purpose. In this context, the use of surrogate microorganisms for process validation has become the most common approach, nevertheless, a number of limitations have been described and need to be overtaken. Although specific surrogates, as *Enterococcus faecium* NRRL B-2354 (ATCC® 8459™), have been widely used in the validation of different low-moisture food products, we cannot talk nowadays about standard or universal surrogates. A good surrogate can be only defined after a complete research program that take into account a number of variables, such as, the target pathogen, the physical-chemical characteristics of the food matrix, the

process to be validated and the specific kill-step, etc. But a question needs to be now answered: Are the food companies prepared to support these research programs and to use the new surrogates developed?

S24 Quality, Safety and Spoilage Issues in the Wine Industry

The use of commercial starter cultures (SCs) by the wine industry has long ensured manageable and safe fermentations by limiting the activity of spoilage microbiota and the production of harmful compounds of microbial origin. However, in the recent years the use of modern technologies in wine production may imply emerging risks for product safety and quality. Exemplificative cases of such novel practices include spontaneous fermentations conducted without the addition of SCs, reduced addition of preservatives or use of SCs for wines of low alcohol levels. These approaches are compatible with consumers' preferences for superior wines of regional characteristics made through natural and organic procedures and also protect consumers' health. On the other hand, these techniques may allow the development of unwanted microorganisms that depreciate quality or spoil wine and pose health risks through the production of harmful compounds, such as biogenic amines. Therefore, there is a need to improve the management of microbial resources in wine production. The current needs in the wine market far surpass the one-fits-all scenario in the use of SCs. Various novel SCs should be developed to appease the existing trends with the assurances of high standards in winemaking. In this section we focus on, but are not limited to, innovative approaches able to conciliate consumer-oriented emerging trends with the high quality and safety standards assured by SC technology.

Shaping Wine Quality by the Use of Native Yeast Microbiota

ASPASIA NISIOTOU, ELGO-'DEMETER', Lycovrissi, Attikis, Greece

The competitive nature of global wine market calls for the production of premium wines endowed with authenticity and typicity. More and more consumers prefer not only superior wines, but also wines made through natural and organic procedures. To fulfill these requirements, modern winemaking adopts alternative technologies which among others include the implementation of spontaneous fermentations, reduced addition of preservatives and production of wines with low alcohol levels. Although such practices may lead to the production of wines with desired characteristics, at the same time musts or wines are susceptible to microbial spoilage. Even more importantly, the development of certain microorganisms may also pose health risks through the production of harmful compounds, such as biogenic amines or ethyl carbamate. Therefore, the microbial community composition should be controlled during the various stages of

wine production. In this respect, the addition of well-selected yeast starter culture (SC) is crucial. Winemakers should adopt precision fermentation technologies taking advantage from the unlimited reservoir of indigenous germplasm and exploiting the biodiversity using the right yeast SC for the particular needs. This, however, entails a deep knowledge of the indigenous microbiota and its activity during winemaking. Here we present the characterization of indigenous yeast isolates from the vineyard as promising novel arrows to the quiver of the current SC technology. Such novel SCs may be applied to appease the existing trends with the assurances of high quality and safety standards SC in winemaking.

Selecting LAB for Use as Starter Cultures in Winemaking

PATRICK LUCAS, University Bordeaux, Bordeaux, France

The use of selected bacteria by the wine industry began in the 1990s and is booming in recent years. Selected LAB help to better control malolactic fermentation kinetics, they limit wine spoilages due to undesirable microorganisms and they ensure wine safety by avoiding the production of harmful biogenic amines that may occur during spontaneous fermentations carried out by indigenous bacteria. At the same time, the development of organic, biodynamic and traditional farming is raising questions about the possible loss of identity of wines produced with selected bacteria. One may wonder whether the selection of bacteria used as malolactic starters -which is now based solely on their technological properties and safety (e.g., no biogenic amine formation) - must also consider their geographical origin, in order to propose starters that match each type of wine. *Oenococcus oeni* is by far the main LAB species that develops naturally in wine and achieves malolactic fermentation. Accordingly, almost all the malolactic starters commercialized today are strains of this species. Numerous studies using diverse molecular techniques have shown that there is a wide variety of *O. oeni* strains in different regions and wines. Today, comparative genomics brings a new light on the diversity of this species, the geographic spread of strains and their adaptation to wine. Although all strains may spread in all regions, it is clear that certain "genetic families" of strains have appeared separately in different regions, representing a genetic adaptation to different types of wines. This presentation will discuss these results and their impact on the selection of malolactic starters.

The Importance of Tailored Starter Cultures to Ensure the Quality and the Safety of "Wild", Organic, Biodynamic, and Typical Wines

VITTORIO CAPOZZI, Pasquale Russo, Giuseppe Spano, Department of Agriculture, Food and Environment Sciences, University of Foggia, Foggia, Italy

The market dynamics of the global wine industry lead to a considerable segmentation of oenological products. Among the different drivers of differentiation, the two macro-categories of 'organic/biodynamic' and 'traditional/typical/artisanal' (including products with a recognized status of Geographical Indications) wines are of a significant worldwide importance. Considering both classes, consistently with the different statuses, the phase of the alcoholic and malolactic fermentations have been receiving growing attention as possible stages of new approaches conceived to improve marketability, such as i) the return to spontaneous fermentations and/or ii) biotechnological solutions able to mimic spontaneous evolution of microbial diversity in the must/wine system. The diversity of indigenous microbial resources and their interactions are of outstanding interest for the general impact on wine quality and safety. The scientific community suggested different approaches to conciliate the needs of differentiation (via alternative management of microbial resources in oenology) and the importance of quality and safety standards in wine productions. This communication provides a review of this topic, encompassing the major trends, the protechnological significance of microbial diversity in oenology, and the potential role of autochthonous resources. Moreover, we will consider the pros and the cons of the main existing approaches, with a particular focus on the 'tailored' starter cultures designed to ensure the quality and the safety of 'organic/biodynamic' and 'traditional/typical/artisanal' wine productions. The social, economic, and environmental sustainability associated with the diverse approaches is also discussed.

Closing Session

The Fallacious Fecal Coliform

MICHAEL BRODSKY, Brodsky Consultants, Thornhill, ON, Canada

The Fecal or Faecal Coliform has been used for > 100 years as an indicator for contamination from a sewage source. There is no such organism. The term "fecal coliform" has no taxonomic significance and bears no definitive relationship to intestinal waste; yet it has been used as the basis for indicating sewage contamination in potable and non-potable water, as well as food and environmental sources by many jurisdictions. The term "Thermotolerant" coliform is biologically correct. Yet many agencies are still reluctant to archive this obsolete terminology. Are there better approaches to assessing the potential

for microbial pathogens in our water and food supplies? New technology and detection systems specifically for *Escherichia coli*, the only definitive thermotolerant coliform associated with sewage, has resulted in the replacement of "fecal" coliform by *E. coli* by most regulatory agencies.

Tree Nuts: Food Safety Risk and Intervention Strategies

LINDA J. HARRIS, University of California, Davis, Davis, CA, USA

Worldwide nut production has expanded rapidly in recent years with a corresponding increase in consumption. Large outbreaks of salmonellosis have been associated with nuts and their products during this same time period, which has resulted in a major shift in the approach used to handle these products. The influence of differences in production, harvest, and processing practices for several types of tree nuts will be discussed in the context of introduction and reduction of foodborne pathogens. Potential routes of pre- and postharvest contamination of nuts with foodborne pathogens will be presented along with an overview of control strategies including current thermal and nonthermal methods and the factors affecting their efficacy.

Beyond Food Safety Management Systems - Food Safety Culture

FRANK YIANNAS, Walmart, Bentonville, AR, USA

Food safety awareness is at an all-time high, new and emerging threats to the food supply are being recognized, and consumers are eating more and more meals prepared outside of the home. Retail and foodservice establishments, as well as food producers at all levels of the food production chain, have a growing responsibility to ensure that proper food safety and sanitation practices are followed, thereby, safeguarding the health of their guests and customers. Achieving food safety success in this changing environment requires going beyond traditional training, testing, and inspection approaches to managing risks. It requires a better understanding of organizational culture, behavioral science, and the human dimensions of food safety.

Think about it. If you are trying to improve the food safety performance of retail or foodservice establishment, an organization with thousands of employees, or a local community, you must change the way people do things. You must change their behavior. In fact, simply put, food safety equals behavior.

While in today's profession, the term food safety culture is often used, what does it really mean? More importantly, are there proven, evidence-based ways to change or strengthen the food safety culture of an organization and influence employee behavior? The good news is the answer is YES!

Come hear an interesting and innovative talk on how to create a food safety culture - not just a food safety program.

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Technical Abstracts



**IAFP'S EUROPEAN
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Technical Session 1 – Applied Laboratory Methods and Novel Laboratory Methods

Wednesday, 11 May – 10.30 – 12.00

* Denotes Developing Scientist Awards Competition.

T1-01 Comparison of Cell-based and PCR-based Assays as Methods for Measuring Infectivity of Tulane Virus

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(2) Shanghai Jiao Tong University, Shanghai, China

Introduction: Human noroviruses (HuNoV) are not culturable viruses. The detection of HuNoV mainly depends on RT-PCR-based assays which cannot measure the infectivity of the virus. We propose that the detection of encapsidated viral genome could be used as an indicator for viral infectivity.

Purpose: Tulane virus was used as a surrogate for HuNoV to compare correlations between the cell-based and the PCR-based assays for measuring viral infectivity.

Methods: Plaque assay and tissue culture 50% infectious dose (TCID₅₀) assay were used as cell-based assays. RNase exposure, porcine-gastric-mucin-mediated *in-situ*-capture qRT-PCR (PGM-ISC-qRT-PCR), and antibody-mediated *in-situ*-capture qRT-PCR (Ab-ISC-qRT-PCR) assays were used as the PCR-based assays to measure for encapsidated viral genome.

Results: Ten batches of viral stocks ranging from 3.41×10^5 to 6.67×10^6 plaque forming units (PFUs) were used for side by side comparison with PFU as a reference. The results indicate that one PFU was equivalent to 6.7 ± 2.3 TCID₅₀ units, 9.8 ± 10.9 RNase-untreated genomic copies (GCs), 2.8 ± 3.1 RNase-treated GCs, 0.07 ± 0.07 PGM-ISC-qRT-PCR GCs, and 0.52 ± 0.39 Ab-ISC-qRT-PCR GCs. We observed that while the cell culture-based assays were consistent with each other, the TCID₅₀ assay was 6.7-times more sensitive than the plaque assay. However, the PCR-based assays were not always consistent with the cell culture-based assays. The very high variations in GCs as measured by both ISC-RT-qPCR assays made them difficult to correlate against the relatively small variations (<20-fold) in the PFUs or TCID₅₀ units as measured by the cell culture-based assays.

Significance: Two cell-based assays were consistent with each other, with the TCID₅₀ assay being 6.7-times more sensitive than the plaque assay. However, there was no significant correlation between the cell-based assays and the PCR-based assays.

T1-02 Evaluation of the European Network for Staphylococcal Enterotoxins Detection in Food Matrice

YACINE NIA, Isabelle Mutel, Adrien Assere, Sabine Messio, Jacques-Antoine Hennekinne and Frédéric Auvray
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Introduction: Staphylococcal food poisoning outbreaks are a major cause of foodborne illnesses in Europe. Their notifications have been mandatory since 2005. The Laboratory for food safety (Anses) has been appointed European Union Reference Laboratory (EURL) for Coagulase Positive Staphylococci (CPS) including *Staphylococcus aureus* (EC No 776/2006 du 23 may 2006). Anses develops reference activities on behalf of the Directorate General Health and Food Safety of the European Commission.

Purpose: Since the validation in 2011 of the European Screening Method (ESM) for staphylococcal enterotoxins (SEs) detection in food matrices, the EURL for CPS organized three Inter-Laboratory Proficiency Testing Trials (ILPT), i.e. in 2013, 2014 and 2015. The objectives were i) to verify the proficiency of the laboratories from the EURL network in implementing the official SE screening method and ii) to ensure the reliability of the results obtained by the participating laboratories.

Methods: Each ILPT was conducted using the ESM with either the commercial VIDAS SET2 or the RIDASCREEN SET Total detection kit. Eight food matrices were used for these ILPT, including cheese, freeze-dried cheese, tuna, mackerel, roast chicken, ready-to-eat food, milk and pastry. Food samples were spiked with four SE types (i.e. SEA, SEC, SED and SEE) at different concentrations. For each ILPT, homogeneity and stability studies were performed.

Results: The results showed that ILPT samples were homogeneous (RSD < 15%) and stable. Overall, 31 participants from 27 European countries analysed 155 blank and 620 spiked samples. The results obtained allowed evaluation of sensitivity (> 98%) and specificity (100%) of each detection kit (VIDAS SET2 and RIDASCREEN SET Total).

Significance: Adding further to the validation study carried out in 2011, the results of three ILPTs assessed the proficiency of the EURL network and the performance of the ESM on a large number of food matrices and samples.

T1-03 Staphylococcal Enterotoxins Detection in Food Matrices from Various Food Poisoning Outbreaks in Europe

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(3) State General Laboratory, Food Microbiology Laboratory, Nicosia, Cyprus

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(6) Dairy Science Laboratory, Co. Kildare, Ireland

Introduction: Staphylococcal food poisoning outbreaks (SFPOs) are a major cause of foodborne illnesses in Europe. Their notifications have been mandatory since 2005 (Commission Regulation (EC) No. 2073/2005 amended by EC No. 1441/2007). Staphylococcal enterotoxins (SEs) represent the first cause of food poisoning outbreaks due to bacterial toxins. In 2014, 12 Member States (MSs) reported 393 foodborne outbreaks caused by staphylococcal toxins. This represents 7.5% of all outbreaks, a small increase compared with 2013 when 12 MSs reported 386 outbreaks.

Purpose: Several outbreaks occurred in MSs over the period 2013-2015 which were characterized in the frame of the European Union Reference Laboratory network. This presentation will focus on a subset of these, including outbreaks that occurred within a transatlantic flight, a nursing home, a prison, a wedding reception, a barbecue and a sandwich take-away restaurant. SEs detected in foodstuffs responsible for these outbreaks will be described.

Methods: SEs were analysed following the European Screening Method and quantified by an in-house ELISA test. SE encoding genes were detected in *S. aureus* strains isolated from food samples by an in-house PCR-based method.

Results: Coagulase positive staphylococci and their enterotoxins were incriminated in each of the outbreaks. For the 5 SEs detected in routine, agreement was established between SE genes identified in *S. aureus* isolates and SEs quantified in the samples. However, some outbreaks were only partially characterized due to the absence of diagnostic tools allowing the detection of other types of enterotoxins such as SEG, SEH and SEI.

Significance: Investigation of SFPOs highlight the need for correct food-handling practice and food storage conditions. Methodology is available for CPS characterization and SE analysis in case of food poisoning events. However, new analytical tools, in particular for SEG, SEH and SEI detection, are needed to enlarge the scope of the methods already available.

T1-04 Certified Reference Materials for the Analysis of *Staphylococcus aureus* Enterotoxin A in Cheese

REINHARD ZELENY¹, Håkan Emteborg¹, Jean Charoud-Got¹, Heinz Schimmel¹, Isabelle Mutel², Yacine Nia², Frédéric Auvray² and Jacques-Antoine Hennekinne²

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Introduction: Staphylococcal enterotoxins (SEs) released into food are causing a large number of foodborne illnesses in the EU and elsewhere (n = 393; 2014). European legislation (Commission Regulation (EC) No 1441/2007) stipulates that, if the food is to be considered safe for human consumption, SEs must not be detected with the so-called European Screening method (ESM) in each of the five

portions to be taken from a food sample. To enable laboratories to provide reliable and accurate results, certified reference materials (CRMs) are pivotal and should be used for method validation and method performance qualification purposes.

Purpose: To certify a set of three reference materials (blank, and two spiked materials with SEA at sub-ng/g cheese) to support laboratories in applying the ESM as regards to presence/absence testing of staphylococcal enterotoxins in cheese.

Methods: A laboratory intercomparison with 15 laboratories was conducted using the ESM with either the commercial VIDAS[®] SET2 or the RIDASCREEN[®] SET Total detection assay. For each material, each laboratory performed 9 independent analyses over 3 measurement days. The obtained data were technically and statistically scrutinized, and certified values were expressed as specificity (blank material) and sensitivity (SE-containing materials) as defined in ISO 16140.

Results: All accepted individual results were correct, i.e., no false positives and no false negative results were reported by the laboratories. The probability of correct classification of these materials was therefore 100%, and one-sided lower confidence limits were estimated assuming a Poisson distribution. In addition, the average value and the intervals of the obtained test values (VIDAS) and absorbance units (Ridascreen) are reported on the certificate.

Significance: The CRMs will support laboratories for the validation of the ESM and serve as QC samples to verify that the method is working correctly. In that respect, they support legislation and improve consumer protection.

T1-05 A Unique Rapid Detection and Quantification Assay for Total Count of Yeasts and Molds in Dairy Products Based on Multiplex Real-time PCR

CHRISTINA HARZMAN, Matthias Giese, Cordt Groenewald and Kornelia Berghof-Jaeger
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Introduction: Ubiquitous in the environment, yeasts and molds can easily contaminate foods and become the predominant spoilers, particularly in dairy products. Thus, their presence and amount are regularly monitored. Conventional methods can take ≤14 days, and current rapid methods require multiple days for an answer. A same-day test would significantly benefit the dairy industry economically with faster product release.

Purpose: Validation of the sensitivity, robustness and specificity of a lyophilized real-time PCR assay (the foodproof *Yeast & Mold Quantification* LyoKit) for detection and quantification of yeasts and molds in dairy products.

Methods: Ten different dairy sample types were diluted 1:10 and spiked with yeasts and molds at specific concentrations of ≤ 6 × 10³ CFU/g. Homogenized sample (800 µl) was treated with Reagent D for live/dead cell differentiation. Only live cell DNA was extracted and real-time PCR performed.

Live/dead differentiation efficiency, sensitivity (DNA and cell spiked samples), specificity (inclusivity and exclusivity), and robustness were determined for the new foodproof *Yeast and Mold Quantification* LyoKit, and compared to the classical ISO-method (ISO 6611).

Results: Genomic DNA from 15 yeast and mold species was tested with 11 replicates. 100% of replicates were positive, even at 0.39 GE in all dairy samples. All sample types spiked with 10^2 and 10^3 CFU/g *Yarrowia lipolytica*, *Aspergillus niger*, *Candida kefyr*, and *Hypopichia burtonii* showed appropriate positive signal. All samples spiked with 6000, 600, 60 and 6 CFU/g showed positive results with a deviation of <1 ct. Thus, quantification was successful (LOD of < 10 CFU/g). Comparison to ISO 6611 showed good correlation independent of matrix type. Specificity results showed 100% success for inclusivity (290 strains from 260 species) and exclusivity testing (> 60 bacteria, plants and mammal cells).

Significance: Validation of the new commercial kit showed results in >4 hours equivalent to or better than ISO 6611 for the detection and quantification of yeasts and molds in dairy products. This kit has proven to be the first rapid same day method available to accurately quantify yeast and molds, particularly in dairy samples.

T1-06 Detection of Distinct Norovirus Genotypes with a Multiplex Qpcr System in Seafood

OLAF DEGEN¹ and Arnt Ebinger²;

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(2) AE, Potsdam, Germany

Introduction: Noroviruses are considered to be the main agent for gastrointestinal diseases. Outbreaks are frequently observed in community facilities (school cafeterias, hospitals). Infection may be direct from person to person or through contaminated food and drinks (seafood, meat, fruits, table water). Genogroups GI, GII and GIV are pathogenic to humans. The most common infecting norovirus is genogroup II genotype 4 (GII.4).

Purpose: The purpose of this study was to evaluate detection and Genogroup differentiation of artificially contaminated shellfish samples by the foodproof Norovirus Detection Kit (GI, GII, GIV).

Methods: RNA of four shellfish samples (pacific oysters and common mussels) and two Lenticules Discs provided by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) as part of the proficiency test 55 (2015) was prepared by the foodproof Virus Sample Preparation Kit. Norovirus detection and genotype identification was performed with the foodproof Norovirus Detection Kit (GI, GII, GIV), a one-step real-time reverse transcriptase-PCR for the multiplex detection of the norovirus genotype I, II, and IV. The assay includes an inhibition/ process control, containing the MS2 bacteriophage.

Results: There were no differences in the detection of norovirus GI and GII in three spiked shellfish samples and two Lenticules Discs. In shellfish

sample 4, no GII could be detected but GI, due to inhomogeneities in the sample materials as well as the spiked virus concentrations below the LOD of the Detection Kit. Highest accuracy was achieved with the artificial spiked shellfish samples of the CEFAS proficiency test 55.

Significance: The foodproof Norovirus Detection Kit shows a high sensitivity in shellfish samples contaminated with norovirus GI and GII with small quantities, and is based on primers, probes, and methods which are mentioned in the ISO/TS 15216. The kit is not limited to seafood but works with minced meat, fruits, and water samples.

Technical Session 2 – Communication Outreach and Education and Other Food Commodities Wednesday, 11 May – 13.30 – 15.00

T2-01 A Food Safety Strategy for Global Logistics Operations: A Global Concept with Local Relevance

NIKOLAOS BESSAS

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Introduction: Food safety and Logistics operations will be an area of great focus for the coming years. New international trading agreements, FSMA and changes in EU legislation will re-shape the international food trade, so there is a greater than ever need to create a Logistics' food safety strategy that will reflect the new market conditions. Logistics' food safety is a part of the supply chain that till today did not have the needed focus and attention. A global food safety capacity development program for Logistics is missing from our global food safety system, a program that will bridge the differences between developed and less developed markets.

Purpose: The purpose of this project was to analyse the current food safety certification status on Logistics of 26 countries of Europe and Asia, with an objective to build a food safety capacity development program that will reflect the individual market needs and conditions and will bridge the gap between developed and less developed markets for Logistics food safety.

Methods: The method for developing such a program was based on the model of the successful program of GFSI-Global Markets.

Results: The initial results from pilot countries have shown that such a program helps in developing the food safety knowledge of Logistics companies and is the tool needed for the step by step development of their food safety system in a cost efficient way.

Significance: The significance of this program is very high because, while Logistics companies are operating today on a globalised economy, in several cases their food safety level is fluctuating and still reflecting more the local food safety level. So a food safety capacity development program for Logistics operations is something that was definitely missing from the market.

T2-02 Consumer Information on the Prevention of Foodborne Microbiological Risks: Improving the Effectiveness of Communication Strategies

PAULINE KOOH¹, Thomas Bayeux¹, Eve Feinblatt¹, Jean Christophe Augustin², Laure Bonnaud³, Olivier Cerf⁴, Michel Gautier⁵, Françoise Gauchard¹, Laurent Guillier¹, Nathalie Jourdan-Da-Silva⁶, Thierry Meyer⁷, Lydiane Nabec⁸, Louis-Georges Soler³, Isabelle Villena⁹, Moez Sanaa¹ and Sandrine Blanchemanche¹⁰

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- (7) Université Paris X, Nanterre, France
- (8) Université Paris Sud, Sceaux, France
- (9) Hôpital Maison Blanche, Reims, France
- (10) INRA, Paris, France

Introduction: Each year, around one third of the foodborne outbreaks reported in France occur in the family environment. Some of these cases are due to inadequate preservation, insufficient cooking or cross contamination. Specific information aimed at consumers could help reduce the risk associated with certain foodborne diseases.

Purpose: This observation led the French Directorate General for Food to make a formal request to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) with the aim of making a substantiated choice from among all the communication strategies to be implemented, in relation to certain food risks and also potential constraints for the sectors in question.

Methods: The scientific expertise was conducted by a multidisciplinary working group including experts in social sciences, biological hazards and risk assessment.

Results: The preliminary work (Opinion of 9 May 2014) helped to identify hazard/food combinations for which a change in consumer practices could result in risk reduction. An inventory of conceivable communication strategies was also conducted. The second report, released in October 2015, deals with the effectiveness of communication strategies for preventing food microbiological risks, focusing on: the identification of the determinants of food-handling behaviors (proposition of health behavior model); the literature review on the effectiveness of various communication strategies (communication campaign, labeling, communication via health professionals, nudges, educative programs); the choice of the targeted population (general or specified population) and the practices, attitudes and characteristics of 3 specific populations (pregnant women, elderly, parents of young children); and the health impact and cost-effectiveness of communication campaigns on microbial food safety (3 cases studies: VTEC/ minced meat, *Listeria monocytogenes*/RTE food, *Campylobacter*/poultry meat).

Significance: The work led to prioritize communication strategies in the area of prevention of food microbiological risks, taking into account the targeted populations, the complexity of the message and the cost-effectiveness of the campaign.

T2-03 Development of Online Teaching and Learning Tools for the Delivery of Poultry Food Safety in the Veterinary Curriculum

RODRIGO J. NOVA; School of Veterinary Medicine and Science, Sutton Bonington Campus, University of Nottingham, Nottingham, United Kingdom

Introduction: The teaching of food safety aspects in the veterinary curriculum at the School of Veterinary Medicine and Science (SVMS), University of Nottingham, is delivered as part of the veterinary public health (VPH) module. Curriculum constraints and logistics of the course resulted in using the full length of the contact time allocated for poultry teaching on practical activities, while the theory was delivered as podcast sessions. Feedback on the practical activities has been consistently positive, but podcast sessions are not considered as an encouraging learning experience by students. As it is recognized that the new generation of students, generation Y, benefits from a higher level of engagement in its learning process it was proposed to produce interactive online resources for the poultry food safety teaching in VPH.

Purpose: To enhance students' learning experience and perceptions by using online tools to deliver the theory of poultry food safety for veterinary students.

Methods: The learning management system, Moodle, was used as the platform for designing and producing the teaching material. The material was embedded with audio visual resources already available on YouTube and pictures from the SVMS photo database in the image hosting website Flickr. A questionnaire was produced and distributed to gather students' feedback.

Results: By providing a more interactive experience students felt more engaged in their teaching/learning process. Additionally, the ongoing access to the study material through the university portal allowed students to organize their own revision times. However, some students felt that they would benefit better from a printable version of the material.

Significance: The new teaching/learning resources produced for the delivery of poultry food safety provided a more fulfilling teaching/learning experience to the students. Additionally, by replacing the podcast sessions with interactive online material, the teaching resources can be modified in a more time-efficient fashion whenever updates are required. Moreover, this format will reduce the reliance of the school on external deliverers.

T2-04 Significance of HACCP Implementation on the Microbiological Quality of Foods and Environmental Hygiene in Mass Feeding Facilities in Greece during the Period 2003 to 2010

Constantin Genigeorgis¹, Niki Thalassinaki² and CHRIS PANOULIS³

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(2) University of Crete, Heraklion, Greece

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Introduction: Conformity to EC178/2002 and 852/2004 demands the establishment of validation procedures and microbiological controls in food production facilities.

Purpose: Microbiological assessment of food production phases in mass feeding facilities as affected by HACCP implementation.

Methods: ISO methods were followed for detection of *Salmonella* spp., *L. monocytogenes*, *E. coli* and *S. aureus*. Presence of *Salmonella* spp., *L. monocytogenes*, >10 CFU/g *E. coli*, and >100 CFU/g *S. aureus* defined an unacceptable sample. Statistical analysis included linear regression.

Results: We surveyed 28 hotels, 8 fast food and 8 catering facilities. Of ingredient samples in HACCP using (group A, 1778, samples) and not using (Group B, 1253 samples) facilities 17.4, 9.4, 10.9 1.6% and 26, 14.4, 18.3, 3.1% were unacceptable for *E. coli*, *S. aureus*, *L. monocytogenes* and *Salmonella* spp., respectively. For ready-to-eat foods in hotels of Group A (1778 samples) and Group B (1258 samples) the respective figures were 4.2, 3.3, 7.7, 0% and 29.4, 27, 8, 31.2 and 2.5%. The figures for Group A and B fast food facilities were 4.5, 1, 2.9, 0% and 9, 10.2, 15.4, 0% and for catering 0.7, 1.4, 4.7, 0% and 14, 13.2, 23.8% respectively. The unacceptable Group A and B environmental samples were 1.3, 2.6, 8.9% and 29.3, 31.1, 32.2% for *E. coli*, coliforms and *L. monocytogenes*, respectively. The difference between Group A and B for all facilities, environmental samples, ready-to-eat foods and agents was significant ($P < 0.01$) but not for entering ingredients ($P 0.2-0.5$). The percent reduction in unacceptable samples after HACCP implementation was calculated and was impressive.

Significance: The findings support universal regulatory enforcement of HACCP in mass feeding establishments.

T2-05 Microbiological Quality and Safety in Mass Feeding Establishments during the Greece Financial Crisis Period 2011 to 2015

CHRIS PANOULIS¹ and Constantin Genigeorgis²

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Introduction: The Greek economy is heavily dependent on tourism. There were over 22 million visitors in 2015. The mass feeding industry should assure food quality and safety as expected by visitors and 11 million Greek consumers.

Purpose: To what extent the economic crisis has affected quality assurance programs in the mass feeding industry needs to be addressed. This study explores the microbiological profile of offered menus and environmental hygiene status in 50 mass feeding facilities which have or have not implemented HACCP.

Methods: ISO 16649-2 for *E. coli*, ISO 6888.1:1999 for *S. aureus*, ISO 11290-1:1996 for *L. monocytogenes* and ISO 6579:2003 for *Salmonella* spp. were used.

Results: A total of 3714 hotel, 1131 catering and 2818 local and multinational fast food facility samples were analyzed. In 26 facilities using HACCP, 3.6, 1.4 and 5.3% of food samples were not in conformity to legislative criteria for *E. coli*, *S. aureus* and *L. monocytogenes*, respectively. In 24 facilities not implementing HACCP, the corresponding figures were 8.9, 8.2 and 10.1%, respectively. Non conformity to guidelines for environmental samples for total coliforms, *E. coli* and *L. monocytogenes* for the first group was 7.9, 5.1 and 9.9% and for the second 27.9, 27.9 and 33.5 % respectively. *Salmonella* was present only in 0.2% (5/2452) of fresh and deli salad samples. Comparison of the present data to those of the 2003-2010 study period to identify trends is in progress.

Significance: Considering the number of facilities and samples analyzed the results are of great significance to regulatory agencies in enforcing HACCP implementation for foreign and local consumer protection.

Technical Session 3 – Microbial Food Spoilage, Meat and Poultry, and Seafood Wednesday, 11 May: 15.30 – 17.00

T3-01 Metagenomics of Spoiled Meat: Meet the Suspects

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Introduction: Meat and meat products are generally subject to fast spoilage. The huge variety in packaging and processing likely results in a variety of dominant spoilage flora as well. Metagenomics now enables analysis of the spoilage flora more cheaply and less labor intensively, inevitably leading to establishment of product segment-associated microbiota.

Purpose: The purpose of this study was to analyze the dominant spoilage flora in several meat product segments.

Methods: We have applied metagenomics to several spoiled samples of vacuum packaged cooked meat, vacuum packaged fresh meat and vacuum and MAP packaged minced meat as well as roast beef. The V1-V4 region of the 16S rDNA gene was amplified, and high throughput sequencing was conducted with the Illumina Myseq. Typically 2000-3000 reads per sample were obtained.

Results: Vacuum packaged hot dogs (3 samples) contained a dominant flora of LAB like *L. sakei* and *Lc. mesenteroides* and was consistent with a previously

established list of top 5 spoiling bacteria by culture dependent methods. Aerobically packaged hotdogs contained 60% of *Brochotrix*. Four vacuum packaged fresh beef pieces slaughtered in different countries and a piece of lamb meat, were invariably dominated by only *L. piscium* and *Lc. inbae*, not found in cooked meat. Both species contributed to 70% or more of the total flora. In the case of minced beef, the type of packaging seemed of some but limited importance. This may be because the minced meat samples showed high bacterial counts from the start. Apart from four different species of LAB present above 10% in different samples, many samples contained at least 20% of *Photobacteria* and occasionally up to 70%, making it an important spoiler of minced beef.

Significance: A better understanding of the dominant spoilage flora will lead to better and specific interventions to elongate shelf life.

T3-02 Reduction of Broiler Chicken and Turkey *Salmonella* Prevalence, Numbers, and Virulence by Diamond V Original XPC

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(2) Iowa State University, Ames, IA

Introduction: Pre-harvest reduction of pathogens results in lower numbers of pathogenic bacteria entering the processing plant.

Purpose: This study was conducted to determine the efficacy of Diamond V Original XPC in reducing pre-harvest *Salmonella* in broiler chickens or turkeys in commercial processing plants, including evaluation of virulence and antibiotic resistance.

Methods: For broiler chickens, 17 houses from three different companies, age approximately 38 d, were selected; 10 were fed a ration that included 1.25 kg/t Diamond V Original XPC (XPC), and seven were fed a typical ration (CON). For turkeys, nine tom turkey houses from two companies were fed either XPC (four houses) or CON feed (five houses) to approximately 139 d of age. One cecum was collected from each of 50 birds from each house (850 chicken samples; 450 turkey samples) during evisceration at commercial processing plants, and analyzed for *Salmonella* prevalence and numbers, with positive samples tested for virulence and antibiotic resistance.

Results: Prevalence of broiler chicken *Salmonella* was significantly ($P < 0.05$) lower for XPC than CON samples (7.6 vs. 46.3%, respectively). Average numbers of *Salmonella* were lower for XPC than CON (28 vs. 417 CFU/g, respectively). Virulence, as measured by cell culture invasiveness, was less in XPC than CON isolates (0.16 vs. 1.18%, respectively). Antibiotic resistance (for florfenicol, enrofloxacin, and ceftiofur) averaged 1.0% for XPC and 1.02% for CON isolates. Prevalence of turkey *Salmonella* was reduced in XPC as compared to CON (5.3 vs. 18.7%, respectively). *Salmonella* numbers were also reduced by XPC vs. CON (8.3 vs. 88.6 CFU/g). Virulence was lower in XPC (0.19%) than CON (1.09%). Resistance

to antibiotics was 3.28% for XPC vs. 13.05% for CON isolates.

Significance: XPC reduced cecal *Salmonella* prevalence, numbers, and virulence in commercial broiler chickens and turkeys. Results show XPC is an effective pre-harvest intervention.

T3-03 Efficacy of Biosecurity Measures for *Campylobacter* Control in Spanish Broiler Farms

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(2) Nutreco Food Research Center, Casarrubios del Monte (Toledo), Spain

(3) National Food Institute, DTU, Søborg, Denmark

Introduction: *Campylobacter* is the leading cause of human bacterial gastroenteritis in the European Union (EU). Handling or consumption of chicken products is considered the main source of infection. It is believed that controlling the infection at primary production could impact the following links along the food chain, and consequently the incidence of human campylobacteriosis. Hence, a reduction in *Campylobacter* prevalence of broiler flocks is a priority in the EU. Currently, the only effective measure available for *Campylobacter* control in poultry farms is a proper implementation of biosecurity at farm and house level.

Purpose: To determine the efficacy of increasing farm biosecurity measures in a field study in 18 Spanish broiler farms during 13 broiler rearing cycles.

Methods: In 12 of these farms, improved biosecurity measures were implemented; in 5 of those, additional fly screens were mounted, once a sufficient level of biosecurity was reached. The remaining 6 farms were control farms, where no changes were made. Weekly boot socks samplings were performed in all farms, and *Campylobacter* detection was performed by PCR.

Results: To date, almost 13 cycles per farm have been monitored. Results show a significant reduction of the number of *Campylobacter*-positive samples in farms with improved biosecurity compared to the control farms, and over a 30% reduction of *Campylobacter* flock prevalence at 5th week of rearing. Moreover, the reduction to date is more marked in the group of farms with fly screens, which provided most influence during the summer months, when the insect population is abundant.

Significance: The implementation and improvement of biosecurity and hygiene measures at farm and house level in Spanish broiler houses reduced *Campylobacter* prevalence significantly. These measures are not costly and can be easily implemented in farms having anteroom.

T3-04 High Pressure Inactivation Kinetics for a Better Understanding of *Listeria monocytogenes* Behaviour in RTE Cooked Meat Products Formulated with Organic Acids

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Introduction: Prepackaged cooked meat products can be submitted to a high pressure (HP) treatment, the efficacy of which has to be validated in each specific type of product, as some food constituents may have a piezo-protective effect. In this regard, formulation with organic acids as antimicrobial agents (AMA), may affect the lethality of HP.

Purpose: The aim of the present study was to evaluate the effect of lactate and diacetate on the HP inactivation of *L. monocytogenes* in cooked ham.

Methods: Cooked ham batches were manufactured with: 1.4% potassium lactate (L14), 2.8% potassium lactate (L28), 0.1% sodium diacetate (D) and 1.4% lactate plus 0.1% diacetate (LD). Once sliced, products were inoculated with *L. monocytogenes* (three different strains, i.e., CTC1011, CTC1034 and ScottA, independently). Vacuum-packed samples were pressurized at 400 MPa for 9 different holding times (0-10 min). *L. monocytogenes* survival was monitored by enumeration on chromogenic medium. Inactivation kinetics was evaluated by fitting primary models to the survival data obtained by each product formulation and strain.

Results: The HP inactivation kinetics observed for *L. monocytogenes* depended on both the product formulation as well as the strain. Overall, the presence of lactate caused a piezo-protection as the inactivation rate was lower in cooked ham formulated with lactate than in the control product. The higher the lactate concentration, the lower the inactivation rate. By contrast, diacetate slightly enhanced HP inactivation of *L. monocytogenes*. A notable difference was denoted in the primary kinetic behavior among the studied strains. A shoulder shape was observed for the product isolates (CTC1011 and CTC1034), while a tail was recorded for the clinical isolate (ScottA).

Significance: The design, validation and implementation of high pressure processing requires a tailor-made approach, taking into account the specific product formulation and also selecting the most appropriate strains for process validation.

T3-05* Distribution of *Salmonella*, ESBL/AmpC-producing *Escherichia coli* and Hygiene Indicator Bacteria on Pig Carcasses after Slaughter

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Introduction: Pig carcasses may get contaminated with *Salmonella* and/or ESBL/AmpC-producing bacteria during different steps of the slaughter process. In the frame of official monitoring programs,

carcass contamination values are expressed by pooling values from different carcass areas; however, not all carcass parts are equally contaminated.

Purpose: The aim of this research was to assess the presence and map the distribution of *Salmonella*, ESBL/AmpC-producing *E. coli* and hygiene indicator bacteria on pig carcasses.

Methods: Seven Belgian pig slaughterhouses were visited twice to collect carcass swab samples after evisceration and trimming. During each visit, 9 carcass areas from 5 randomly selected carcasses were swabbed using cellulose sponges: foreleg, head, pelvic duct, sternum, belly, throat, distal part of the foreleg, ham and loin. All samples were analyzed using direct plating to quantify *E. coli*, *Enterobacteriaceae* and the total aerobic count. Furthermore, swab samples were analyzed for the presence of ESBL/AmpC-producing *E. coli* and *Salmonella*.

Results: Overall, the average total aerobic count varied between 3.1 (loin and pelvic duct) and 4.5 log₁₀ CFU/cm² (distal part of the foreleg) while median numbers for *Enterobacteriaceae* and *E. coli* ranged from values under the detection limit (ham respectively loin, foreleg and ham) up to 1.65 log₁₀ CFU/cm² respectively 1.18 log₁₀ CFU/cm² (both distal part of the foreleg). *Salmonella* was recovered from 4% (foreleg and ham) to 33% (distal part of the foreleg) of the samples. ESBL/AmpC-producing *E. coli* were found in none of the loin swabs and up to 20% of the head swabs. Respectively 53% and 41% of all carcasses were contaminated with *Salmonella* and ESBL/AmpC-producing *E. coli*, varying between slaughterhouses from none to all carcasses contaminated.

Significance: The large variations observed between slaughterhouses and carcass areas indicate the need for risk categorization of slaughterhouses, carcasses and pork cuts along the production chain.

Technical Session 4 – Sanitation and Antimicrobials Thursday, 12 May – 8.30 – 10.00

T4-01 Synergistic Effect of Nitric Oxide Donors in Association with Sanitizers in Dispersing Biofilms of Industrial Interest

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(4) University of Chile, Santiago, Chile

Introduction: Biofilms formed in post-harvest production facilities are recalcitrant reservoirs of pathogens, which are difficult to control. Pathogens in biofilms are resistant to common disinfectants and their mechanical removal is only partially effective. Therefore, novel approaches for controlling biofilms are needed. Recent discoveries of the function of nitric oxide in dispersing preformed biofilms offer an opportunity to test the feasibility of using this gas in industrial applications.

Purpose: Determine the dispersal effect of several nitric oxide donors (molsidomine, MAHMA-NONOate and diethylamine NONOate sodium salt hydrate) in association with disinfectant on biofilm of *Salmonella enterica*, *Escherichia coli* and *Listeria innocua* pre-formed on surfaces of industrial interest.

Methods: Biofilms were pre-formed in appropriate media for 24 hours or 1 week in 96-well plastic plates (polypropylene, polystyrene). After incubation, medium was removed and nitric oxide donors were resuspended in Phosphate Saline Buffer (PBS) and added to the wells using concentrations of 10 μ M, 10nM and 10pM. Biofilms were exposed to the donors for 6 hours at 22°C and 4°C to mimic the post-harvest environments. Biofilms were further treated with selected disinfectants (DiQuat, H₂O₂, peracetic acid and Pheno-Tek II) according with the manufacturer's recommendations. Dispersal was measured by staining the remaining biofilms using crystal violet or using a GFP labeled *Salmonella* strain. The donors were also tested in association with a cellulose nanocrystals hydrogel (CNC).

Results: Molsidomine and MAHMA-NONOate alone were able to disperse at least 50% of the biofilms pre-formed by human pathogens *Salmonella enterica* 14028 and *Escherichia coli* O157 (EHEC). The association of the two nitric oxide donors with cellulose nanocrystals was also effective in dispersing *Salmonella* preformed biofilms on polypropylene up to 15% of the total biomass. In addition, the effectiveness of peracetic acid was enhanced up to 25% by pre-treating *L. innocua* biofilms with the donor diethylamine NONOate sodium salt hydrate.

Significance: Our results show that nitric oxide donors expand the toolset of proactive solutions for removing industrial biofilms.

T4-02 Decontamination of Dry and Powdery Food Products by Vaporized Hydrogen Peroxide (VHP)

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Introduction: Dry and dehydrated products are widely used in the food industry. However, their microbiological quality is not always satisfactory, especially for spore forming bacteria. Moreover, these ingredients are difficult to decontaminate, according to their high sensibility to process parameters such as moisture and high temperatures.

Purpose: In canning industry, there is a strong need of alternative technologies able to decontaminate heat-resistant spores in dry products. We investigated a gaseous biocide technology with industrial isolates inoculated as spores.

Methods: An automated dispersion system is used to vaporize H₂O₂ liquid solution in dry air stream, in order to obtain a uniform distribution of the sporicidal agent (Vaporized Hydrogen Peroxide or VHP), in the enclosure. This chemical decontamination process is achieved at moderate temperature (< 60°C) with a low relative

humidity (less than 5%). First, the efficiency was tested with biotest and secondly, inoculated foods were used. Species were selected for their specific interest in the canned food industry (*Moorella*, *Thermoanaerobacterium*, *Geobacillus stearothermophilus* and *Bacillus coagulans*).

Results: In a first step the ability of the VHP process to destroy highly resistant spores deposited in dry state on biological indicators (stainless steel samples) and then exposed to different VHP treatments was determined. More than 5 logs of spores were decontaminated for *G. stearothermophilus* while only 3 logs of destruction were observed with *Moorella*, known to be the most heat resistant spore species. In a second step, 5 different kinds of dry food products - spices or dehydrated vegetables - artificially contaminated with the same species spores were exposed to VHP. Nature of the dry food (i.e., garlic powder) impacted the decontamination efficiency.

Significance: This study shows that the VHP treatment is effective for the reduction of heat resistant spores even at low temperature and may be suitable for the treatment of dry and dehydrated products with limited impact on the food characteristics.

T4-03 Prevention and Reduction of Bacterial Cross-contamination by Natural Antimicrobials during the Washing of Ready-to-Eat Lettuce

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Introduction: Microbiological safety of fresh-cut produce is not guaranteed as shown by the current increase in the number of outbreaks due to its consumption. Since processing water is the main vehicle for the spread of bacterial contamination, its quality needs to be maintained.

Purpose: Evaluation of the antimicrobial properties of natural products accepted as food additives found in vegetables as isothiocyanates (ITCs) and in crustacean shells as chitosan (CHS), and their potential application in wash waters of fresh-cut produce to prevent cross-contamination.

Methods: Antimicrobial properties of allyl ITC (AITC) and benzyl ITC (BITC) (5; 50; 100 μ g/mL), and CHS (0.01; 0.1; 0.5; 1.0 %) against *Salmonella* were evaluated by standard plate count and impedance technique. The washing process of 25 g of fresh-cut iceberg lettuce in 1 L of tap water with CHS (0.1%) or BITC (75 μ g/mL) took place for 5 min, and was repeated twice within 2 subsequent days with reused water. Bacterial concentration was quantified in water and in the produce before and after the washing treatment.

Results: Increasing the concentration of CHS and BITC leads to a decrease in viable *Salmonella* of up to 2 and 3 logs, respectively. After the first washing cycle, 10² CFU/mL of natural bacteria present in lettuce is transferred to the water. The initial addition of BITC leads to a complete removal of bacteria in the wash water after 24 h, before starting the second cycle. CHS does not affect the viability of natural bacteria, but diminishes *Salmonella* concentration in

water up to 2 logs after the 2nd cycle of washing.

Significance: BITC and CHS displayed a potential antibacterial activity against total coliforms and *Salmonella*, respectively, being able to effectively reduce and even completely inactivate bacteria during washing of fresh-cut produce, reducing cross-contamination and risk of outbreaks.

T4-04 Tolerance to Quaternary Ammonium Compounds May Enhance Growth of *Listeria monocytogenes* in the Food Industry

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Introduction: *Listeria monocytogenes* is frequently isolated from the food industry despite daily cleaning and disinfection. The genes *qacH* and *bcrABC*, encoding transporters belonging to the small multidrug resistance (SMR) protein family, have been proposed to contribute to persistence as they are linked to low level tolerance against the disinfectant benzalkonium chloride (BC).

Purpose: Examine prevalence of genes encoding tolerance to disinfectants and their role under practical food industry conditions.

Methods: Isolates of *Listeria monocytogenes* (n = 101) from Norwegian meat- and salmon-processing plants were tested for the presence of *qacH* and *bcrABC*. The growth inhibitory effects of BC in suspension and bactericidal activity in biofilm (50 ppm) and suspension (10 ppm) were tested. The presence of BC was analysed in water remaining on surfaces in the food industry after sanitation.

Results: 23 and 8 of the isolates contained *qacH* and *bcrABC*, respectively. Isolates with *qacH* and *bcrABC* genes could multiply in higher concentrations of BC compared to isolates lacking these genes, however, they did not show increased tolerance to the biocidal effect of BC. Residues of BC were detected in all eight analysed samples of water residues collected from surfaces after sanitation. Two collected water samples with high concentration (>100 ppm) of BC had a bactericidal effect against *L. monocytogenes*, while a water sample with about 10 ppm BC had had different growth inhibition effects against *L. monocytogenes* with or without the *qacH/bcrABC* genes.

Significance: Low level tolerance to BC resulting from the presence of genes *bcrABC* and *qacH* may be advantageous for growth of *L. monocytogenes* in niches with residual disinfectant after practical cleaning and disinfection.

T4-05 Impact of Enrofloxacin Treatment on Fecal Populations of *Campylobacter* spp. in Calves

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Introduction: Emergence of antimicrobial resistance (AMR) in zoonotic pathogens colonizing food animals has major public health implications. Significant attention has been paid to antimicrobials used as growth promoters. However, limited information is available on AMR outcomes from therapeutic use of antibiotics in food animals.

Purpose: This study examined impacts of therapeutic regimens for administration of enrofloxacin (a fluoroquinolone antibiotic) in calves on AMR of *Campylobacter*.

Methods: Three calves (5-mo old steers) were treated subcutaneously, first with low (5mg/kg for 3 days) and after 10 days with high (12.5 mg/kg once) doses of enrofloxacin. Fecal samples were monitored for *Campylobacter* prior to the first treatment (period T1), between first and second treatment (T2) and subsequent to the second treatment (T3). *Campylobacter* spp. were analyzed by enrichment, direct plating and enumerations on selective media (mCCDA), and characterized for resistance to the (fluoro) quinolones ciprofloxacin and nalidixic acid. To detect *gyrA* mutations associated with resistance, a *gyrA* fragment was amplified and sequenced.

Results: Resistance prevalence differed among sampling periods ($P < 0.0001$), with T1 isolates being *C. jejuni* susceptible to nalidixic acid and ciprofloxacin, while most T2 and T3 isolates (100 and 75%, respectively) were nalidixic acid-resistant (Nal^R). However, 96.84% of these Nal^R isolates were ciprofloxacin-susceptible and 94.74% appeared to be *C. fetus*, reported previously to be frequently Nal^R but susceptible to ciprofloxacin. Their *gyrA* sequence was highly conserved with *C. fetus gyrA* and lacked mutations associated with (fluoro)quinolone resistance in *C. jejuni*. These findings suggest a shift in *Campylobacter* spp. populations in response to enrofloxacin treatment, with Nal^R campylobacters, primarily *C. fetus*, predominating subsequent to the administration of the antibiotic.

Significance: The data suggest complex outcomes to therapeutic antibiotic administration in food animals. These data will contribute to development of science-based recommendations for food animal veterinarians to maximize antibiotic efficiency while minimizing AMR.

T4-06 Maleic Acid Enhances Acid Sensitivity of *Listeria monocytogenes* through Inhibition of the Glutamate Decarboxylase Activity

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Introduction: *Listeria monocytogenes* is the most deadly foodborne pathogen, causing listeriosis which is responsible for most deaths from food poisoning than any other pathogen. The Glutamate Decarboxylase (GAD) system is the most important mechanism of acid resistance in *L. monocytogenes* allowing it to survive in acidic foods or pass through the stomach.

Purpose: To investigate the effect of various compounds on the GAD system activity and acid resistance of *L. monocytogenes*.

Methods: The Minimum Inhibitory Concentrations of various compounds on *L. monocytogenes* strains 10403S and EGD-e were identified and subsequently, their role in survival under lethal acidic conditions (pH 3, HCl) and on GAD activity was assessed with the use of mutants in GAD decarboxylase genes (stationary phase, BHI, 37°C). Extracellular γ -amino butyric acid (GABA_e) and intracellular γ -amino butyric acid (GABA_i) was studied in all strains and mutants in the presence or absence of all compounds, under sublethal acidic conditions to assess the effect of the compounds in the activity of each decarboxylase. Furthermore, the effect of these compounds on the GAD activity in protein lysates was investigated. Finally, it was assessed the ability of each of the compounds to remove and/or inhibit biofilms formation

Results: Out of dozens of compounds, maleic acid was able to increase the acid sensitivity of both 10403S WT and EGD-e. GadD1 did not play any role in acid resistance while GadD3 played a role but was not affected by maleic acid. GadD2 was the major determinant of GAD activity and its activity was inhibited in the presence of maleic acid as was also shown in protein lysates.

Significance: Maleic acid can significantly modulate the acid resistance of *L. monocytogenes* through the inhibition of GAD activity and used in disinfection regimes to eliminate this pathogen from foods and lower the incidence of disease.

Technical Session 5 – Risk Assessment Thursday, 12 May – 10.30 – 12.00

T5-01* Risk Assessment as a Tool for Evaluating Temperature Requirements in Handling Area of Chill Food Distribution Centers

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Introduction: Temperature management in the cold chain recently began to receive attention in Taiwan. Recently, the government is considering setting 15°C as a temperature requirement in handling area for chilled food distribution centers. However, a decision cannot be made without information about the average risks exposed to consumers.

Purpose: This study aimed to evaluate temperature requirements in handling areas by developing a risk assessment of *Bacillus cereus* associated with ready-to-eat rice balls produced and consumed in Taiwan and suggest temperature requirements and effective intervention steps to control the risks.

Methods: Data on the prevalence, concentration and growth of *B. cereus* in ready-to-eat rice balls were collected through literature and sample analysis. Actual questionnaires were sent to chilled food distribution centers and retail shops by mail to obtain actual temperatures and time related information in different operational steps in summer seasons. Probability distributions were then selected to describe these data. A Monte Carlo simulation model was created using @risk.

Results: Preliminary results indicated prevalence and initial concentration of *B. cereus* in ready-to-eat rice balls during food factory were 2.5 log CFU/g. By soliciting 100 food distribution centers, we received 7 responses by 1 March 2016. Average actual handling temperatures and time spent at chilled distribution centers for chilled foods were 7.79°C and 5.41 hours. As for retailers, actual handling temperatures and time were 24°C and 0.29 hours. Predictive microbiology models simulated that final number of *B. cereus* at 6.59 log CFU/g, and we also found that by controlling handling temperatures at 15°C both at distribution center and retail, *B. cereus* can be reduced one log CFU/g. The most influential interventions on the risk of illness were handling temperature and storage temperatures in distribution centers.

Significance: The findings could be used by the food regulatory agencies in Taiwan to establish food safety policies and develop risk management strategies to reduce food safety risk associated with the consumption of ready-to-eat foods.

T5-02 A Quantitative Microbiological Exposure Assessment Model for *Bacillus cereus* Group IV in Couscous Consumed on a Weekly Basis or during Collective Events

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Introduction: The couscous, widely consumed in Algeria, has been associated with several outbreaks. Couscous semolina may contain heat-resistant spores of *Bacillus cereus* groups IV, these bacteria are mesophilic and potentially producer of emetic toxin.

Purpose: The present work aimed at predicting (i) the prevalence and concentration of *B. cereus* during couscous preparation at consumer's place, and (ii) the number of consumers exposed to 10⁵ CFU/g or more *B. cereus* in Algeria, due to couscous consumed on a weekly basis or during collective events.

Methods: The couscous semolina preparation was divided into three modules (i) the contamination at retail, (ii) the inactivation of *B. cereus* during couscous steaming and (iii) the growth during storage before serving. Each module was built using a combination of newly gathered data, literatures studies, predictive models and expert's opinion information. Algeria was split into four regions: North, South, High plateau and Kabylie. The main differences between each region were the seasonal temperature and the consumption pattern. In absence of a dose-response model, a concentration of 10⁵ CFU/g of *B. cereus* was considered as a "risky" concentration.

Results: Per year, a total of 62,477 people (0.15% total population) and 41,602 people (0.10%) were estimated to be exposed to risky concentration due to weekly and collective consumption, respectively. The highest exposure came from for couscous

semolina kept at ambient temperature, in south of Algeria during summer.

Significance: Cold storage and consumption within the first 12 h after steaming are conditions which will limit the exposure to *B. cereus* in couscous.

T5-03 Phenotypic Behavior of 35 *Salmonella enterica* Serovars Compared to Epidemiological and Genomic Data

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Introduction: Despite a declining trend, *Salmonella* is with 82,694 confirmed human cases in 2013 still the second most important zoonosis in the EU. The Netherlands reported 992 laboratory confirmed cases of salmonellosis corresponding to an estimated number of 28,000 cases in the general population of 16.8 million people. The Dutch food and consumer product safety authority (NVWA) monitors the presence of *Salmonella* according to EU-regulation 2073 (2005), which on several occasions reveal serovars that differ from those most frequently found in human reported cases. This raises questions about the current *Salmonella* regulation in relation to the potential hazard of these infrequently found serovars.

Purpose: Put the virulence of infrequently found *Salmonella* serovars in relation to the potential hazard of more frequently found laboratory confirmed serovars from human cases in The Netherlands.

Methods: We investigated the phenotypic behavior of 70 strains of 35 different *Salmonella* serovars in an *in vitro* gastro-intestinal tract (GIT) system as proxy for virulence. Virulence was expressed as the probability of infection, P(inf), i.e., fraction of the overnight culture invading into Caco-2 cells after GIT passage. In addition, the phenotypic *in vitro* GIT results are put into perspective of human cases and molecular virulence properties related to these serovars.

Results: Results show that the (average) P(inf) of *Salmonella* serovars ranges from 1.7 10⁻⁸ (*S. Kedougou*) to 5.3 10⁻⁵ (*S. Typhimurium*). In general, the P(inf) corresponds to available epidemiological and virulotypic data. Still, individual exceptions exist and it is hypothesized that the public health risk from *Salmonella* is associated with exposure (prevalence, dose and/or acquired immunity) rather than difference in virulence.

Significance: Knowledge about the relative phenotypic virulence of different *Salmonella* serovars in relation to epidemiological and genomic data may provide guidance to policy makers for future regulation strategies.

T5-04 Meta-analysis of the Inactivation Effect of High Hydrostatic Pressure on *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*

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Introduction: Since the late 1980s, High Hydrostatic Pressure (HHP) has been widely used to preserve foods as an alternative technology to thermal treatments. Microbial inactivation induced by HHP is generally recognized as depending on many factors such as temperature, microbial species, physiological state, medium, etc.

Purpose: The aim of the study was to identify the main influencing factors and to predict survival rates for three major pathogens: *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*.

Methods: In contrast with thermal treatments, HHP inactivation kinetics do not always follow first-order kinetics but show tailing and the use of D_p values is consequently often unadapted. Therefore a dataset of 120 inactivation curves achieving at least three log₁₀ reductions of the selected pathogens, was collected from the literature and analysed with reparameterized Weibull model to estimate t_{3D} values, time needed to reach 3-log₁₀ reduction. Bayesian hierarchical modeling was used to develop within a meta-regression analysis, a mixed-effect linear model with log₁₀ t_{3D} values (s) as response and pressure intensity and temperature as explicative variables. Treatment medium was considered as a co-variable having fixed effects whereas species, strain and study were considered as random factors. Several models differing by their description of variability on log₁₀ t_{3D} and including combinations of factors were tested.

Results: Based on goodness-of-fit and parsimony, a model was chosen. Resistance to HHP was found to be the highest in meat products and the lowest in fruit juices. The determination of Z_{HP} values, increase of pressure necessary to decrease log₁₀ t_{3D} by 10, enabled to rank the three pathogenic species according to high-pressure resistance. *L. monocytogenes* appeared to be the most resistant to HHP, then followed by *S. aureus*.

Significance: The model developed could be profitably used by food manufacturers to set appropriate HHP processing parameters to assure food safety.

T5-05 Risk Ranking of Chemical Hazards in Spices and Herbs

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Introduction: Spices and herbs bring flavour and nutrients to cuisine; however, they may expose consumers to various contaminants that pose a risk to human health.

Purpose: To rank the risks of chemical hazards in selected spices and herbs that have the potential for contamination as input for setting up monitoring programs.

Methods: A risk ranking toolbox for food and feed related issues was used to systematically select a ranking method. The method was applied to rank chemical hazards in paprika/chili, black pepper, nutmeg, basil, thyme, and parsley leaves. The severity and probability of the hazards were scored as low, medium, high, or severe. Literature and data were collected from scientific publications, alerting and (national) monitoring data, and other relevant European Union reports and databases to determine the scores.

Results: A risk matrix approach was selected to rank various chemical hazards including natural contaminants such as mycotoxins and plant toxins, environmental contaminants such as pesticides, dioxins, heavy metals, and polycyclic aromatic hydrocarbons, and deliberate contaminants such as dyes. The risk ranking showed that the following hazards were seen as the riskiest with respect to human health: the mycotoxins aflatoxins and ochratoxin A, the pesticides chlorpyrifos and triazophos, and the dye Sudan I.

Significance: A risk matrix provides a transparent risk ranking approach based on available data without the intensive demands required by risk assessments. These results can assist European Union initiatives focusing on sampling strategies for monitoring programs with respect to chemical contaminations in spices and herbs.

T5-06 Large-scale Molecular Risk Assessment of Shiga Toxin-producing *Escherichia coli* (STEC) by Whole Genome Sequencing

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(3) Istituto Superiore di Sanità, Rome, Italy

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are potentially lethal zoonotic pathogens with a clinical spectrum including non-bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). The most common STEC serotype associated with human disease is O157:H7, but there is a growing recognition of over a hundred non-O157 STEC serotypes that also may cause human illness. The large variation of serotypes and genetic contents of STEC associated with severe disease results in the current inability of designating individual strains as pathogens, which has negative impact for both clinical care and food safety measures.

Purpose: To predictively classify the clinical and/or epidemic potential of a STEC isolate it is crucial to associate its virulence and other distinctive features to the ability to cause disease. This study aimed at identifying associations between serotype, virulence factors, phylogeny, isolation source and severity of disease in order to gain an increased understanding on the complex epidemiology of STEC infections.

Methods: Nearly 400 STEC isolates (including clinical, animal and food isolates of multiple serotypes) were whole-genome-sequenced and serotypes, phylogenetic groups, MLST, and virulence profiles were determined *in silico* from the assembled genomes and used in a multi-dimensional scaling (principal component analysis).

Results: The results clearly showed distinct groups of STEC based on their virulence profiles, with STEC isolated from pigs, plants and imported meat being clearly separated from the group of clinical human isolates. These non-clinical isolates lacked many of the known STEC virulence markers. Interestingly, the classical Karmali's seropathotype classification is visible in the multidimensional scaling based on the virulence profiles. In contrast to the virulence profiles, the phylogeny based on single-nucleotide-polymorphisms (SNPs) was not informative for the risk analysis.

Significance: WGS followed by *in silico* virulence profiling provides ample opportunities to assess the potential public health risk associated with STEC strains.

Technical Session 6 – Pathogens and Produce Thursday, 12 May – 13.30 – 15.00

T6-01 Inactivation of Norovirus in the Presence of Soil Loads Simulating Actual Conditions of Viral Transmission

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Introduction: Human norovirus is recognized as very persistent in the environment. Attempts to prevent its transmission by inactivating may fail if the protective effect of the medium in which it is naturally shed (feces and/or vomit) is not taken into consideration. In laboratory studies, a "soil load" should be used to mimic the vehicle of transmission.

Purpose: The aim of this study was to compare the protective effects of different soil loads on a norovirus surrogate that can be cultured *in vitro* under three different inactivation conditions.

Methods: Artificial feces, real fecal matter, ASTM tripartite soil load and fetal bovine serum were tested as soil load for murine norovirus (MNV) in three inactivation conditions: chemical disinfection in suspension, chemical disinfection on surfaces, and heat treatment. The viruses were recovered after each treatment and detected by plaque assay.

Results: Soil load had no significant impact on inactivation of MNV in suspension by peroxyacetic acid. The ASTM medium and real fecal matter did not offer any protection to virions dried onto stainless steel against the antiviral effect of sodium hypochlorite solution. However, two types of Feclone™ and real fecal matter (10% and 20%) limited the loss of infectivity to 1 log cycle following heat treatment at 63°C for 2 min. Feclone BFPS-4 showed the most similarities to real fecal matter and thus appeared to be the best soil load. The

consistency of the fecal matter seems to have an impact on the efficacy of an inactivation treatment.

Significance: These results will be helpful for studies in which a soil load is used to approximate actual conditions of human norovirus transmission and inactivation.

T6-02* Non-protective Role of *sigB* against Oxidative Stress in *Listeria monocytogenes*

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Introduction: *L. monocytogenes* is a foodborne pathogen causing listeriosis, a life-threatening disease, that has to cope with extreme conditions, such as oxidizing environments, in order to survive and cause disease. Oxidative stress occurs during aerobic respiration, disinfection regimes (oxidative disinfectants) and during the infectious process. *sigB* is a well-known transcriptional regulator involved in regulating the expression of numerous stress- and virulence-related genes, whose role in oxidative stress is still controversial.

Purpose: To clarify the role of *sigB* against oxidative stress during different stages of the cell cycle.

Methods: *L. monocytogenes* 10403S, wild type (WT) and *sigB* mutant ($\Delta sigB$), were challenged with H₂O₂ in mid-exponential and stationary phases of growth. Bacterial viability was assessed by quantifying the colony forming units during the 60 minutes of challenge. In parallel, the levels of dissolved oxygen (DO) and the catalase activity were determined using a novel quantitative method.

Results: During stationary phase, the *sigB* mutant was significantly more resistant to H₂O₂ than the WT strain (~5 logs difference, $P < 0.05$), despite both strains presenting similar DO levels (6.41 ± 0.12 for WT and 6.50 ± 0.22 for $\Delta sigB$). However, during mid-exponential phase both WT and *sigB* mutant showed similar resistance to oxidative stress (~4 logs reduction after 60 min of challenge). Interestingly, the levels of DO during mid-exponential phase were significantly lower (~0 mg/L) for both WT and *sigB* mutant. During mid-exponential phase the catalase activity was very low both in WT and $\Delta sigB$ strains, however after 13/14 hours of growth, the $\Delta sigB$ presented stronger catalase activity than the WT.

Significance: We hypothesise that the presence of *sigB* gene has a non-protective effect against oxidative stress, which is probably mediated by the presence of oxygen. These findings will help us understand in depth the oxidative stress resistance mechanisms of this pathogen and thus reduce the occurrence of disease.

T6-03 Analysing the Microbial and Sensory Quality of Fresh Produce Following the Application of Ultrasound Decontamination

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Introduction: There is an imminent concern about food safety due to increased notifications of food contamination by pathogenic microorganisms. Leafy green vegetables are presented as the priority for the food sector regarding their safety and quality control. It is therefore evident that the effective implementation of decontamination technologies in the production of ready-to-eat vegetables is a prerequisite to achieve the production of safe produce. Even though the treatment of healthy produce such as green vegetables is necessary, this treatment should not reduce the appealing sensory characteristics of the products.

Purpose: The objective of this study is to evaluate the impact of ultrasound treatment on the shelf-life and sensory properties of lettuce leaves based on the evaluation of a number of safety and quality indices.

Methods: The shelf-life of lettuce leaves was analysed following the application of an ultrasonic system operating at 26kHz, 90 μ m, 200W in continuous mode for 5 minutes. Total aerobic count, psychrotrophic bacteria, *Salmonella*, Enterobacteria, yeasts and moulds levels were evaluated by using appropriate selective media. Sensory aspects such as appearance, browning, texture and odours were assessed by a semi-trained panel. Multivariate analysis was applied (i.e., principal component analysis followed by a partial least squares analysis) in order to better understand the relationships between all the experimental variable inputs (i.e., different bacteria, sensory scores and processes/preservation conditions).

Results: Overall, it was observed that storage time had the major influence on the bacterial growth, except for *Salmonella* for which temperature had the main impact. The ultrasound technology can be efficient against pathogens (*Salmonella* spp.) specifically at lower temperatures. Regarding the sensory characteristics of lettuce leaves, ultrasound yields some negative impact on the overall quality of the product towards the end of its shelf-life.

Significance: This work proposes an alternative technology for the decontamination of lettuce leaves during the application of ultrasound processing. Ultrasound could guarantee the quality and safety for the consumers under specific processing and storage conditions. The potential of this technology is highlighted based on the obtained safety and sensory assessments.

T6-04* *Salmonella*/Salad Interactions

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Introduction: Fresh produce such as salad leaves are an important part of a healthy diet but in recent years have been associated with infection by enteric pathogens such as *Salmonella enterica*. So far, studies have concentrated on improving the hygiene of production and have not considered what happens to the behaviour of *Salmonella* when they enter the actual salad bag. It is known that salad leaves become damaged during processing and that juices are released, so bacteria residing in a salad bag will be bathed in leaf juice.

Purpose: The intention of our research is to investigate the effect of juices released from damaged salad leaves on the growth, virulence and salad leaf colonisation of *S. enterica*. Our aim is to use this information to develop ways of preventing enteric pathogen attachment to fresh salad produce.

Methods: *Salmonella* responsiveness to salad juices was analysed in water, to reflect the salad bag environment, and in serum-media to model the co-consumption of pathogen and salad leaf. We used assays that measured the effect of salad leaf juice on *S. enterica* growth, motility and biofilm formation. Light and scanning electron microscopy were used to visualise juice effects on *Salmonella* colonisation of salad leaves and the salad container.

Results: Salad juices at more than 1:50 dilutions stimulated *Salmonella* growth in all media tested. In serum-media, juices enhanced growth by several logs via provision of host iron from serum-transferrin. In water, leaf juices from all salad leaves tested significantly increased *Salmonella* biofilm formation and its capacity to colonise and persist on salad leaves, and the salad bag container.

Significance: Our study shows that even very dilute salad juice can contribute to *Salmonella* colonisation of salad leaves and re-emphasises the importance of preventing enteric pathogen of fresh produce.

T6-05* The Influence of Pre-wash Chopping and Storage Conditions of Parsley on the Efficacy of Disinfection against *Salmonella* Typhimurium

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Introduction: Initial chopping of parsley before washing for subsequent processing into ready-to-eat foods later in the day is common in some restaurants.

Purpose: The aim was to evaluate the influence of pre-wash chopping on the *S. Typhimurium* decontamination by common washing and disinfection methods.

Methods: Vinegar (4%, v/v, acetic acid), 0.25g/l sodium dichloroisocyanurate (NaDCC), and water combined with rinsing and manual agitation were applied for 15 minutes.

Results: This study demonstrated limited efficiency of applied methods and that holding pre-wash chopped leaves at 30°C reduced the effectiveness of all washing solutions. Scanning electron microscopy (SEM) imaging indicated initiation of biofilm formation after 24 h at 5°C with noticeable adhesion of cells to inaccessible folds of the vein on the leaf surface. NaDCC was shown to be the most effective solution achieving log reductions of 1.92 – 3.12 on intact parsley leaves. The latter being on those held at 5°C for 4 h; however, its effectiveness was reduced by 0.73 – 0.93 and 1.19 log CFU/g on chopped leaves at 5°C and on both intact and chopped leaves at 30°C, respectively.

Significance: In conclusion, strict temperature control and avoiding pre-wash chopping are highly recommended during handling of parsley for the optimal elimination of pathogenic microorganisms.

T6-06 Determination of Fatty Acid Composition of *Pistacia vera* Selected from the Valley of River Platani (Sicily)

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Introduction: The nutritional value and the beneficial effects of pistachio have been demonstrated. The growing area attributes specific properties to the nut trace element profile. The Platani river valley has a soil composition that could be responsible for specific fatty acid (FA) nut contents.

Purpose: This study determines the total FA composition in *Pistacia vera* (cultivar Napoletana) harvested in a restricted geographical area of Sicily as a guarantee of the typicality of the pistachio.

Methods: Pistachio nuts were harvested at the end of August from different sites located in the Platani river valley. Fat extraction from nut oil was carried out using an Accelerated Solvent Extraction system. The esterification of FAs to FA methyl esters was performed using an alkylation derivatization reagent, and analysed by a gas-chromatographic technique with FID detector using a 100% polyethylene glycol capillary column. The relative percentage of each FA was calculated on the basis of the peak area of a FA species to the total peak area of whole FA in the oil sample.

Results: The classes of monounsaturated, saturated and polyunsaturated FAs were determined. Our data showed that the samples were composed of oleic (69.9%), palmitic (9.8%), linoleic (18.0%) and stearic (1.2%) acids, as well as palmitoleic (0.8%), linolenic

(0.4%) and arachidonic (0.1%) acids. Oleic acid is the main FA representing more than 69% of the total oil content.

Significance: These results highlight the richness of unsaturated fatty acids in *Pistacia vera* nuts, underscoring its high nutritional value. We suggest that the FA composition of nuts is increased under the influence of pedological and environmental factors affecting the fruit quality. The antioxidant effects and nutritional properties of this pistachio will be further investigated.

Technical Session 7 – General Microbiology and Non-microbial Food Safety Thursday, 12 May – 15.30 – 17.00

T7-01 Assessment of the Biofilm Formation Interactions between *Cronobacter sakazakii* and *Bacillus subtilis*

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Introduction: *Cronobacter sakazakii* is an opportunistic foodborne pathogen that is commonly isolated from powdered infant formula and represents a significant risk to the health of neonates. At the same time, milk powder products are also frequently contaminated with the spore-forming species of the genus *Bacillus*. Since one of the most common sources of microbial contamination of food is through biofilms formed on surfaces and equipment used in food industries, *C. sakazakii* and *Bacillus* spp. are likely to co-exist within biofilm communities.

Purpose: The aim of this study was to evaluate the biofilm-forming ability of *C. sakazakii* and *Bacillus subtilis* under mono- or mixed-culture conditions.

Methods: Biofilm formation was studied on stainless steel (SS) coupons utilizing a two-step procedure: SS coupons were initially incubated in saline bacterial suspensions at 15°C for 3 h (attachment step) and afterwards, coupons carrying strongly attached bacteria were further incubated at 37°C for 72 h or at 20°C for 144 h. Viable biofilm bacterial populations were determined, using the bead vortexing method, after initial attachment, and at 24-h and 48-h intervals during incubation at 37°C and 20°C, respectively.

Results: Biofilm formation was found to be influenced by time, bacterial species and culture conditions (mono- or mixed-culture). Regarding the mono-culture conditions, *B. subtilis* exhibited a significantly ($P < 0.05$) lower biofilm formation compared to *C. sakazakii* for the first 48 h of incubation at 20°C. Co-culture with *B. subtilis* within a dual-species community significantly ($P < 0.05$) enhanced the biofilm-forming ability of *C. sakazakii* at 37°C; nonetheless, no corresponding difference was observed with regard to biofilm formation at 20°C.

Significance: Multi-species biofilms are dynamic systems with variable interactions among the

different species, and the above results reinforce the aspect that bacteria have developed different strategies to adapt to environmental conditions and exist within biofilm communities.

T7-02* Electron Beam Processing Improves the Microbiological Safety and Retains the Sensory Qualities of Alfalfa Sprouts

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Introduction: There has been a significant increase in the consumption of fresh produce in the U.S. This increased consumption puts consumers at risk of pathogen exposure since there is no pathogen kill step for sprouts. The hypothesis was that electron beam (eBeam) technology can significantly reduce pathogen exposure via alfalfa sprouts without impairing sensory qualities.

Purpose: The objective was to demonstrate the effectiveness of eBeam (at a FDA approved dose of $\leq 1\text{kGy}$) as a non-thermal pathogen kill technology to improve alfalfa sprouts' microbiological quality without impairing its sensory qualities.

Methods: Alfalfa sprout samples were inoculated with a cocktail of non-O157 STEC strains and processed using the eBeam technology (dose $\leq 1\text{kGy}$). The eBeam treated and control samples were compared in terms of the reduction of natural bioburden as well as the levels of the inoculated non-O157 STEC pathogens. The treated and control samples were also compared in terms of texture, color, and electrolyte leakage for sensory attributes.

Results: eBeam beam processing of alfalfa sprouts at doses $\leq 1\text{kGy}$ achieved a 4-log reduction of the non-O157 STEC strains. The natural bacterial and fungal bioburden levels were reduced by 2.2 and 2.1 logs respectively over a 21 day period in refrigerated storage. There was no significant ($P \leq 0.05$) difference in texture, color, or electrolyte leakage between eBeam treated and (untreated) control samples.

Significance: eBeam treatment of alfalfa sprouts at doses $\leq 1\text{kGy}$ is an FDA approved technology to extend shelf life and has a major collateral benefit of achieving a 4-log reduction of a key bacterial pathogen associated with alfalfa sprouts without impairing the sensory qualities.

T7-03* Behaviour of Psychrotrophic *Bacillus cereus* during the Life Cycle of Food Products for Elderly People

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Introduction: The aim of the FP7-OPTIFEL project is to create new food products based on fruits and

vegetables, supplemented with specific nutrients to fight elderly's nutritional deficiencies. The microbial safety of these products is of great importance due to the high risk of foodborne disease in the elderly population. The foodborne pathogen *Bacillus cereus* can resist pasteurization treatments, psychrotrophic strains can multiply during food storage at low temperatures, and some may produce the heat resistant emetic toxin cereulide.

Purpose: Understanding *B. cereus* behaviour during the life cycle of the food products.

Methods: We determined the growth ability of two psychrotrophic *B. cereus* strains KBAB4 and ADRIA I21 in BHI medium at various pH values between 5.0 and 7.0, with and without oxygen, at 8°C and 10°C. We also investigated the possibility for the two emetic psychrotrophic *B. cereus* MC 67 and BtB2-4 strains to produce the emetic toxin (cereulide) from 8°C to 25°C and at various pH values. We finally analysed the heat-resistance of the vegetative cells of six psychrotrophic and two thermophilic strains of *B. cereus* during food reheating.

Results: Combination of anaerobiosis, pH < 5.7 at 10°C, or anaerobiosis at any pH and temperatures < 8°C prevented psychrotrophic *Bacillus cereus* growth. The LC-MSMS analysis showed that the production of cereulide began during the stationary phase of bacterial growth and that low pH and low temperatures limited its production. At 48°C, D value of vegetative cells of psychrotrophic strains varied from 3 to 8 min. For all strains, the heat-sensitivity (z-values) varied between 1.6°C and 5.6°C.

Significance: These data on *B. cereus* cell behaviour will be used as guidance to help new product development.

T7-04 Contribution of the Certified Reference Materials to Food Safety

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European Commission, Geel, Belgium

Introduction: The main purpose is simple, to get safe food on our plates. However the mechanisms in place at the EU to protect its citizens and ensure Food Safety are in fact complex. The process starts by identifying molecules that pose adverse health effects, determined through risk analysis and is followed by regulating the presence of maximum levels of related compounds in foodstuffs. Accordingly, the analytical laboratories measure the compounds for control and monitoring of the levels to ascertain compliance with the legislation before the food products are safely placed in the market. Food contaminants, natural or human-made are analysed daily by numerous commercial and official laboratories, using a large variety of analytical methodologies. The laboratories need to provide results that are reliable; hence, they need to demonstrate the good performance of their methods. This may be done by the analysis of certified reference materials (CRMs). When a CRM is measured following the laboratory's protocol

and the result obtained is statistically equivalent to the value certified in the material, the laboratory is automatically demonstrating the satisfactory quality of their analyses according to internationally accepted standards that also provide traceability and commutability of their processes.

Regulated contaminants such as polycyclic aromatic hydrocarbons (PAHs), mycotoxins or pesticides, and emerging contaminants, still not regulated in food, notably perfluoroalkyl substances (PFASs) or acrylamide, are examples of compounds of high interest whose levels are measured in the EU. Given their relevance, the European Commission develops dedicated CRMs to provide quality control tools to laboratories that facilitate the task of implementing accurate methods and contribute to the harmonisation of data results.

Purpose: The role of CRMs in Food Safety and the proper use of CRMs will be discussed using some examples of reference materials for contaminants in food recently developed at the Institute for Reference Materials and Measurements (IRMM).

Methods: Simple statistical tests will be applied to a number of CRMs to assess the statistical significance of the method bias taking into account the relevant uncertainty contributions.

Results: The outcome of the bias test will be discussed and interpreted according to different possible scenarios

Significance: Accurate analytical results are key during decision making processes on compliance with legal limits set for contaminants in food. CRMs can be used to demonstrate the accuracy of analytical methods, hence ensuring the quality of analytical data, and consequently contributing to Food Safety by proper establishment of compliance, or not, with the legal limits.

T7-05 Mycotoxins Impact Prediction in Food Due to Climate Change Using Big-Data Analysis in Korea

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Introduction: The growth of mycotoxigenic fungi and mycotoxin production level is predicted to be affected greatly due to average temperature increase caused by global warming and massive weather anomaly. Since high temperature and high humidity by climate change accelerates growth of mycotoxigenic fungi in food and raises risks of mycotoxins, the accompanying countermeasures of food safety are required. This study was conducted to predict the types of mycotoxins with potential risk in the future and the vulnerable regions to mycotoxins by collecting and executing spatial-temporal analysis of big-data on the climate change, monitoring data on mycotoxin contamination level in food, and cultivation distribution of food in Korea.

Purpose: To analyze climate vulnerability of mycotoxins in food, to deduct high priority risk indicator, and to develop scientific basis for the

decision making on regional priority of national monitoring sampling.

Methods: The impacts of each mycotoxins were predicted in this study by analyzing the change of the occurrence of days on optimum climatic conditions for mycotoxin production using Korea weather history data and future climate change scenarios (RCP scenarios). The regions vulnerable to mycotoxins in Korea were selected in this study by generating big-data via connecting national monitoring data and agricultural cultivation data. As a big-data analysis method, Time Series Analysis and SNA (Social Network Analysis) were particularly used.

Results: The results obtained with analysis of the change on occurrence of days for each 13 kinds of mycotoxins activity based on climate change in Korea suggested Ochratoxin A has increased most apparently since 1999, while Aflatoxin has not shown any change, and the other hazards have increased overall and showed radical increase since 2010. Also, Gyeongsangbuk-do, Gangwon-do and other east part of Korea are indicated as vulnerable regions by the results of analyzing risk rate calculated from the number of days on optimum climatic conditions for mycotoxin, detection rate of mycotoxin, and cultivation measurement of mycotoxin detected food. Precise forecast is requested on detail analysis of effect of food for further studies.

Significance: The effects of mycotoxins in food in medium and longer term according to climate change were forecast in this study, and Ochratoxin A in addition to the existing monitored mycotoxins such as Deoxynivalenol, Fumonisin and Zearalenon is required to be monitored in the level of food production. Also, sampling plan applied weighting on regions vulnerable to mycotoxins is suggested to be established to execute effective risk management within limited budget.

T7-06 Efficacy of Aqueous Chlorine Dioxide on *Escherichia coli* Inactivation during Fresh-cut "Lollo Rossa" (*Lactuca sativa*) Washing at the Pilot Scale

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Introduction: Controlling water quality is critical in preventing cross-contamination during produce washing. Wash water disinfection strategies may help prevent cross-contamination within the washing water and, subsequently, between produce batches.

Purpose: The aim of this study was to evaluate the efficacy of aqueous chlorine dioxide (ClO₂) on the reduction of supplemented, non-pathogenic *Escherichia coli* in the washing water and on processed "Lollo rossa" lettuce at the pilot scale.

Methods: A 3.5 m³ washing line was continuously supplied with ClO₂, until approximately 5 ppm, before and during 800 kg of lettuce washing (~90 minutes). Then, the lettuce input and the ClO₂ supply halted. A non-pathogenic, overnight cultured (37°C), *Escherichia coli* non-typable strain, which was isolated from surface water, was added to the tank resulting in an approximate final concentration of 10⁶ CFU/ml. Wash water and produce samples for microbiological and chemical analyses were taken before and after the input and supply halted.

Results: No detectable levels of *E. coli* were determined within 1 minute after supplemented *E. coli* entered the ClO₂ containing washing water. A log 2 reduction was achieved on leaf samples, compared to control experiments without aqueous ClO₂. ClO₂ concentrations quickly decreased after introduction of the strain, yet a residual concentration (>2 ppm) remained present in the washing water.

Significance: Results demonstrated that ClO₂ application at the pilot scale was able to reduce the load that entered the process wash water. However, this treatment was not able to prevent attachment to the lettuce. Nevertheless, the use of ClO₂ decreased the possibility for cross-contamination between batches in comparison to no disinfectant. Overall, cross-contamination prevention via the washing water remains critical and application at the industrial scale is attainable.

Technical Session 8 – Modelling, Beverages and Microbial Food Spoilage Friday, 13 May – 8.30 – 10.00

T8-01 Designing a Food Matrix Ontology for Supporting a Predictive Microbiology Database

Salavador Cubero¹, FERNANDO PEREZ-RODRIGUEZ¹, Elena Carrasco¹, Antonio Valero¹ and Matthias Filter²

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- (2) BfR, Berlin, Germany

Introduction: Predictive microbiology models are intended to represent microorganism behavior in food matrices and provide accurate mathematical predictions. In this field, food matrices are usually reported in unstandardized fashion, thus limiting its application and/or validation when models are required for specific food products.

Purpose: The main objective was to design an ontology for food matrix to be applied to predictive microbiology.

Methods: Food matrices from the Open Food Safety Model Repository (Open FSMR) were used in this project. An ontology prototype was generated considering ontologies available at *Biportal* through *Ontomaton*. Then, the prototype was reviewed and refined using the software *Protégé*. Food matrices were categorized according to logical criteria based on its origin and composition, and in the case of foods containing multiple ingredients, allocating it in multiple categories.

Results: More than 700 food matrices were mapped (i.e., cross-referenced with other ontologies at *Bioportal*) and categorized. Few matches were found when compared with existing ontologies at *Bioportal*. The main reason was due to differences in the type of food matrix used in biomedical science (*Bioportal*). Therefore, in this work, several categories had to be created from scratch. As an example, salads were categorized as “salad” within “group of vegetables”, provided that salads have a vegetable base in the recipe. Moreover, depending on the rest of the ingredients, it could be included at the same time into other food categories (i.e., multiple categorization).

Significance: Further steps in this project will consist of integrating this ontology into *PMMLab program* so as to provide with a more accurate re-implementation and application of predictive microbiology models.

T8-02 Using Genome-scale Metabolic Models of Foodborne Pathogens to Address Human Disease and Food Safety

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Introduction: Using a combination of computational techniques and laboratory methods, genome-scale metabolic models (GEMs) can be created, validated, and used to identify differentiating metabolic capabilities of microbes of interest within the same genus.

Purpose: With projects sequencing the genomes of >100,000 important foodborne pathogens currently underway, the objective of this study was to generate GEMs for numerous foodborne outbreak strains of *Salmonella* spp. and *Listeria monocytogenes*, and to compare nutrient utilization predictions of these models to *in vitro* results.

Methods: Genomes of six *L. monocytogenes* and five *Salmonella* spp. strains were taken from the NCBI database and uploaded to KBase, a semi-automated computational resource used to generate the GEMs. These models were then used to generate strain-specific nutrient utilization predictions for 95 sources of carbon under aerobic and anaerobic conditions using General Algebraic Modeling System (GAMS) software. *In silico* predictions were then compared to *in vitro* experiments performed using Biolog phenotypic microarray plates. Following this comparison, GEMs were manually curated to increase agreement between *in silico* predictions and *in vitro* results.

Results: Carbon source utilization agreement between *in silico* predictions and *in vitro* results was strong and significant (Pearson correlation test statistic yields $P < 0.001$) and ranged from 80% to 90% for *L. monocytogenes* and from 90% to 98% for *Salmonella* strains. Once validated, *L. monocytogenes* and *Salmonella* GEMs were used to simulate environments of numerous food matrices and host-niches to identify differentiating metabolic pathway capabilities and 100's of essential metabolic reactions required for growth and viability for the strains from each genus.

Significance: Since many of these pathogenic bacteria continue to emerge in foods and have large global impacts to human health and the economy, this research has demonstrated new post-genomic era approaches to identify new targets to treat human disease and make foods safer from *Salmonella* spp. and *Listeria monocytogenes*.

T8-03 Validation of a *Vibrio parahaemolyticus* Growth Model for Application in a TTI-based Seafood Safety Management System in Oysters

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Introduction: It has been reported that infections caused by pathogens commonly transmitted through food have declined, or are approaching targeted national levels, with the exception of *Vibrio* infections. Growth of *Vibrio* spp. in shellfish after harvest is a typical time-temperature relationship which can be used as a predictive model for growth. A Time Temperature Integrator (TTI) based system may be used for a realistic control of the chill chain and efficient management of either shelf life, quality changes, risk assessment and/or bacterial growth prediction. The current TTI technology and a scientific approach with regards to quantitative study of safety risk in foods allow the undertaking of the next important step, i.e., the application of TTIs to manage safety risks of foods. Vitsab has initiated a development program for suitable enzymatic *Vibrio*-TTI formulations.

Purpose: The aim of the study was to validate existing predictive models for *Vibrio parahaemolyticus* growth and to evaluate the applicability of the Vitsab *Vibrio*-TTI for monitoring Vp risk in oysters during distribution and storage.

Methods: Oysters (*Grassostrea gigas*) were inoculated with Vp placed in plastic trays with attached Vp-TTI labels and stored at controlled isothermal conditions (0-30°C) and at variable conditions. Vp load at predetermined times was estimated based on the response of the TTI and was compared to actual measured Vp enumeration.

Results: The comparison between the experimental (actual) and predicted values by the TTI microbial load was based on the accuracy and bias factors.

Vibrio spp. risk in oysters, using validated kinetic models, can be estimated at any point of the chill chain if the temperature history is known.

Significance: The results of the study indicate that the developed Vp-TTIs can be a powerful and a cost effective tool in validating improved handling and cooling procedures and monitoring the distribution of oysters locally as well as for longer transports.

T8-04 Fungi in Juices: Survey on the Use of Homogenization and Ultrasound as Efficient Preserving Tools

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Introduction: Fungal spores represent a threat for juices, as they can survive traditional thermal treatments. However, the use of higher temperature and/or time is not advisable due to the strong impact on nutritional and sensory scores.

Purpose: This research proposes some promising ways to control fungi in juices.

Methods: *Fusarium oxysporum*, *Penicillium italicum* and *Aspergillus nidulans* were studied in relation to their resistance to homogenization (single or multiple pass-treatments up to a 150 MPa pressure) and ultrasound (by modulating the duration of treatment and pulse, and power) both in model systems and in juices (pineapple, orange, and tomato juices). These treatments were also combined with Na-benzoate and citrus extract. The experiments were planned and performed by using the theory of the Design of Experiments.

Results: Homogenization could affect fungal spores only when a multiple-pass treatment was used; moreover, it reduced the number of spores but did not control survivors. Similar results were found for ultrasound, although the managing parameters (power, pulse, duration of the treatment) played a different role. The combination of citrus extract improved the effect of the treatment, reduced the targets below the detection limit, and allowed a reduction of the amount of Na-benzoate. In addition, homogenization also caused a macroscopic variation on *Penicillium*, as the colonies turned to white and lost the green pigment.

Significance: The use of alternative approaches to thermal treatment and the reduction of benzoate in food are some primary goals of food technology. This research proposes a critical evaluation of two possibilities by highlighting their limits and benefits.

T8-05 *Alicyclobacillus acidoterrestris* from Soil and Pear Juice: Do Some Strains Move from Soil to Other Environments?

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Introduction: *Alicyclobacillus acidoterrestris* includes thermo-acidophilic and spore-forming bacteria able to spoil fruit juices and acidic drinks. Soil has been referred as the primary source for juice contamination.

Purpose: The main topic of this research was to address the similarity/dissimilarity between soil and juice strains and pinpoint a “quasi-species,” i.e., an intermediate between different evolutionary states.

Methods: 23 strains from soil and two strains from spoiled pear juice (CB-1 and CB-2) were characterized at biochemical (growth range for pH, temperature, resistance to salt, effect of anaerobiosis, amino acid and sugar utilization, enzymatic patterns) and molecular levels (16S rDNA sequencing and RAPD).

Results: Data of soil-borne strains pinpointed that they could be divided into three blocks, represented

by soil strains and by strains moving from soil to other niches. In this context, phenotyping and genotyping did not group the strains in the same way and many strains phylogenetically different showed the same phenotypic trend, thus suggesting that *A. acidoterrestris* could exist as a genomovar. In addition, the strain CB-1 was distant from other alicyclobacilli, although it possessed the same traits as the other isolates from juice (CB-2); therefore, it is probably a fast-clock organism or the in beginning of an alternative pathway in alicyclobacilli evolution.

Significance: The characterization of *A. acidoterrestris* represents the first step to design an efficient approach to inhibit and/or control spores in juices. The results from this research showed that the classical ideas of genus and species, as well the international cut-off point for 16S rDNA (95 and 98%), could not satisfactorily describe *A. acidoterrestris*.

T8-06* Understanding the Fate of Bacterial Transference in a Simulated Wash Process of Fresh-Cut Lettuce

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Introduction: Inadequate quality of water used in the washing of fresh-cut produce has the potential to be a direct source of contamination and a vehicle for spreading bacteria.

Purpose: Understand the uptake of bacterial cross-contamination during the washing step of fresh-cut lettuce by evaluating the accumulation of microorganisms in the water and their transfer from the water to the fresh-cut produce and, vice versa.

Methods: Twenty-five g of fresh-cut iceberg lettuce were washed in 1 L of tap water under stirring at 260 rpm for 5 min at room temperature, and repeated twice within 2 subsequent days with reused water. Bacterial concentration was quantified in water and in the produce before and after the washing treatment. *Salmonella* Enteritidis was also inoculated in water. Uncut lettuce was used to evaluate internalization in tissues. The influence of temperature, pesticides (20 ppb) and NaOCl (300 ppm), time (5-30 min) and speed of stirring (150-300 rpm) were evaluated.

Results: Similar bacterial concentration (10^5 CFU/g) is found in both types of lettuce. However, bacterial concentration in fresh-cut lettuce notably increases in subsequent days (10^7 CFU/g). Total coliforms are transferred from the lettuce to the water ($5 \cdot 10^2$ CFU/mL). *Salmonella* remaining in wash water reaches different batches of produce (from 10^5 up to 10^1 CFU/g depending on its concentration in water). No growth of bacteria in water occurs between cycles when using water at 4 and 25°C but *Salmonella* remains in water at 4°C. NaOCl completely removes bacterial concentration in water but they still remain in the produce after washing.

Significance: Development of a deeper knowledge of the washing process is required to design an adequate water treatment to provide a barrier to cross-contamination, together with the minimization in water and energy consumption of the process, reducing costs for the vegetable processing industry.

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Poster Abstracts



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P1-01 Antifungal Activity of Various Bacterial Species against *Botrytis cinerea*, *Fusarium pallidoroeseum*, and *Fusarium moniliforme*

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Introduction: *Botrytis cinerea*, *Fusarium pallidoroeseum* and *Fusarium moniliforme* are fungal plant pathogens that can cause significant produce loss, both pre- and post-harvest. Typical fungal control requires the application of chemical fungicides that may negatively impact the environment and human health. Alternatively, the use of biological control agents has shown potential for fungal control; however, commercial use is limited. Due to development of fungicide resistance in pathogen populations and the demand for more environmentally sustainable solutions, further research on the identification and development of biocontrol methods that could extend produce shelf life is merited.

Purpose: The purpose of this study was to screen 22 bacterial isolates for antifungal activity against *Botrytis cinerea*, *Fusarium pallidoroeseum*, and *Fusarium moniliforme*.

Methods: Bacterial isolates were individually spot-inoculated onto Tryptic Soy Agar, Potato Dextrose Agar, or *Lactobacillus* MRS agar, depending on isolate growth requirements, and a 9 mm plug of fungal-colonized agar was placed onto the center of the isolate-inoculated plate. Plates were incubated at 24°C for 10 days. Fungal growth was evaluated daily, beginning on Day 3, by measuring the diameter of the fungal colony.

Results: Nine of the 22 isolates, including *Bacillus megaterium*, *Bacillus coagulans*, *Bacillus amyloliquefaciens*, and *Serratia plymuthica*, inhibited all three fungi; fungal inhibition ranged from 51–62% for *B. cinerea*, 60–68% for *F. pallidoroeseum*, and 40–61% for *F. moniliforme*. Three Lactic Acid Bacteria species—*Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*—and three *Bacillus* species—*Bacillus thiaminolyticus*, *Bacillus firmus*, and *Bacillus clausii*—inhibited *B. cinerea* only by 30–56%. Seven isolates showed no suppression of any of the three fungi.

Significance: This study identified nine bacterial isolates capable of suppressing the growth of *B. cinerea*, *F. pallidoroeseum*, and *F. moniliforme* *in vitro*. Evaluation of antifungal efficacy on produce (in planta) is required to determine an isolate's potential use as a biocontrol agent.

P1-02 Quantification of Nisin A Gene Expression in Graviera Cheese during Real Cheese Manufacturing Conditions

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Introduction: Nisin-producing (Nis+) lactic acid bacteria (LAB) have high potential for use as starter and/or protective adjunct cultures in order to improve the safety of fermented foods. However, the expression of nisin genes in traditional Greek cheeses by using Nis+ LAB as starter and/or protective adjunct cultures remains unknown.

Purpose: This study evaluated the *in situ* expression of the nisin A gene (*NisA*) of the novel NisA+ *Lactococcus lactis* subsp. *cremoris* M78 strain during Graviera cheese processing.

Methods: First, survival and growth kinetics of a mixed LAB commercial starter culture (CSC) against strain M78 were evaluated in synthetic media and milk. The inhibitory activity of strain M78 was evaluated individually against each CSC strain assigned to *Streptococcus thermophilus*, *Lactobacillus helveticus* and *Lc. lactis* subspecies *lactis* and *cremoris*. Then Graviera cheeses were manufactured without (CSC) or with strain M78 (CSC+M78) added to thermized ewes' milk under commercial conditions. The cheeses were sampled on the day of manufacture (day-0) and after 24 h (day-1). Total RNA was directly extracted from cheeses and the expression of *NisA* was evaluated by real-time reverse transcription-PCR.

Results: Growth kinetics of each CSC strain in co-culture with strain M78 showed that while lactococcal and thermophilic lactobacillic strains were inhibited strongly, streptococcal strains were inhibited weakly by nisin A *in vitro*. Importantly, *NisA* expression was detected in CSC+M78 cheese only, with its expression levels being 0.11- or 1.62 times higher in the curd (day-0) or 24-h cheese (day 1) compared to those of the reference gene, respectively.

Significance: This is the first report on quantitative expression of the *NisA* gene in Graviera cheese during real cheese manufacturing conditions using a wild, rarely isolated NisA+ *Lc. lactis* subsp. *cremoris* genotype as a bioprotective co-starter culture.

P1-03 Control of *Listeria monocytogenes* in Heat-treated Milks by *Enterococcus faecium* KE82, a Multiple Enterocin-producing and Potential Probiotic Strain

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Introduction: Enterococci comprise a major part of the natural microbiota of traditional cheeses. Enterocins may contribute to minimize survival or growth of *Listeria monocytogenes* in milk and cheese.

Purpose: *Enterococcus faecium* KE82, a multiple enterocin-producing strain isolated from traditional Greek Graviera cheese was assessed for antilisterial activity in milk, and possession of other desirable technological and probiotic properties.

Methods: Sterile milk (SM)-used as a model system, or thermized (63°C, 15 sec) milk (TM) were inoculated with 3 to 4 log CFU/ml of *L. monocytogenes*. Contaminated milk portions (50 ml) were further inoculated with ca. 6 log CFU/ml of a commercial starter culture (CSC) and/or strain KE82. All milks were incubated at 37°C for 6 h and then at 18°C for an additional 42 h.

Results: *Listeria monocytogenes* increased by 5 log units in SM, whereas in SM+CSC it increased by 2 log units within the first 12 h, but its further growth was minimized. In SM+CSC+KE82, a complete growth inhibition followed by progressive inactivation of *L. monocytogenes* (0.6 log CFU/ml at 48 h) populations was noted. *L. monocytogenes* increased by 2 log units in TM, whereas in TM+CSC and TM+KE82 its growth was retarded within 6 to 12 h, and then ceased. In TM+CSC+KE82 the pathogen could not grow. When detected by PCR the enterocin genes A, B, and P gave a stronger signal in TM+KE82 than in TM samples, but weaker in TM+CSC+KE82 samples. Strain KE82 showed high survival under various challenging conditions simulating those of the gastrointestinal tract.

Significance: Thus, *E. faecium* KE82 constitutes a promising adjunct culture in traditional cheese making since it concomitantly produces enterocins A, B and P *in situ* in milk, and exhibits a high probiotic potential.

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P1-04 Anti-listerial Activity of *Enterococcus hirae* ST57ACC in Reconstituted Skim Milk

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Introduction: Bacteriocinogenic strains present antimicrobial activity, which may be useful for industry to control pathogenic and spoilage microorganisms in food. The research of new strains with these properties present new opportunities for food biopreservation.

Purpose: This study aimed to evaluate the ability of bacteriocinogenic isolates obtained from raw milk cheese to control *Listeria monocytogenes* co-cultures in skim milk.

Methods: Overnight cultures of bacteriocinogenic *Enterococcus hirae* ST57ACC was inoculated at concentration of 2% in flasks containing 10% of reconstituted skim milk. An overnight culture of *L. monocytogenes* 422 was also inoculated in the flask, at concentration of 0.1% to mimic conditions normally expected in food contamination scenario. *L. monocytogenes* 422 was also inoculated as a single culture and in the same concentration in skim milk as control. The flasks were incubated at 37°C for 48 h and at 3 h intervals aliquots were taken to determine changes in cell numbers of *L. monocytogenes* 422 and to monitor bacteriocin production, by using agar spot method.

Results: *E. hirae* ST57ACC was capable of reducing cell numbers of *L. monocytogenes* 422 from 4 log CFU/ml (time 0) to undetectable levels after 48 h. Production of bacteriocin was observed during all periods evaluated, reaching a stable level of production after 12 hours (3,200 AU/mL), remaining until the end of the experiment. In the control flask, *Listeria monocytogenes* 422 counts varied from 5 log CFU/mL (time 0 h) to 8 log UCF/mL (time 48 h).

Significance: The obtained results indicated that *E. hirae* ST57ACC was able to eliminate *L. monocytogenes* in milk after 48 h of inoculation, demonstrating the potential for biocontrol of this pathogen in milk.

P1-05 Safety Aspects of Bacteriocinogenic *Pediococcus acidilactici* ET34 Strain Isolated from Smoked Salmon

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Introduction: *Pediococcus acidilactici* is a wide spread bacterium in fermented food products and its potential in producing bacteriocins represents an important opportunity for exploration as tools for food biopreservation. Safety assessment for presence of virulence and antibiotic resistance genes in lactic acid bacteria (LAB) is an important task to be evaluated in order to select these strains as beneficial commercial cultures.

Purpose: The aim of this study was to explore safety aspects of bacteriocinogenic *P. acidilactici* ET34 based on presence and expression of genes related to the virulence factors, production of biogenic amines and antibiotic resistance.

Methods: *P. acidilactici* ET34 were isolated from smoked salmon, identified based on their biochemical and genetic characteristics including PCR with species-specific primers, and characterized as bacteriocin producers against some food spoilage microorganisms and foodborne pathogens. The strain was subjected to molecular and phenotypical tests to assess the presence of more than 50 genes related to virulence factors, production of biogenic amines and antibiotic resistance.

Results: *P. acidilactici* ET34 produced class IIa bacteriocin with 3.5 kDa, with bactericidal activity against *Staphylococcus* spp., *Enterococcus* spp. and *Listeria* spp., including *L. monocytogenes* from various serological groups. *P. acidilactici* ET34 presented low virulence profile, indicated by the presence of few genes related to antibiotic resistance and surface proteins, based on genetic and physiological tests.

Significance: Besides the beneficial properties studied for various LAB, most considered as safe, special attention needs to be paid to the possible presence of virulence factors, production of biogenic amines and antibiotic resistance. Horizontal gene transfer of virulence factors between pathogenic and LAB, including probiotics, is a highly possible scenario in the case of uncontrolled application of probiotics or starter cultures.

P1-06 Studying the Individual and Combined pH, NaCl and Propolis Limits for Growth of *Penicillium expansum*

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Introduction: Although industrial standards have been greatly improved in the last years, food spoilage by fungi is still a major concern for the food industry. The development of visible mycelia on food products before the end of their shelf life is one of the most significant quality problems for food products with low pH and water activity values. Since contamination of such products with fungal spores in many cases cannot be avoided, antimicrobial agents are employed. Nevertheless, due to the consumers' demands for minimally processed and healthier food products, it is essential to study and introduce the natural substances as antimicrobial agents. One promising substance for this purpose can be considered propolis, a resinous substance, collected by honeybees.

Purpose: Given the above, in this study the effect of propolis, pH and NaCl on the formation of visible mycelia by *Penicillium expansum*, previously identified as a spoiler of dairy products, was assessed.

Methods: Mycelium formation was evaluated in tryptone soy broth (TSB) at 25°C and at different combinations of propolis (0 – 300 µl/ml TSB), pH (2.0 – 4.75) and NaCl concentrations (0% – 18% w/v). In total, 625 visible mycelium formation tests in 125 propolis, pH and NaCl combinations were carried out in polystyrene microtiter plates.

Results: A probabilistic model predicting the *P. expansum* mycelium formation boundaries was developed.

Significance: The information provided from the developed model can be used as a useful tool in order to control the growth of moulds in foods with specific characteristics (pH, a_w) with the use of a natural substances like propolis. Further research objectives of great value for the control of moulds in foods, especially with the use of propolis, include the incorporation of this natural compound in biobased polymer packaging materials, such as edible films or coatings.

P1-07* The Effect of Oregano Essential Oil Encapsulated in Liposomes and High-pressure Processing on the Survival and Heat Tolerance of *Escherichia coli* O157:H7 in Ground Beef

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Introduction: Mild preservation technologies and natural antimicrobials become increasingly popular in food safety, especially in the context of multiple hurdles.

Purpose: To investigate the impact of oregano essential oil (OEO), free or encapsulated in liposomes, and High Pressure Processing (HPP) on: (i) the survival of *Escherichia coli* O157:H7 during refrigerated storage of ground beef patties, (ii) the heat tolerance of the pathogen and (iii) the organoleptic characteristics of the patties.

Methods: Ground beef patties were formulated with (a) no antimicrobials, (b) 0.5% v/w OEO, (c) 0.5% v/w OEO encapsulated in liposomes (bilayer capsules). All samples were inoculated (10⁵ CFU/g) with *E. coli* O157:H7, vacuum packaged, received or not HP treatment at 250 and 400 MPa and stored at 5°C for 30 days. On day 0, 15 and 30, samples were cooked in a preheated oven to internal temperature of 65°C.

Results: Treatments reduced *E. coli* O157:H7 from minimum 1 log CFU/g (OEO only) to below the enumeration limit of 10 CFU/g (OEO and HPP) during refrigerated storage. OEO addition caused an initial reduction of 1 log CFU/g and additional decrease by 2.5 log CFU/g till the end of storage. OEO-loaded liposomes showed no remarkable reduction until the first 15 days; however inactivation by 1.5–2.0 log CFU/g was observed on day 30. HP treatment (400 MPa) combined with free OEO showed the highest synergistic effect, although affected the redness of ground meat negatively. OEO alone or in combination with HPP increased susceptibility of *E. coli* O157:H7 to heat resulting in additional reductions of 2.5 log CFU/g, compared to heated control (without OEO and HPP) samples.

Significance: Combination of EOs with HP treatment may be promising interventions for ground beef safety. Liposomes, as encapsulation systems, confer controlled release of the EOs while markedly improving the sensory properties (juiciness) of ground beef.

P1-08 Efficacy of the Combined Application of High Pressure Processing and Oregano Essential Oil-based Antimicrobial Edible Films for the Control of *Listeria monocytogenes* on Ham Slices

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Introduction: The emergence of *Listeria monocytogenes* in ready-to-eat meat products is constantly referred to as a result of post-process contamination. Consumers' demand for minimally processed foods has prompted industries to apply alternative or innovative technologies such as active packaging and High Pressure Processing to contribute to the products' safety.

Purpose: The objective of the present study was to evaluate the efficacy of Na-alginate films containing 1% oregano essential oil to inhibit *Listeria monocytogenes* growth in ham slices with or without High Pressure Processing.

Methods: Ham slices inoculated with 4 log CFU/g of *Listeria monocytogenes* (3 strains) were packaged anaerobically in all cases (with or without HPP at 500 MPa for 2 minutes, in presence of alginate film with or without 1% oregano essential oil) and stored at 3 temperatures, 4, 8 and 12°C. Samples were microbiologically tested and pH measurements were taken.

Results: In ham slices without HPP, *Listeria monocytogenes* counts in control samples (without edible film) were not different than in samples with edible film containing no essential oil, while in the presence of 1% oregano essential oil 1–2 log CFU/g reduction was caused at all temperatures. With the use of HPP, 1 log CFU/g reduction in pathogen's population levels was observed, but at the end of storage counts were below detection limit in cases without essential oil. HPP with 1% oregano essential oil in films caused no pathogen detection from the middle of storage period or earlier depended on temperature.

Significance: The results of this study are promising for controlling pathogens with innovative methods, which can be applied by food industries, to assure food safety and protect public health.

P1-09 Assessment of Antimicrobial Activity of *Satureja thymbra* Essential Oils and Extracts against Food Spoilage and Pathogenic Species

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Introduction: Nowadays, there has been an increased interest in essential oils from various plant origins as potential antimicrobial agents due to the rising number and severity of food poisoning outbreaks worldwide along with the recent negative consumer perception against artificial food additives.

Purpose: The aim of the present study was to investigate the antimicrobial properties of *Satureja thymbra* essential oil (EO), as well as water (W), ethanol (E) and ethyl acetate (EAc) extracts and assess their commercial potential in the food industry.

Methods: EO and the extracts were analyzed by GC/MS and LC/MS-DAD, respectively. The antimicrobial properties were evaluated by the disk diffusion assay and the minimum inhibitory and non-inhibitory concentration values were determined using a sophisticated model.

Results: The main constituents identified in EO were γ -terpinene (40.1%), carvacrol (30.8%), p-cymene (9.3%) and trans-caryophyllene (7.6%), whereas the extracts were rich in phenolic compounds. EO was effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Pseudomonas fragi*, *Saccharomyces cerevisiae* and *Aspergillus niger*. In contrast, E and EAc extracts were active only against the bacteria species, but not against *S. cerevisiae* and *A. niger*, while no antimicrobial activity was observed for W extract. The antilisterial efficiency of E and EAc extracts was further evaluated in oil-in-water emulsions deliberately spiked with *L. monocytogenes*, which served as food model system used as the basis for sauces, dressings, etc. Noticeably, the results showed a significant reduction of the pathogen viable counts during storage at 4°C.

Significance: Overall, the data indicated that *S. thymbra* EO, E and EAc extracts are noteworthy growth inhibitors with industrial potential in food technology.

P1-10 In Vivo Screening of the Effect of 3 Essential Oils against Ochratoxin A by *Aspergillus carbonarius* Isolates in Grapes

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Introduction: *Aspergillus carbonarius* has been defined as one of the most important pathogens in grapes since it has the higher incident of ochratoxin-producing isolates within the *Aspergillus* spp.

Ochratoxin A (OTA) is a fungal secondary metabolite which is considered to be a prevalent mycotoxin.

Purpose: In this work, three different essential oils were evaluated for their anti-ochratoxigenic effect against two toxigenic *A. carbonarius* isolates that originated from Greek vineyards.

Methods: Grapes were inoculated with 10³ spores and treated with 200 ppm EOs of *Eugenia caryophyllus* (clove), *Cinnanomum cassia* (cinnamon) and *Cymbopogon flexuosus* (lemongrass) in a contact assay and a volatile assay. Grapes were incubated at 25°C and removed for OTA determination by HPLC after 5 days.

Results: The analysis led to diverse differences within the different EOs but also between the two isolates. In contact assay, similar results were observed for both isolates, even though EOs efficacy differed by approximately 40% of toxin reduction by clove, 73% by cinnamon and 58% by lemongrass. On the contrary, volatile assay revealed an isolate dependent effect. Clove oil reduced OTA production by 44% and 90% and cinnamon by 41% and 98%, in Ac29 and Ac28, respectively. Lemongrass had a strong effect in both isolates amounting to 92% and 98%. In conclusion, all EOs used in this study had a great influence on toxin production. However, in comparison of the two EOs treatments, volatiles showed the highest impact on *A. carbonarius* growth and eventually on OTA production.

Significance: The above findings suggest that natural compounds such as essential oils could be a promising approach to control mycotoxin production against chemical protection strategies.

P1-11 Efficacy of Multi-spectral Imaging and FTIR Spectroscopy as Methods for Detection of Frozen-then-Thawed Minced Beef

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Introduction: In recent years, sensors for hyper/multi-spectral image and spectral acquisition have been used for the assessment of meat quality in terms of safety, spoilage and fraud detection. A common fraudulent practice is frozen-then-thawed meat sold as fresh.

Purpose: The aim of this work was to develop a rapid, cost-effective procedure for the detection of frozen-then-thawed minced beef using multispectral imaging and FTIR spectroscopy either separately or in parallel.

Methods: In this study, freshly-ground beef was purchased and divided in ~75g-portions on seven separate occasions. Fifteen samples per case were placed in Petri dishes, multispectral images of the first five were immediately acquired and then, ~3g-portions were used for FTIR measurements. The remaining samples were frozen (-20°C) and stored for 7 and 32 days (5 samples/case). Samples were thawed for 4h at 4°C and subsequently subjected to similar data acquisition. Data analysis methodologies, i.e., Partial Least-Squares Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM), were utilized for model building. While five meat batches were used for calibration and internal validation, models were further validated with independent data from the last two batches to avoid over-optimistic results. In total, 105 multispectral images and FTIR spectra were collected, along with microbiological measurements per batch as background information.

Results: Results showed a clear separation of fresh vs. frozen samples, as all samples were classified correctly during calibration and validation in the case of SVM. However, it was more difficult to separate frozen samples depending on storage time.

Significance: In conclusion, this study proves that sensor data could be used in a rapid quality control/assurance system for the detection of frozen-then-thawed minced beef. While some studies have explored the use of sensor data previously, this study not only uses two different sensors, but also utilizes a validation scheme that proves its effectiveness when applied to independent data.

P1-12 Effect of Rocket Extract on MRSA Proteome: Metabolic Adjustments in Plant-based Media and Defense Mechanisms against Plant-derived Antibacterials

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Introduction: The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in food has provoked a great concern about the presence of MRSA in associated foodstuff, nowadays. Although MRSA is often detected in various retail meat products, it seems that food handlers are more strongly associated with this type of food contamination. Taking this information into account, any food could be contaminated with this pathogen in an industrial environment or in household and cause food poisoning.

Purpose: This study aims to examine the effect of rocket extract on MRSA growth and proteome.

Methods: A comparative study of the MRSA strain COL proteome, cultivated in rocket extract versus laboratory medium (LB) was designed. The differential expressed proteins in the rocket extract were monitored using 2-DE analysis and bioinformatic tools

Results: It was shown that MRSA growth was delayed in rocket extract compared to LB medium. In addition, proteome analysis using 2-DE method showed that MRSA strain COL is taking advantage of the sugar-, lipid- and vitamin-rich substrate in the liquid rocket extract, while defending against the antimicrobial agents found in the plant extract (flavonoids, terpenoids or oxidative agents).

Significance: This work could initiate further research about bacterial metabolism in plant-based media and defense mechanisms against plant-derived antibacterials.

P1-13 Biofilm Formation by *Salmonella enterica* on Abiotic Substrata in the Presence of Cell-free Culture Supernatant of *Hafnia alvei* Containing or Not Ahl Signal Molecules

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Introduction: The presence of food spoilage microorganisms capable of producing cell-to-cell communication signal molecules is possible to influence the attachment and biofilm-forming ability of pathogens on food-contact surfaces. Particularly, acylated homoserine lactone (AHL) signal molecules produced by many Gram-negative bacteria may play an important role on these processes.

Purpose: Evaluation of the effect of AHLs produced by *Hafnia alvei* on the ability of *Salmonella* Typhimurium to form biofilm on two abiotic substrata.

Methods: *S. Typhimurium* was left to form biofilm on stainless steel (SS) and polystyrene (PS) surfaces for 12, 24 and 48 h in either the absence, or presence (50% v/v) of cell-free culture supernatant (CFCS) of *H. alvei*. To do this, the growth media used to support biofilm development were based on tryptic soy broth (TSB), which also contained CFCS of either the AHL-producing *Hafnia alvei* 718 or the AHL-lacking isogenic mutant. Biofilm formation on SS was evaluated by cell detachment and colony enumeration, while the crystal violet binding assay was used to do the same for the PS surfaces.

Results: Biofilm formation increased as incubation time increased, regardless of the growth medium composition and the surface. The presence of CFCS containing AHLs seemed to reduce the quantity of strongly attached/biofilm cells recovered from SS after 12 h of incubation, compared to pure TSB or TSB containing CFCS of the mutant strain. No such inhibitory effect was observed on the PS surface following the same incubation period. For both surfaces, no further differences on biofilm formed under the different treatments were observed following 24 and 48 h of incubation.

Significance: The present study provides some data on the influence of cell-to-cell communication on biofilm formation by an important foodborne pathogenic bacterium.

P1-14 Detection of Dapsone in Muscle by Ultra-high-Performance Liquid Chromatography–tandem Mass Spectrometry

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Introduction: Dapsone is a sulphonamide with bacteriostatic and bactericidal activity. It is used as an antibiotic and to prevent diseases. The administration of dapsone in livestock is prohibited in the European Union in accordance to the Directive 90/2377/EC so it is necessary to check the illegal use of this substance according to the Decision no. 2002/657/EC.

Purpose: A rapid screening method for the analysis of dapsone by U-HPLC-MS/MS is described and it is able to reveal the dapsone recommended concentration of 5 µg kg⁻¹.

Methods: Samples are extracted with 0.1 M EDTA and acetonitrile, which is then evaporated under a stream of nitrogen and reconstituted in 0.1% formic acid in deionized water. An aliquot is analysed by U-HPLC-MS/MS using positive electrospray ionisation and multiple reaction monitoring. Mobile phases used are 0.1% formic acid in water and 0.1% formic acid in methanol. Validation is according to Commission Decision 2002/657/EC and was carried out for bovine and porcine species.

Results: Sulphadimetoxin-d6 is used as internal standard. Molecular weight of Dapsone is 249 kDa. The product ions are 156 (qualifier) with 14 of collision energy and 92 (quantifier) with 24 of collision energy. The specificity of the method was tested analyzing twenty blank samples. A result of $r^2 > 0.99$ indicated a good linearity in the concentration range studied (2.50–050 µg/L). Trueness and precision were evaluated analyzing recovery on fortified blank samples spiked with dapsone.

Significance: A rapid method was developed for the survey of dapsone. It can be used on bovine and porcine tissue samples using liquid chromatography tandem mass spectrometry. This method is in accordance with Commission Decision 2002/657/EC.

P1-15 Evaluation of Commercial Serological ELISA Kits for the Detection of *Toxoplasma gondii* Antibodies in Meat Juice of Pig

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Introduction: *Toxoplasma gondii* is considered as one of the most important biological hazards in the context of meat inspection of pig according to the European Food Safety Authority (EFSA). Meat juice serology at slaughter can be used as a tool for targeting measures to control *Toxoplasma gondii* in pork.

Purpose: The purpose of the study was to evaluate four different commercial enzyme-linked immunosorbent assays (ELISA) for detection of antibodies against *Toxoplasma gondii* in meat juice samples from 90 naturally exposed slaughter pigs.

Methods: Meat samples (about 10 g of muscle from the diaphragm) from 90 slaughter pigs were collected. Samples were analyzed for *Toxoplasma gondii* antibodies using modified agglutination test (MAT) and four commercial ELISA kits. The results from the meat juice samples tested with the ELISA kits were evaluated against the results obtained with the MAT, which was considered as a reference method.

Results: Differences in the sensitivity (Sn), specificity (Sp) and accuracy (Ac) between the ELISA kits were detected and results were dependent on the cut-off level used: ELISA I with cut-off value 0.15: Sn 93.4 %, Sp 74.7 %, Ac 80.7 %; ELISA I with cut-off value 0.2: Sn 92.4 %, Sp 86.3 %, Ac 88.2 %; ELISA II: Sn 86.4 %, Sp 99.1 %, Ac 94.4 %; ELISA III: Sn 71.2 %, Sp 93.7 %, Ac 85.3 %; ELISA IV: Sn 3.6 %, Sp 100 %, Ac 70%.

Significance: The differences between ELISA I, II and III are mainly resulting from different cut-off values provided by the manufacturers, otherwise they seem to detect *Toxoplasma gondii* antibodies in meat juice samples from naturally infected finishing pigs comparably. The sensitivity of the ELISA IV was poor and further testing on meat juice from naturally infected animals is required.

P1-16 Development of an Asymmetric Five-primer Loop-mediated Isothermal Amplification for the Detection of *Sarcocystis* spp. in Humans

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Introduction: *Sarcocystis* comprises *Apicomplexan* species with a two-host life cycle and humans may be both intermediate and definitive hosts for some of these species. *Sarcocystis* undergoes sexual reproduction in the intestinal epithelium and oocysts are subsequently shed in host stool in definitive hosts.

At least two species, namely, *Sarcocystis hominis* and *Sarcocystis suibominis*, exist with humans as definitive hosts and hence as potential causes of human intestinal sarcocystosis, with cattle and pigs as intermediate hosts, respectively. Notwithstanding the potential for intestinal sarcocystosis to afflict a large number of people, extent and importance of human sarcocystosis remain largely unknown.

Purpose: The objective of this study has been the development of a new molecular diagnostic tool (LAMP method) to facilitate the detection of the parasites in stool samples and the understanding of the epidemiology and clinical significance of *Sarcocystis* infections in humans.

Methods: A set of five primers were designed according to the conserved region of the 18S rRNA gene of *S. hominis* and *S. suibominis* by using the software PrimerExplorer V4. Alignment of *Cystoisospora belli* and *Cryptosporidium hominis* were used to ensure the specificity of the assay. The LAMP products were monitored using 2% agarose gel electrophoresis and also visually detected by adding 2 µL of the amplification product to 1:1000 diluted fluorescent dye EuroSafe to the reaction tubes, visualized under UV light. Samples from human stools (n=10), positive to *S. hominis*, and pig muscular tissue harbouring *S. suibominis* (n = 5), were used as positive samples. Stool samples that tested negative with the conventional PCR for *Sarcocystis* spp. (n = 20) were included as negative samples.

Results: The new LAMP protocol confirmed the results of the conventional PCR for *Sarcocystis* spp., demonstrating, at least, the same sensitivity and specificity.

Significance: The new assay is therefore a rapid, simple and specific method for the detection of zoonotic *Sarcocystis* in humans.

P1-17 Optimization of Microbiological Recovery from Surfaces for Environmental Monitoring

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Introduction: The FLOQSwab is a specimen collection device recognized worldwide for its superior performance in the clinical diagnostics. The aim of this work was to evaluate the FLOQSwab for the recovery of microbiological samples from surfaces compared to the traditional swab (rayon tipped swab) as per ISO 18593:2004 standard.

Purpose: The FLOQSwab, thanks to the innovative manufacturing technology, allows improvements to the efficiency of recovery and release of analyte. The study has been divided into 2 experiments.

Methods: In the first experiment the two swabs were evaluated for their capacity to recover and release the analyte (three different bacterial loads of *E. coli*). In the second experiment, the two swabs were evaluated for their capacity to recover three different bacterial loads of *E. coli* from two different surface materials (stainless steel and polypropylene).

Results: In all experiments the flocked swab demonstrated a higher recovery rate compared to the traditional rayon tipped swab.

Significance: The data obtained from this preliminary study demonstrated that the FLOQSwab can be a good food surfaces collection device that improves the recovery of the analyte and thus produce accurate results. Based on the outcomes of the study, a larger field study is in progress using the FLOQSwab for sample collection to improve both environmental monitoring and the efficacy of the hygiene controls for food safety.

P1-18 Growth Potential of *Listeria monocytogenes* in Feta Sauce Packed under Aerobic Conditions and Stored at 4°C

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Introduction: According to Commission Regulation (EC) No 2073/2005, food business operators (FBOs) that produce ready-to-eat (RTE) foods shall conduct studies, if necessary, so as to demonstrate whether their food products are able to support the growth of *Listeria monocytogenes*.

Purpose: The objective of the present study was to evaluate *L. monocytogenes* growth in cheese-based (Feta) sauce, by conducting a challenge test for the assessment of growth potential for the pathogen.

Methods: Samples of Feta sauce (ca. 1% NaCl, 900 ppm sorbic acid), were inoculated with a two-strain mixture of *L. monocytogenes* (ATCC 19115, wild type strain) and stored in the air at 4°C for 30 days. Uninoculated samples served as controls and were used for enumeration of aerobic plate count (APC), lactic acid bacteria (LAB) and yeasts and moulds (Y/M) at the beginning of storage (day 0) and at the end of shelf-life (day 30). Sampling units (10 g) were analyzed in triplicate for pH and *L. monocytogenes* enumeration at days 0, 3, 5, 10, 15, 20, 25, 30, 35 and 40. The growth potential (i.e., difference between the log₁₀CFU/g at days 0 and 30) was evaluated. Two batches of the product were tested.

Results: There was no natural *L. monocytogenes* contamination in Feta sauce. Average initial pH was 4.6 and APC, LAB and Y/M populations (in log₁₀ CFU/g) were 4.6, 4.5 and 2.6, respectively; pH dropped to 4.0 at day 30, while APC, LAB and Y/M were 5.8, 6.6 and 3.5 log₁₀ CFU/g, respectively. Starting from average concentrations of 2.3 and 2.2 log₁₀ CFU/g, *L. monocytogenes* count decreased to 1.1 log₁₀ CFU/g in both batches at the end of shelf life; maximum growth potential of *L. monocytogenes* in Feta sauce was, therefore, -1.2 log₁₀ CFU/g (pathogen

reduction). At days 35 and 40 pathogen counts were below the limit of detection (<0.5 log₁₀ CFU/g).

Significance: Findings of this study should be useful to regulatory authorities and FBOs manufacturing acidic cheese-based spreadable products, as they consider *L. monocytogenes* contamination and safety of RTE products.

P1-19 Microbiological Hygiene Indicators and *Campylobacter* Enumeration by Four Different Plating Procedures in Naturally Contaminated Chicken Meat Samples

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Introduction: The genus *Campylobacter* consists of significant thermophilic bacterial foodborne pathogens. The International Organization for Standardization (ISO) recommends the use of modified charcoal cefoperazone deoxycholate agar (mCCDA) for *Campylobacter* enumeration (ISO 10272-2:2006), nevertheless alternative plating methods encompassing the use of chromogenic media have to be considered for easy reading and improved observation of presumptive *Campylobacter* colonies.

Purpose: The present work aims to determine the distributions for the hygiene indicator microorganisms most commonly found in poultry meat, as well as to describe the performance of four culture media used for *Campylobacter* enumeration in chicken meat.

Methods: Samples of naturally contaminated raw chicken meat with skin ($n = 47$, ca. 100 g per sample) were obtained from broiler carcasses, which were purchased from meat retailers within the metropolitan area of Athens, Greece. Sampling units (10 g) were analyzed for *Campylobacter* spp. (acc. to ISO 10272-2) and for aerobic plate count (APC), *Enterobacteriaceae*, and *Escherichia coli* enumeration. Analysis for *Campylobacter* enumeration involved two chromogenic media, besides the charcoal-based selective agars mCCDA and Karmali Agar (KA), named, *Campylobacter* Selective Agar (CASA) and Brilliance *Campy*Count Agar (BCCA). *Campylobacter*s on the latter media appear as dark “brick” red colonies.

Results: Plating for *Campylobacter* produced countable results (>0.92 log₁₀ CFU/g), at least in one medium, in all samples tested. *Campylobacter* spp. was enumerated onto mCCDA, KA, CASA and BCCA in 91.5% (43/47), 87.2% (41/47), 95.7% (45/47) and 93.6% (44/47) of total tested samples, respectively. Confirmed *Campylobacter* spp. counts (mean log₁₀ CFU/g ± SD) were 2.63 ± 0.76, 2.48 ± 0.82, 2.44 ± 0.86 and 2.65 ± 0.83 for mCCDA, KA, CASA and BCCA, respectively. The distributions (mean log₁₀ CFU/g ± SD) for the hygiene indicator organisms in chicken meat were 5.37 ± 0.92 (APC), 2.92 ± 0.87 (*Enterobacteriaceae*) and 2.16 ± 0.83 (*E. coli*).

Significance: The results demonstrate the microbiological hygiene condition of chicken meat and highlight the ability of the chromogenic media used in this study (CASA and BCCA) to perform equally ($P > 0.05$) when compared to mCCDA medium, recommended by ISO. Therefore, CASA and BCCA can be considered as suitable alternatives for easy *Campylobacter* enumeration in chicken meat.

P1-20 Quantification of Cow's Milk Percentage in Dairy Products with a Novel Lateral Flow Device

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Introduction: Higher priced milk is commonly and fraudulently adulterated with lower price cow milk used in direct human consumption or in cheese manufacture. In addition, cow's milk allergy is one of the most common food allergies. To date, the percentage of cow milk in other species' milk has been detected by lateral flow rapid tests and quantified by ELISA with total procedure time at least 10 and 90 minutes, respectively.

Purpose: The purpose of this work was to combine the quantification capability and the immunochromatography swiftness into a lateral flow device that detects bovine milk in other species' milk.

Methods: An indicative lateral flow strip consisted of a nitrocellulose membrane with a high affinity monoclonal primary antibody test line and an anti-species control line, a conjugate pad with a colloidal gold conjugated secondary monoclonal antibody and an absorbent pad. Monoclonal antibodies against bovine IgG produced after mice immunisation, preparation of hybridomas, clones screening and protein G purification. The colloidal gold nanoparticles (40nm) were produced by reduction of chloroauric acid and the conjugate with the secondary antibody was carried out by pH-modulated absorption. The milk sample was diluted five times with a running buffer before strip immersion and the percentage of bovine milk was determined by a novel quantification system using a high technology scanning device.

Results: This lateral flow strip requires one drop of milk and the procedure total time is 10 minutes. The combination of the high affinity antibody pair and the advanced quantification system contributed to high range standard curve. According to scanning device findings, the type of graph was close to ELISA method levels.

Significance: This lateral flow device constitutes a very valuable tool in the rapid quantification of cow milk in other species' milk by combining the fast protocol and the significance of the result.

P1-21 Optimization of Total Aflatoxin Recovery Levels in Overparticular Matrices Using a Twenty-minute ELISA by Dilution Normalization and Two Incubation Steps

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Introduction: There have been many problems with total aflatoxin recovery levels using most commercial competitive ELISAs for grains. Especially, corn germ, corn silage, cottonseed, ginger, paprika and walnuts require clean-up pre-columns because when extracted, overparticular matrices are formed. Extracts usually constitute one-third of the incubated mixture affecting negatively the HRP and competitive step. Although most immunoassays run better after pre-column treatment, unsatisfactory recovery levels are avoidable.

Purpose: The purpose of this work was an alternative way to improve the recovery levels of total aflatoxin in overparticular matrices avoiding the pre-column treatment.

Methods: Fifty grams of the above samples were extracted with 70% methanol and after filtration the total aflatoxin was determined by a direct competitive ELISA with or without pre-column treatment. Microplates were coated with a high affinity monoclonal antibody produced after mice immunisation, preparation of hybridomas, clones screening and protein G purification. The immunoassay had two incubation steps (in contrast with other tests), the first incubation of 20% extract and 80% HRP-free buffer mixture for 10 minutes followed by washing steps, the second incubation of aflatoxin-HRP for 5 minutes followed by washing steps and the addition of chromogen for 5 minutes followed by the addition acidic solution. The aflatoxin-HRP and the immunogens were produced by organic modification of hapten, active ester biochemical conjugation, optimization of conjugates molarity and gel filtration purification.

Results: The immunoassay requires only 20% of extract and the sensitivity remains in increased levels giving the maximum range of quantification. Due to increased dilution normalization of matrix and enzyme protection from methanol by using two incubation steps, the recovery levels were more acceptable without pre-column step.

Significance: This ELISA constitutes a very valuable tool in the total aflatoxin analysis that optimizes the recovery levels and is an effortless and rapid immunoassay without pre-column treatment.

P1-22* Association of Targeted Metagenomic Analysis and Classical Microbiology for *Clostridium difficile* Detection and Microbial Ecosystem Mapping of Surfaces, Hands and Foodstuffs in a Meat Processing Plant

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Introduction: Proper hygiene practices in meat processing plants are essential for prevention of foodborne disease outbreaks. Metagenetics is a culture independent based strategy allowing for the identification of bacterial populations present in a large panel of samples.

Purpose: This study aimed to evaluate the hygienic level of a meat processing plant and to identify possible sources of cross contamination. A microbiological detection scheme was performed along with an overall microbial biodiversity study of the samples by 16S metagenetic analysis. Detection of the pathogenic bacteria *Clostridium difficile* was also performed.

Methods: The production line of two Belgian meat products (pork tomato sauce and white pudding) was monitored. Samples from operator hands (n = 8), surfaces (n = 9) and products (n = 11) were collected at different points of the production. All samples were analysed by classical microbiology to determine the levels of total aerobic viable counts of *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus* and to detect the presence of *C. difficile*. Metagenetic analysis was targeted on the V1-V3 hyper-variable region of 16S rDNA. The taxonomical assignment of the populations was performed with Mothur and Blast algorithms.

Results: None of the samples were positive for *C. difficile*. Using international standards, all of the samples contained acceptable levels of the other bacteria studied. Metagenetic analysis revealed the presence of some taxa in the final products that were not detected in the intermediate products, including populations from the *Acinetobacter*, *Proteus* and *Staphylococcus* genera isolated in some environmental samples. A large proportion of sequences did not belong to known bacterial species.

Significance: Results indicated that *C. difficile* contamination of the meat products studied was unlikely. High-throughput sequencing reveals a cross contamination in the production line. Further studies are needed to improve taxonomic identification.

P1-23 Performance Assessment of the Thermo Scientific SureTect *Cronobacter* Real-time PCR Assay Kit According to the ISO 16140-2 Standard for *Cronobacter* spp. Detection in Infant Formula and Environmental Samples

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

Purpose: An independent study was conducted to validate this new method in comparison to the ISO/TS 22964 standard, as part of the NF Validation approval process and according to the ISO 16140-2 standard.

Methods: The alternative method includes single step enrichment in BPW for 16 h to 20 h at 37°C for infant formula, and in BPW supplemented with vancomycin for 18 h to 22 h at 37°C for environmental samples. After DNA extraction, PCR is run in the Thermo Scientific PikoReal Real-time instrument.

Results: 64 infant formula samples with and without probiotics, and 64 environmental samples, were analyzed in a relative trueness study. The relative detection levels were determined in infant formula, infant formula with probiotics and process water. The results demonstrate equivalent performance between the alternative method and the ISO/TS 22964 method. The inclusivity and the exclusivity of the alternative method were assessed with 50 target and 30 non target strains. The alternative method was also evaluated in a ring trial involving 11 laboratories. The results of the calculated acceptability limits clearly show that the alternative method precision is equivalent to the ISO ISO/TS 22964 standard.

Significance: The Thermo Scientific SureTect *Cronobacter* Real-time PCR Assay Kit is a reliable method for *Cronobacter* spp. detection in infant formula with and without probiotics, as well as environmental samples, and offers important economic savings by reducing time to result and handling time.

P1-24 A Preliminary Study for Alternative Methods for Staphylococcal Enterotoxins (SEs) Extraction and Concentration in Food Matrices

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Introduction: *Staphylococcus aureus* strains produce several enterotoxins (SEs) with demonstrated emetic activity, which are a major cause of food poisoning occurring after ingestion of contaminated foods. The concentrations of SEs

able to produce symptoms can be lower than the limit of detection (LOD) of methods used for SEs detection in food control laboratory. For this reason, a concentration step is usually performed, before the detection assay. To date, the dialysis concentration protocol is used in accredited laboratories for official controls on foods. The evaluation of different extraction/concentration methods in various food matrices will possibly reduce performing time and improve LOD of SEs.

Purpose: Our aim is to evaluate new protocols for extraction/concentration of SEs in order to increase the performance of detection step and to reduce the response-time of laboratory.

Methods: Three different proteins extraction and/or concentration methods were tested in parallel with the dialysis concentration method. The alternative protein precipitation protocols were: trichloroacetic acid (TCA), gel phosphate buffer (TGF), and Tris Glycine Beef Extract with polyethyleneglycol (TBGE/PEG). Each concentration protocol was used on milk and water samples contaminated with SEB from *Staphylococcus aureus* (SIGMA-ALDRICH). Milk samples were spiked at concentrations from 50 ng/ml to 1 ng/ml and water samples from 50 ng/ml to 0,1 ng/ml. After extraction/concentration, all samples were tested with RIDASCREEN SET Total ELISA kit.

Results: The TCA protein precipitation showed a lower efficiency compared with the other methods and was dismissed for further tests. Our results, based on the ELISA test, confirm that other investigated extraction/concentration methods are suitable for improving SEs detection step and can be the object of further investigation.

Significance: The dialysis protocol is to date recognized as the more suitable method for SEs concentration. Due to its long incubation period, efforts are required to evaluate new and less time consuming methods.

P1-25 Evaluation of a TaqMan Salmonella Triplex PCR Assay for *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* Compared to ISO 6579:2002 Method in Raw Poultry, Raw Pork and Environmental Samples

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Introduction: Thermo Scientific™ TaqMan™ Salmonella Triplex Assay with lysis is a PCR-based test for the simultaneous detection and differentiation of *Salmonella* species, *Salmonella* Enteritidis and *Salmonella* Typhimurium in poultry, pork and environmental samples. The TaqMan Salmonella Triplex Assay workflow includes a simple 15 minute sample lysis step followed by a 40 minute PCR run on Applied Biosystems™ 7500 Fast Real-time PCR instrument.

Purpose: The purpose of the study was to compare performance of the Salmonella Triplex Assay with a range of different raw poultry, raw pork and

environmental samples and compare the results to those obtained with the ISO 6579:2002 – Horizontal method for the detection of *Salmonella* spp.

Methods: An unpaired study was conducted by testing 24 samples of 25 g comprising eight different raw poultry, raw pork and environmental matrices. Samples were spiked in duplicate with <2 CFU of *Salmonella* Typhimurium or *Salmonella* Enteritidis. Unspiked samples were also tested to screen for natural contaminants of *Salmonella*. Samples were diluted 1-in-10 with BPW+12mg/l novobiocin or BPW and enriched for 16 hours at 37°C for the candidate method and reference method respectively. Analyzes were conducted according to the protocol described by the Salmonella Triplex PCR Assay with lysis and according to ISO 6579:2002.

Results: The Salmonella Triplex Assay yielded comparable results against the ISO 6579:2002 reference method in 22/31, 26/31 and 29/31 of tested samples for *Salmonella* spp., *S. Typhimurium* and *S. Enteritidis*, respectively. Similarly 4/31, 3/31 and 1/31 of samples yielded positive deviation and 5/31, 2/31 and 1/31 of samples yielded negative deviation when the Triplex Assay was compared to the ISO 6579:2002 method.

Significance: The study demonstrated that the TaqMan Salmonella Triplex Assay offers a rapid, easy-to-use and reliable workflow for the detection of *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* in raw poultry, raw pork and environmental samples.

P1-26 Evaluating Food Enrichment Methods Using the Illumina MiSeq

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Introduction: Culture-based methods, critical for identifying pathogens from contaminated foods, vary depending on the commodity type and target pathogen and can take up to one week to complete. This study surveyed enrichments throughout the culture process using 16S rRNA gene sequencing to analyze pathogen recovery and population dynamics, including species missed by traditional culture-based methods.

Purpose: This work uses 16S rRNA gene sequencing on the Illumina MiSeq to evaluate enrichment protocols for the detection of *Salmonella enterica* and *Listeria monocytogenes* from food.

Methods: Store-bought cilantro (n = 5) and mung bean sprouts (n = 4) were inoculated with *Salmonella* (2–5CFU/25g cilantro) and *Listeria* (50–100CFU/25g sprouts), aged, and cultured using modified FDA BAM protocols. Genomic DNA, taken from 24 and 48-hour cultures, was prepared for 16S rRNA gene amplification and sequenced in multiplex using the MiSeq platform. High-quality 16S rRNA sequences (normalized to 25,000 reads per sample) were analyzed using Resphera Insight software.

Results: In 24-hour cilantro cultures, we observed an average proportional abundance of 50% for *Enterobacteriaceae*, 0.2% (49 reads) being *Salmonella*. *Salmonella* increased to 37% (9,336 reads) and 93% (23,268 reads) after selective enrichment in Tetrathionate (TT) and Rappaport-Vassiliadis (RV) broths, respectively. Unlike *Salmonella*, the average proportional abundance of *Listeria* in 48-hour sprout cultures only reached 0.15% (38 reads). Other families present in 48-hour TT cilantro cultures included *Planococcaceae* and other *Enterobacteriaceae*, mainly *Lysinibacillus* (22%) and *Proteus* (15%) species, respectively. *Streptococcaceae* and *Enterobacteriaceae* families dominated sprout cultures with abundances of 80% (19,883 reads) and 9% (2,366 reads) at 24-hours, respectively, shifting to 52% (13,013 reads) and 28% (7,052 reads) at 48-hours.

Significance: High abundances of nonpathogenic species in cilantro and sprouts demonstrate the need for robust commodity-driven culture methods that favor pathogens such as *Salmonella* and *Listeria*. 16S rRNA gene sequencing can provide valuable information to improve detection methods for food safety.

P1-27 Improved Real-time PCR Detection of *Listeria* in Environmental Samples after Rapid Single Step Enrichment

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Introduction: Finished product testing for *Listeria monocytogenes* often provides limited information on the safety status of a food by neglecting recontamination risks during slicing and packaging steps and because in real world contamination events the individual portion prevalence rate in a production lot can be low. Implementation of an effective environmental monitoring program is becoming an increasingly relevant proactive food safety strategy for controlling *L. monocytogenes* in food processing facilities.

Purpose: The goal of this study was to evaluate a sensitive and rapid method for monitoring *L. monocytogenes* and *Listeria* genus in environmental samples using a rapid, single-stage enrichment in Actero *Listeria* Enrichment Media followed by processing with the BAX System real-time PCR assays for *Listeria* and *L. monocytogenes*.

Methods: Environmental samples were collected from food contact (stainless steel and plastic) and non-food contact (ceramic, sealed concrete and rubber) surfaces. Stressed overnight by desiccation, five different *Listeria* species (*L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri* and *L. welshimeri*) were used alone or together with high numbers competitive background flora (*E. faecalis* or *P. aeruginosa*) to artificially inoculate surfaces. Samples

were enriched in Actero *Listeria* Enrichment Media, then processed with the DuPont BAX System real-time PCR assays.

Results: A total of 930 environmental sponge samples were evaluated in two large-scale, unpaired validation studies in comparison with the USDA-FSIS and Health Canada reference methods, including 570 samples analysed by certified independent laboratories. Enrichment phase optimization studies allowed for reduction by up to 20 hours the time required to detect *Listeria*. The method comparison studies demonstrated no false positive results and a false negative rate of 0.3%. According to the Probability of Detection statistical model, the candidate method showed equivalent or superior performance to the reference methods.

Significance: Thus, a reliable and rapid alternative method has been developed and validated that can be successfully used as a monitoring tool for controlling *Listeria* in the food industry.

P1-28 Development of Novel Peptide Nucleic Acid Melting Array for the Detection and Genotyping of *Toxoplasma gondii*

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Introduction: Although reasonable numbers of PCR and real-time PCR assays have been developed to identify *T. gondii* in animals and their meat products, the methods for genotyping *T. gondii* strains (type I, II and III) are limited to serological tests. Thus, there is a need for fast and reliable sequence-based assay for differentiating most virulent type I strain from less virulent type II and III strains.

Purpose: The purpose of this study was to detect *T. gondii* in food and environmental samples and differentiate genotypes using novel peptide nucleic acid (PNA) melting array.

Methods: Genomic information of *T. gondii* type I, II and III obtained from NCBI was aligned and SNP (single nucleotide polymorphism) analysis was performed to identify targets of PCR amplification and PNA melting array. Prior to the PNA melting array, conventional PCR amplified the GRA6 gene of *T. gondii*. After the amplification, PNA melting array was carried out using two different PNA hybridization probes labeled with fluorescent dyes (FAM and HEX) and quencher. Then melting temperature of each probe was analyzed to finalize genotype and possible mutation.

Results: Conventional PCR confirmed amplification of 214-bp region of GRA6 gene in *T. gondii*. Total of 8 *T. gondii* strains (3 type I, 3 type II and 2 type III) tested for the specificity showed exact genotypes with PNA melting array. Non-*T. gondii* strains including 14 different food-borne pathogens as well as 3 protozoan parasites such as *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica* showed no amplification for the prior PCR, suggesting high specificity of the assay.

Significance: Although this is only a proof-of-concept study, the assay showed promising results for fast and reliable genotyping of *T.gondii* isolated from food and environmental samples.

P1-29 An Innovative Rapid Two-incubation Immunoassay to Optimize the Recovery Levels of Ochratoxin A in Overparticular Matrices by Dilution Normalization

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Introduction: Most commercial competitive immunoassays have problems with Ochratoxin A (OTA) recovery levels when particular samples of cereal, grains, spices and animal feeds are analyzed. When extracted, these samples form overparticular matrices for most ELISAs, since they usually constitute one-third of the incubated mixture affecting negatively the competitive step and the HRP itself. One way to overcome the matrix issue is by using clean-up pre-columns but unsatisfactory recovery levels are unavoidable.

Purpose: The purpose of this work was an alternative way to improve the recovery levels of OTA in overparticular matrices avoiding the pre-column treatment.

Methods: Fifty grams of the above samples were extracted with 70% methanol and after filtration the OTA was determined by a direct competitive ELISA with or without pre-column treatment. Micoplates were coated with a high affinity monoclonal antibody produced after mice immunisation, preparation of hybridomas, clones screening and protein G purification. The immunoassay had two incubation steps (in contrast with other tests), the first incubation of 20% extract and 80% HRP-free buffer mixture for 10 minutes followed by washing steps, the second incubation of OTA-HRP for 5 minutes followed by washing steps and the addition of chromogen for 5 minutes followed by the addition of acidic solution. The OTA-HRP and the immunogens were produced by active ester biochemical conjugation, optimization of conjugates molarity and gel filtration purification.

Results: Although the immunoassay requires only 20% of extract, the sensitivity was increased giving the maximum range of quantification. Due to increased dilution normalization of matrix and enzyme protection from methanol by using two incubation steps, the recovery levels were more acceptable without pre-column treatment.

Significance: This immunoassay is a very valuable tool in the OTA analysis without pre-column treatment, which optimizes the recovery percentage and is an effortless and rapid procedure.

P1-30 Development and Validation of a Lateral Flow Test Kit for Detection of Native and Deamidated Gliadin Residues

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Introduction: Gluten intolerance disorders, including celiac disease, are relatively common and necessitate strict limits in dietary intake. Consequently, the gluten content of foods labeled “gluten-free” is regulated in the U.S. under Federal law. However, accurate determination of gluten levels in food is problematic because deamidated wheat gluten is difficult to detect due to chemical alterations in glutamine (Q) residues located in the diagnostic epitopes.

Purpose: To improve upon current gluten-detection capabilities, Pi Bioscientific (Pi Bio) has developed and validated a highly rapid diagnostic tool specifically designed to detect deamidated gliadin in foods, on environmental surfaces, and in personal hygiene products.

Methods: Monoclonal antibodies (mAbs) were raised against a synthetic variably deaminated tandem repeat of the peptide recognized by the widely used R5 mAb: L{Q/E}P{Q/E}{Q/E}PFP{Q/E}{Q/E}L{Q/E}P{Q/E}{Q/E}PFP{Q/E}{Q/E}A and chemically deamidated gliadin, screened against a panel of prolamins, purified on a protein G column using FPLC, and used to develop a sandwich lateral flow immunochromatographic (LFD) assay. Sample extraction buffers and running buffers were developed that enable rapid and highly sensitive operation of the LFD. The kit and test method were validated.

Results: The Pi Bio deamidated gluten lateral flow test method had a sensitivity of 0.1 µg protein/swab and 1.0 µg protein/mL of food extract for both deamidated and native gliadin (prolamins) in under 20 min (sample preparation and LFD operation). Specificity analysis indicated cross-reactivity with proteins from teff and selectivity analysis using a panel of problematic matrices revealed no matrix effects on the LOD.

Significance: The development of a highly sensitive and rapid test method capable of accurately detecting trace amounts of deamidated gliadin in under 20 min should aid food manufacturers, contract laboratories and regulatory entities in monitoring for gluten derivatives that have previously proved challenging and will provide more accurate measurements of the gluten content of foods.

P1-31 Validation of Food Allergen Lateral-flow Devices in the Presence of Hygiene Chemicals

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Introduction: A principle cause for food recalls in the U.S. is the presence of undeclared food allergens. One common reason for these recalls is allergen cross-contact that occurs during manufacturing. Allergen control measures, such as cleaning, can help prevent or minimize cross-contact, but rely on the use of rapid screening methods to verify that cleaning was effective. Lateral Flow Devices (LFDs) are ideally suited for rapid, qualitative screening of environmental surfaces to verify cleaning efficacy. However, LFD function can be inhibited by various factors including residual cleaning agents.

Purpose: To validate the sensitivity of the ELISA SYSTEMS (ES) AllergenControl™ Total Milk and Egg LFDs in the presence of 3 common cleaning agents commonly used in the food industry.

Methods: The effects of cleaning chemicals on the ES LFD kits were determined using BioSide HS 15% (Enviro Tech Chemical Services, Inc. Modesto, CA), Power 99 Plus Degreaser (Morgan Gallacher Inc. Santa Fe Springs, CA), and Blend Foam Cleaner (Univar Inc., Redmond, WA). Each of these was diluted in water to reach the concentration levels chosen to be grossly higher than those expected from normal carry-over based on the application directions for each. 100 µL of each prep was mixed with 900 µL of LFD extraction buffer containing NFSM or egg white protein (EWP) at either 1.1X or 2.1X the reported kit limit of detection (LOD). 100 µL of this mixture was applied to the sample port of the LFD. Line intensity values were measured using a Qiagen ESE reader at 15 min, and the averages and StDv (parenthetically) for the sandwich test line results are reported for triplicate tests.

Results: The Total Milk LFD kit demonstrated an analytical limit of detection (LOD) of 0.02 ppm milk protein at the highest concentrations of sanitizer tested (200–1,000 ppm). The Egg LFD kit demonstrated an LOD of 0.1 ppm EWP at the highest concentration of each sanitizer tested (200–1,000 ppm).

Significance: The application of a highly sensitive and rapid test method capable of detecting trace amounts of allergens in the context of residual hygiene chemicals (even if inadequately removed) will aid food manufacturers and regulatory agencies in monitoring for allergen residues present on environmental surfaces and improve existing allergen control measures.

P1-32 Development and Validation of a Rapid Qualitative Test Kit for Detection of Raw and Cooked Pork Meat and Gelatin Residues

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Introduction: Reports over the past years show that pork adulteration, accidental or intentional, in beef retail products is relatively common. To broaden the application of rapid testing for meat authentication, we developed two Lateral Flow Devices (LFDs) to detect raw and cooked pork, as well as pork gelatin, in beef.

Purpose: To develop a highly specific detection kit that can rapidly detect raw and cooked pork meat and gelatin residues in beef.

Methods: Goat antibodies against porcine serum albumin (PSA) and thermal stable meat protein (TSMP) were used to prepare a lateral flow immunochromatographic assay (LFIA or LFDs). Sample extraction buffers and running buffers were developed to enable detection of raw porcine meat, cooked meat, and gelatin from beef samples. These kits and test methods were validated for sensitivity, specificity and dynamic range. Method concordance was assessed using a PCR-based meat authentication method (IEH).

Results: The Pi Bioscientific pork meat LFD has a limit of detection (LOD) of 0.01% spiked raw pork meat (into beef meat), 1% spiked cooked pork meat (into cooked beef meat), and 0.1-2.5% spiked gelatin (depending on whether it was buffer or candy gelatin). Specificity analysis revealed no cross-reactivity with meats derived from chicken, turkey, horse, beef, lamb, and goat. The assay was able to detect gelatin residues where PCR methods failed due to the inability to recover DNA from gelatin.

Significance: The development of a highly specific test system to detect trace amounts of both raw and cooked pork meat as well as gelatin in ~40 min can become an important tool for food safety authorities, contract laboratories and food producers in their continued efforts to monitor the purity of meat products.

P1-33 Cross-reactivity to Mahaleb Spice in a Subject with Allergies to Almond and Other Tree Nuts

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Introduction: In 2014 and 2015, a number of food recalls occurred worldwide due to contamination of ground spices with peanut and tree nut residues. A subset of these recalls associated with putative almond contamination in cumin were later rescinded in Canada and the UK when confirmation testing attributed the contamination to mahaleb residues. Routine immunodiagnostic-based and PCR-based methods for almond determination were unable to discriminate between almond residues and closely related mahaleb proteins. Liquid chromatography-tandem mass spectrometry and PCR-based techniques were later employed by the UK FOOD Standards

Agency and the Canadian Food Inspection Agency to identify mahaleb-specific markers in these cumin samples.

Purpose: In view of the inability of routine testing to distinguish between almond and mahaleb, it is important to know whether mahaleb proteins present a risk for almond-allergic consumers. To begin assessing this risk, we assessed mahaleb sensitivity in an individual with multiple tree nut allergies.

Methods: Indirect ELISA and skin prick testing were used to assess mahaleb sensitivity in a subject with severe nut allergies. Western blot analysis and indirect ELISA were used to assess in-vitro cross-reactivity between almond and mahaleb proteins.

Results: Skin prick testing and indirect ELISA performed using the subject's IgG revealed significant reactivity to mahaleb and pistachio, a tree nut unrelated to almond or mahaleb for which the subject had a history of severe allergy. Antigenic similarity between almond and mahaleb was confirmed using indirect ELISA as well as Western Blot analysis using an anti-almond pAb generated by PIBio, whereas little or no activity was seen against pistachio proteins, indicating that the commercial antibody was highly specific for prunus genus seed material.

Significance: The high level of cross-reactivity between almond and mahaleb and the clinical presentation of the study subject highlights the need for regulatory bodies and food manufacturers to address the potential for mahaleb contamination in food, particularly foods that are imported from areas where mahaleb is used as a spice.

P1-34 ELISA Systems Allergen Control Soy LFD Kit for Detection of Modified Soy Protein Residues

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Introduction: Soy is commonly used in many foods as a plant-based protein source and as a texture-enhancing agent. This means that soy proteins are frequently treated by heat, pressure, and extreme pH which modifies the overall conformation of the individual soy proteins and their antigenicity. A direct outcome of these process-induced changes is a reduction in the ability of soy protein detection systems, including sandwich ELISA, to detect and quantify modified soy proteins.

Purpose: To develop and validate a soy lateral flow device (LFD) that can detect commonly modified soy bean proteins.

Methods: The ELISA Systems (ES) AllergenControl™ Soy LFD kit was developed by using pAbs raised against modified soy proteins. The kit was validated using distinct soy protein sources, including native and modified proteins and cross-reactivity analysis was carried out against a panel of key commodities. A method comparison was performed by using the Romer Labs AgraStrip® Soy.

Results: The ES Soy LFD kit demonstrated matrix-free analytical sensitivity of 0.1 ppm native soy protein and 0.1 ppm modified soy protein, minimal cross-reactivity against key commodities, and an improved detection of soy protein present in soy protein isolate, soy protein concentrate, and soy milk relative to the Romer Labs AgraStrip® Soy.

Significance: Development of a LFD capable of detecting modified soy protein should be an important and rapid tool for food manufacturers and regulatory entities in their allergen control management.

P1-35 Optical Biosensing Using Functionalized Gold Nanoparticles for Simultaneous Detection of *Salmonella* spp. in Food and Environmental Samples

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Introduction: *Salmonella* spp., a major foodborne pathogen, is transmitted to humans through contaminated meat, poultry products and vegetables, causing outbreaks and illnesses such as gastroenteritis.

Purpose: The objective of this study was to develop a method to determine the contamination of various *Salmonella* spp. strains in poultry, produce and environmental samples by employing an efficient pooling and immunomagnetic separation (IMS) system coupled with rapid optical biosensing detection using functionalized-gold nanoparticles (AuNPs).

Methods: Pairs of single stranded thiol-modified oligonucleotides (30-mer) were immobilized onto AuNPs and used as probes to capture *ttrRSBCA* region (192-bp) from asymmetric polymerase chain reaction (asPCR). DNA samples from pure cultures, inoculated chicken and blueberries, and natural environmental samples were sandwich hybridized with AuNP-oligo probes at optimal conditions (55°C, 40 minutes). A complex was formed from the hybridized AuNP-probes and target DNA fragment which allowed retention of the initial red color of the target reaction solutions following an increase in salt concentration. For non-target DNA, a color change from red to purplish-blue was observed. Shortened enrichment (6 hours) and pooling of samples using Pathatrix IMS system were utilized to ensure detection of viable cells.

Results: From a total of 55 individual samples (chicken = 25; blueberry = 25; environment = 5), 40 samples tested positive for *Salmonella* spp. All environmental samples tested negative. The assay had a superior detection limit of 1 log CFU/g with 100% specificity and required less than one hour to complete after DNA sample preparation. Transmission electron microscope (TEM) images confirmed AuNP-DNA hybridization while spectrophotometric data

supported the color discrimination based on the occurrence of molecular aggregation.

Significance: The significant features of this study took advantage of the unique colorimetric properties of AuNPs, shortened enrichment with robust sampling and pooling systems for rapid detection of *Salmonella* spp. even at a low contamination level.

P1-36* *In Vitro* Gene Expression of *Listeria monocytogenes* after Exposure to Human Gastric and Duodenal Aspirate

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Introduction: *Listeria monocytogenes* is a pathogenic microorganism with high penetrating ability that causes severe human illness (uterine infections, bacteremia and central nervous system infections) after consumption of contaminated food.

Purpose: To study *L. monocytogenes* population changes and to determine the expression of its virulence-associated genes, after *in vitro* exposure to human gastric and duodenal aspirate.

Methods: Gastric and duodenal human fluids were collected endoscopically. *L. monocytogenes* cultures (16 h) of the strain LQC 15257 (serotype 4b) were inoculated in gastric fluid at 9 log CFU mL⁻¹. Samples were incubated at 37°C for 100 min and then centrifuged. The precipitate was resuspended in duodenal fluid and samples were incubated at 37°C for 2 h. Population changes and gene expression were studied by culture-based methods and RT-qPCR, respectively.

Results: *L. monocytogenes* population was decreased in all studied cases; the microorganism was not detectable after its exposure to gastric aspirate in 11/12 patients with gastric pH < 2.9 in contrast to 1/3 patients with gastric aspirate pH > 2.9 ($P=0.022$). In 3/4 of gastric aspirates from patients who had previously received proton pump inhibitors (PPIs) the microorganism was also undetectable, versus 9/11 gastric aspirates from patients who had no previous PPIs intake ($P = 0.021$). *bly* and *inlC* genes were over-expressed in 8/12 aspirates from patients with gastric pH < 2.9 as compared to 1/3 with pH > 2.9 and in 10/12 aspirates from patients with gastric pH < 2.9 as compared to 1/3 pH > 2.9, respectively. However, changes in the expression of *prfA*, *sigB*, *plcA*, *plcB*, *InlA*, *InlB*, *InlJ*, *lmo2470* and *lmo2672* genes were not associated with the pH of the gastric aspirate.

Significance: For the first time, a study simulated real exposure conditions using gastroduodenal human aspirates. Results indicated that conditions increasing gastric pH value, such as PPIs intake, may predispose patients to listeriosis.

P1-37* Effect of Co-culture with Enterocinogenic *Enterococcus faecium* on *Listeria monocytogenes* Key Virulence Gene Expression

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Introduction: The reason of this study was to improve understanding of *Listeria monocytogenes* physiology under stress conditions.

Purpose: The aim of the present study was to assess *L. monocytogenes* key virulence gene transcription during co-culture with an enterocinogenic *Enterococcus faecium* strain.

Methods: *L. monocytogenes* strain LQC 15108, serotype 4b, and *E. faecium* strain LQC 20005 were inoculated in BHI broth at 7 and 4 log CFU mL⁻¹, respectively, and incubated at 5 and 37°C. Sampling took place after 8 and 24 h of incubation, corresponding to the maximum and minimum of enterocin production, respectively. After collection of the biomass, RNA extraction, cDNA synthesis as well as real-Time qPCR were performed using commercially available kits.

Results: During growth at 5°C, co-culture resulted in downregulation of *prfA*, *plcA*, *plcB*, *inlA* and *inlC* after 8 and 24 h of incubation and *bly*, *inlB* and *inlJ* only after 24 h of incubation. On the other hand, during growth at 37°C, after 8 h of incubation co-culture resulted in upregulation of *bly* and *inlC* and after 24 h of incubation in downregulation of *prfA* and upregulation of *bly*, *sigB* and *inlJ*.

Significance: Exploitation of regulatory and response mechanisms of *L. monocytogenes* is important for the design of effective intervention strategies.

P1-38 Occurrence of Different *Salmonella* Serotypes in Food Products of Plant-origin

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Introduction: *Salmonella* is one of the most important causes of foodborne outbreaks and diseases. Although salmonellosis is often related to animal-origin food products, reports of *Salmonella* outbreaks related to plant-origin foods are increasing.

Purpose: To evaluate the presence of *Salmonella* in plant-origin food products mainly imported to Greece and identify the different serotypes.

Methods: 1687 raw or processed food products of plant-origin were tested for the presence of *Salmonella* spp. in the accredited laboratory of

the Institute of Technology of Agricultural Products during the period June 2008–December 2015. The majority of them (1575) were products tested to get permission for importing to Greece, whereas the remaining 112 were produced by Greek food enterprises. For the detection of *Salmonella* spp. the ISO 6579 (4th ed. 2002-07-15/Cor.1:2004) was applied. Further confirmatory and identification tests to species, subspecies and serovar level were performed in the National Reference Laboratory for *Salmonella* (Hellenic Ministry of Rural Development and Food) accredited for the serotyping.

Results: 128 samples were found positive for *Salmonella* spp., while 6 samples were false positive. Specifically, *Salmonella* was detected in 92 out of 1091 sesame samples (92/1091), 18/160 pine nuts, 6/89 coconut, 4/5 paan leaves, 3/15 cocoa beans, 1/39 almond kernel, 1/5 chickpeas, 3/20 paprika samples. 127 isolates were identified as *S. enterica* subsp. *enterica* and 1 as *S. enterica* subsp. *salamae*. The *S. enterica* subsp. *enterica* isolates designated by their antigenic formula included *Salmonella* Virchow, Typhimurium, Kuesel, Wien, Oranienburg, Agona, Mikawasima, Hvittingfoss, Bredeney, Anatum, Senftenberg, Orion, Havana, Paratyphi B var. Java, Mbandaka, Liverpool, Telhashomer, Idikan, Brunei, Westhampton, Tennessee, Poona, Mountpleasant, Tilene, Kentucky, Amsterdam, Tilburg, Ruivu, Fresno, Bredeney, Montevideo, Ekotedo, Hongkong, Dallgow, Bergen and Kastrup.

Significance: The study indicated that several food products of plant origin constitute a potential hazard for human consumption.

P1-39 The Impact of Co-cultivation on Growth, Expression of Virulence Genes and *In Vitro* Virulence Potential of *Listeria monocytogenes*

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Introduction: The interactions between *Listeria monocytogenes* and food-associated bacteria are critical for the growth of the microorganism in food environments and often related to under-detection of *L. monocytogenes* during enrichment. However limited information exists on the impact of food microorganisms on the virulence of the pathogen.

Purpose: The study investigated: i) growth, ii) expression of virulence genes and iii) *in vitro* virulence potential of *L. monocytogenes* in the presence of four different food-related microorganisms.

Methods: Growth of *L. monocytogenes* (ScottA) was evaluated as monoculture or in co-culture with *L. innocua*, *Bacillus subtilis*, *Lactobacillus plantarum* and *Pseudomonas aeruginosa* in Tryptic Soy Broth (10°C/10 days and 37°C/24 hours). The transcription of 9 key virulence genes (*inlA*, *inlB*, *inlC*, *inlJ*, *sigB*, *prfA*, *hly*, *plcA*, *plcB*), in addition to invasion efficiency (45 min) and intracellular growth (4 h) in Caco-2 cells, were determined for *L. monocytogenes* grown singly or in co-culture previously incubated for 3 days at 10°C or 9 hours at 37°C.

Results: Significant differences in growth between single and co-cultures of ScottA were observed when grown with *L. innocua* at 37°C or 10°C (e.g., lower final populations) and *B. subtilis* at 37°C (e.g., growth cessation after 9 h). ScottA revealed considerably increased invasion efficiency when co-cultured with *L. innocua* but attenuated, efficiency in the presence of *B. subtilis*. Intracellular growth of *L. monocytogenes* in Caco-2 cells was reduced up to 35 folds compared to monoculture, when grown in co-cultures. The key virulence genes of *L. monocytogenes* were under-expressed after co-cultivation with *B. subtilis* at both temperatures while co-cultivation with *L. innocua* at 37°C, increased the overall gene expression levels of ScottA (e.g., 7-fold increase of *prfA*).

Significance: Investigating the impact and mechanisms of microbial interactions on growth and virulence of *L. monocytogenes* expands our understanding on the survival and infection potential of the pathogen in the gastrointestinal environment.

P1-40 Identification and Characterization of *Listeria monocytogenes* in Fish and Fish Products in Poland

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Introduction: *L. monocytogenes* may be present in different types of food including fish and fish products. It is considered a major public health concern responsible for many cases of human infections.

Purpose: The aim of this study was to determine the prevalence of *L. monocytogenes* in fish and fish products as well as molecular characterization and antimicrobial resistance of the bacterial isolates.

Methods: A total of 100 samples of fresh fish (n = 26) and fish products (n = 74) purchased in local retail shops and investigated for the presence of *L. monocytogenes* using the EN-ISO 11290 standard. The fresh fish represented 9 species (cod, flounder, halibut, crucian, bream, salmon, panga, roach, trout) whereas the samples of fish products (smoked fish) were cod, halibut, bream, salmon, mackerel, sprat, and herring. Molecular serotyping and virulence genes (*inlA*, *inlC*, *inlJ*, *lmo2672*, *llsX*) were identified with PCR. Antimicrobial susceptibility of the isolates to

17 antimicrobials was determined using the MIC (Minimal Inhibitory Concentration) method.

Results: *L. monocytogenes* was found in 5 (19.2%) samples of fish and in 11 (14.9%) fish product samples. In all samples the number of *L. monocytogenes* was below 100 CFU/g. In all strains the *inlA*, *inlC*, *inlJ*, *lmo2672*, *plcA*, *bly*, *mpl*, *actA*, *plcB*, *inlB* virulence markers were identified; none of the *L. monocytogenes* strains were positive *lssX*. The presence of the *flaA* gene was identified only in strains of the 1/2a serotype. It was found that the most contaminated species was salmon, especially smoked salmon. The antibiotic resistance analyses revealed that *L. monocytogenes* were sensitive to most of the antibiotics used. Single isolates were only resistant to ceftriaxone (1 strain) and to oxacillin (2 strains); some strains showed an intermediate resistance to clindamycin (9 strains), ceftriaxone (6 strains) or ciprofloxacin (3 strains).

Significance: The data show that some fresh and smoked fish available on the Polish market were contaminated with *L. monocytogenes*. The isolates possessed virulence markers that make them potentially pathogenic for humans.

P1-41 Modeling the Growth Response of Pathogenic *Escherichia coli* at Various Storage Temperatures in Kimchi

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Introduction: Kimchi is a Korean traditional fermented vegetable product and is gaining popularity as a functional food. Although this type of food is generally recognized as safe due to its high salinity (> 2.0%) and low pH (< 4.5), recent microbiological safety issues have thrown this assumption into doubt. Several large outbreaks of pathogenic *Escherichia coli* in South Korea have been attributed to Kimchi. In South Korea in 2012, 1,642 people were infected by pathogenic *E. coli* after eating Kimchi products.

Purpose: The objective of this study was to develop a predictive model of the growth of pathogenic *E. coli* in Kimchi as a function of storage temperature.

Methods: We investigated that growth of the cocktail of five pathogenic *E. coli* (EAEC, EHEC, EIEC, EPEC, and ETEC) strains inoculated in Kimchi at different storage temperatures (5, 10, 15, 20, 25, 30, and 35°C) for a maximum of 12 days.

Results: Fitted into the Baranyi model to generate the growth parameters including specific growth rate (SGR) and lag time (LT) with high coefficients of determination ($R^2 > 0.95$) except 10°C ($R^2 < 0.90$). The obtained SGR and LT were employed to develop secondary exponential equation models to evaluate the effects of storage temperature on the growth

kinetics of pathogenic *E. coli* in Kimchi. The values of bias factor (1.000–1.004) and accuracy factor (1.004–1.070), which were regarded as acceptable, demonstrated that the obtained models could provide good and reliable predictions.

Significance: The developed predictive model could be suitable for the purpose of microbiological risk assessment of pathogenic *E. coli* in Kimchi.

P1-42 Temporal Expression of Genes for Initiation of the Sporulation in Anaerobiosis in *Bacillus cereus*

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Introduction: *Bacillus cereus* is a foodborne pathogenic bacterium that can live and survive (spore and vegetative cells) in many environments. It is important to know if ingested spores are able to germinate and produce their toxins in the intestine where the atmosphere is reducing. Our previous studies showed that in anaerobiosis, *B. cereus* strains displayed lower sporulation ability than in aerobiosis.

Purpose: To seek the cause of this low sporulation in absence of oxygen, we followed during growth the main genes (*kinA*, *kinB*, *spo0F*, *spo0B*, *spo0A*) involved in sporulation process.

Methods: Cells were grown in controlled batch cultures to study gene expressions by real-time RT-PCR using SYBR green technology on a light cycler instrument. A chemical defined medium was used (MODs).

Results: These experiments have shown that the expression of genes *kinA*, *spo0F*, *spo0*, *spo0B* had the same kinetics with overexpression at the beginning and the end of the anaerobic growth compared to aerobic conditions. During growth, it was noted an under-expression of the gene *kinA* while *spo* genes showed no difference in expression under anaerobic conditions compared to aerobic conditions. In addition, *kinB* (*kinB1*, *kinB2*, *kinB3*) had a reverse trend of expression than previous genes. They showed no difference in expression compared to anaerobic aerobic early (2h) but also at the end (9h) of growth. After 2h of culture, the expression of genes *kinB* increased gradually until it reached expression peaks at 4h (*kinB3*) and 6h (*kinB1* and *kinB2*) growth. We also note that the genes *kinA* and *kinB* have inverse expression levels. When *kinA* was overexpressed, *kinB* was subexpressed and vice versa. These results could assume that the role of *kinB* here is secondary or complementary to *kinA*.

Significance: This work thus provides new data on sporulation process in this food-born pathogenic bacterium

P1-43 *Kudoa septempunctata* Was Recognized by Toll-like Receptor 2

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Introduction: In Japan, food poisoning outbreaks associated with the consumption of raw olive flounder have increased, with more than 50 cases reported per year. The causative agent of these outbreaks is *Kudoa septempunctata*, a myxosporean parasite. *K. septempunctata* dwells in the trunk muscles of olive flounder. The lag phase of disease caused by *K. septempunctata* is 1–20 h and symptoms include transient but severe diarrhea and emesis. One characteristic of this foodborne disease is a rapid recovery, and the disease has a good prognosis. The rapid recovery following ingestion of the parasite is thought to be associated with the immunity of the host, particularly innate immunity. Our previous studies showed that when macrophages were incubated with *K. septempunctata*, macrophages strongly secreted TNF- α and several chemokines, such as IP-10, MIP-1 β , and MIP-2. However, the receptor on macrophage that recognize *K. septempunctata* remains unknown.

Purpose: Macrophages express Toll-like receptors (TLRs), and each TLR has a specific ligand. The ligands of TLRs are conserved motifs that are found predominantly in microorganisms, and TLR signaling enables macrophages to recognize pathogens and subsequently produce cytokines. The aim of this study was to identify the TLR receptor that recognize *K. septempunctata*.

Methods: To identify the macrophage receptor of *K. septempunctata*, the activation of HEK 293 cells expressing each TLR (from TLR1 to TLR9) was measured using NF- κ B-dependent reporter assay.

Results: TLR2-expressing HEK 293 cells were strongly activated following stimulation with *K. septempunctata*.

Significance: Our study demonstrated that *K. septempunctata* was recognized by TLR2. This result suggests the mechanism that *K. septempunctata* is recognized by TLR2 on macrophages and TLR2-signaling leads to the production of cytokines.

P1-44 Virulence Gene *ail* Present in *Yersinia enterocolitica* Biotype 1A Strains

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Introduction: The pathogenicity of *Yersinia enterocolitica* strains varies widely: biotypes 1B and 2–5 are pathogenic, whereas biotype 1A strains are considered as nonpathogenic due to the lack of important chromosomal virulence genes, like

ail gene coding for adhesion to epithelial cells, and the virulence plasmid. The virulence gene *ail* is also commonly used target for PCR detection of pathogenic *Y. enterocolitica*.

Purpose: The carriage of virulence gene *ail* in *Y. enterocolitica* biotype 1A strains isolated from various sources was studied to evaluate the prevalence of the gene in nonpathogenic *Y. enterocolitica* strains and the usefulness of this gene in PCR detection of the pathogenic strains.

Methods: Total of 420 *Y. enterocolitica* 1A strains isolated from samples originating from packaged leafy vegetables (11), raw milk (34), sheep feces (17) and feces of wild rodents (358) were investigated. The presence of gene *ail* was studied with real-time PCR method based on SYBRGreen. The *ail* genes from six strains isolated from rodents were also partly sequenced.

Results: From 420 *Y. enterocolitica* biotype 1A isolates studied, 94 (22.4%) were carrying the virulence gene *ail*. Isolates of sheep origin were most commonly *ail*-positive (58.8%). Total of 82 (22.9%) isolates of rodent and two (18.2%) of salad origin were *ail*-positive. None of *Y. enterocolitica* 1A isolates from raw milk samples were carrying the *ail* gene. All six partially sequenced *ail* genes were identical to each other and with 98.8 % similarity to previously sequenced *Y. enterocolitica* 1A *ail* genes.

Significance: The virulence gene *ail* of *Y. enterocolitica* is frequently carried by biotype 1A strains regarded as nonpathogenic. The presence of *ail* gene in nonpathogenic *Y. enterocolitica* strains questions the usefulness of this gene alone in the PCR detection.

P1-45 Influence of Bacterial Foodborne Diseases in South Korea by Climatic Factors

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Introduction: Climate change may have both direct and indirect impact on the food safety at various stages of the food chain. Especially bacterial foodborne disease were confirmed as one of major impact parts due to climate change by the results of previous studies in Korea. Nevertheless the correlation between conditions of climate change and outbreak of foodborne disease is still poorly understood. Therefore, the reliable estimate on the impact were very important to develop the strategy on adaptation to climate change for food safety in Korea.

Purpose: Therefore, the aim of the study was to investigate the relationship between bacterial foodborne pathogens (BFP) and climatic conditions in Korea, and then to establish a simulation model, based on QMRA (Quantitative Microbial Risk Assessment), that can be used to estimate the prospective impact of climate change on food safety.

Methods: To select climatic sensitive BFP, correlation analysis identifying the relationship between 8 climatic variables and 13 BFP in Korea during the period 2002-2013 were conducted with SPSS Ver. 12 (Data Solution). Climatic and epidemiological data on FBDOs were obtained from the KMA (Korea Meteorological Administration) and the KMFDS (Korean Ministry of Food and Drug Safety), respectively. Also, the future incidence trend of FBDOs due to climate change in Korea were predicted by QMRA simulation model, developed using national monitoring data and meta analysis (review manager Ver 5.1 (RevMan) in this study, combining new climate change (RCP 4.5 and 8.5) scenarios produced in KMA based on the RCP scenarios.

Results: The results of correlation analysis shown that *Vibrio parahaemolyticus* (VP) had the highest positive correlation coefficient with climatic variables, followed by *Campylobacter* spp., Pathogenic *E. coli* (EHEC), and *Salmonella* spp (SA). The incidence rates of FBDOs due to the 4 identified pathogens are shown in relation to mean monthly temperature, relative humidity, and precipitation. QMRA simulation model estimated the incidence rate of BFD caused by 4 pathogens in 2050 and 2100 will be higher from 5.7% (SA in 2050 under RCP 4.5) to 114.6% (VP in 2100 under RCP 8.5) than the present. The Incidence rate of BFD caused by 4 pathogens in the future will be increased from 5.7% (SA in 2050 under RCP 4.5) to 114.6% (VP in 2100 under RCP 8.5)

Significance: The study was significant for the development of QMRA simulation model as a useful tool on food safety to strengthening its capacity to be proactive in addressing threats to food safety to a changing climate. And results of the study will be assisted the development of the strategy on adaptation to climate change for food safety management, and build up the national adaptation capacity on food safety in Korea.

Acknowledgment: This research in conducted within Korean Ministry of Food and Drug Safety (KMFDS) project "System development for food safety management to support adaptation to climate change."

P1-46 EFSA's Guidance Document on Uncertainty in Scientific Assessment

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Introduction: Identifying and characterising uncertainties in the scientific assessment process, and explaining their implications for assessment conclusions, are crucial parts of the European Food Safety Authority's responsibility to provide robust and transparent scientific advice.

Purpose: Development of a guidance document

providing a harmonised framework applicable to all relevant working areas of EFSA on how to characterise, document and explain uncertainties in the various steps of risk assessment.

Methods: The EFSA Scientific Committee set-up an overarching Working Group with representatives from all EFSA Panels and the support of relevant external experts. During the development of the GD, the WG consulted the EFSA Panels, risk managers from DG SANTE, national authorities, EU sister agencies, and international organisations, and published the document for public consultation. In light of all the comments received a revised version of the GD is currently being tested by all EFSA Panels on selected opinions. At the end of the testing phase the GD will be further revised and finalised.

Results: The GD proposes a methodological framework for analysing uncertainties in all EFSA scientific assessments and provides a tool box of qualitative and quantitative methodologies that can be used to perform the analysis. The approach aims to be sufficiently flexible to adapt to the needs of the different EFSA Panels and to the circumstances of each assessment, e.g., from urgent advices to longer-term comprehensive reviews of all available scientific knowledge.

Significance: The GD will contribute to increase transparency in the risk assessment process carried out by EFSA and further strengthen the basis for an informed decision-making process in the area of food safety.

P1-47 EFSA's Knowledge Networking Activities on Emerging Risk Identification, 2010–2015

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Introduction: EFSA's activities in the area of identification and characterization of emerging risks in food and feed are required by Article 34 of the EU General Food law.

Purpose: In 2010, the European Food Safety Authority (EFSA) established an Emerging Risks Exchange Network (EREN) and a Stakeholder Consultative Group on Emerging Risk (StaCG-ER) to exchange information on possible emerging risks for food and feed safety between EFSA and the Member states (MSs) and between EFSA and the civil society and private sector, respectively.

Methods: EREN is composed of delegates from 21 MSs and Norway. The StaCG-ER comprises 19 members belonging to consumer associations and organisations representing the food, livestock and agricultural industry. The two groups contribute to the identification of emerging issues, the collection of data and its assessment. The two networks meet twice a year and assess identified issues using a standard template.

Results: Between 2010 and 2015 EREN and StaCG-ER assessed a total of 86 signals of potential emerging issues. Out of these, 39 were identified by EFSA, 37 by MSs and 10 by civil society. The issues discussed were

mainly microbiological and chemical hazards, but also food safety issues such as those resulting from illegal activity, new consumption trends, biotoxins, new technologies and processes, allergens, animal health, environmental pollution, new analytical methods, new food packaging technology and unknown hazards. Based on the available evidence, the networks recommended whether an issue merited follow up actions, such as generation of new data, a full risk assessment and/or consultation with other bodies. The methodology developed to preliminarily assess signals of potential emerging issues is presented and discussed with two case studies.

Significance: The increasing number of issues identified over the years shows the value of such knowledge networks as information sources to identify emerging risks.

P1-48 Expert Systems for Food Safety – Unleashing Its Full Potential

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Introduction: Computer-based solutions have become important tools supporting decision making in business operations, governmental authorities and even the consumer sector. The exponential growth of available experimental and process data as well as improved sensor technologies applied during food production, processing and distribution open new horizons for the development of decision support systems (DSS) or expert systems (ES) in the food sector.

Purpose: This research aimed at identification of issues hindering the full exploitation of ES's potential in the food safety domain. The gained results served then as a basis for the development of a strategy to overcome existing development hurdles.

Methods: A literature review on ES developed and applied in the domain of food safety was performed covering the period from 2013 to 2015. General structural ES components, stakeholders to-be-involved as well as lessons to-be-learned for ES development were identified from literature reports and own practical experiences.

Results: A key finding of this research is that the proper technical, structural and algorithmic system design addressing the issue of knowledge base updating is crucial for successful ES development. Even though this sounds trivial, evidence from several scientific publications prove that this feature is still not available in many ES. This is caused frequently by the fact that applied knowledge generation algorithms do not support the incremental update of math-based knowledge representation. In the light of this finding we illustrate how the establishment of community driven food safety model repositories could support ES development and which practical steps are needed to develop them.

Significance: Application of ES will become increasingly important in all areas related to food safety. Collaborative efforts to setup shared knowledge repositories have thus a high potential to

facilitate math-based knowledge exchange specifically between academia and business and/or governmental institutions. Here we also illustrate that the critical technical and formal foundation for community driven knowledge repositories is already emerging.

P1-49 Identification of Factors Associated with Technical Accreditation and Food-safety Compliance in Food and Drink Manufacturing and Processing Businesses in Wales, UK

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Introduction: Technical accreditation and food-safety compliance are essential for food and drink manufacturing and processing businesses (FDMPBs) to enable brand protection and due diligence in compliance with UK food law. Such accreditation schemes may enable FDMPBs to supply commercial sectors. Ultimately, such schemes ensure product safety for consumers. Currently, data detailing the associated factors of technical accreditation/food-safety compliance of FDMPBs in Wales are lacking.

Purpose: The study aimed to identify factors associated with FDMPBs' technical accreditation/food-safety compliance in Wales.

Methods: As part of the 'Wales Food and Drink Survey 2015–16', online questionnaires were completed by FDMPBs in Wales ($n = 103$) to obtain baseline information. Collated data were statistically analysed to determine significant associations with accreditation.

Results: The leading product sectors included bakery (26%), dairy (19%) and alcoholic drinks (18%). The majority (60%) reported having technical accreditation/food-safety compliance, including British Retail Consortium (BRC; 24%), Safe and Local Supplier Approval (SALSA; 16%) and Soil Association (12%). Statistical analysis determined that the alcoholic drink sector was less likely to have accreditation ($P < 0.05$). The cereals & snacks sector was more likely to have SALSA ($P < 0.01$) and the dairy sector most likely to have Soil Association accreditation ($P < 0.001$). Micro-FDMPBs with <10 employees/turnover < £250,000 were associated with not having accreditation ($P < 0.001$). SALSA was most frequent among small/medium-FDMPBs, and BRC accreditation was associated with large-FDMPBs with > 250 employees/turnover > £50M ($P < 0.001$). FDMPBs with accreditation were more likely to supply wholesale ($P < 0.05$), major retailers ($P < 0.001$), major food service ($P < 0.001$) and the public sector ($P < 0.001$); those without accreditation were associated with selling direct to the public ($P < 0.005$).

Significance: Significant associations were determined between sector, size and accreditation. However findings indicate a need to identify the barriers to micro/small-FDMPBs in Wales obtaining

technical accreditation/food-safety compliance. Such data may inform the development of support mechanisms to enable increased accreditation and accelerate food sector growth in line with Welsh Government aspirations.

P1-50 Auditing of Official Control in Finnish Red Meat Slaughterhouses

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Introduction: The quality of meat inspection and food safety inspections (including verification of the slaughterhouses' own-check systems) performed by the official veterinarians (OVs) in the slaughterhouses is important for meat safety. The EU member states have to ensure that official control in slaughterhouses is performed according to the regulations. Every high-capacity slaughterhouse in Finland has a chief OV who has the main responsibility for the meat and food safety inspections. In Finland, the Finnish Food Safety Authority (Evira) performs internal audits to verify the quality of the official control in slaughterhouses. In 2009–2013, Evira performed on average 9 audits of meat or food safety inspections in high-capacity slaughterhouses annually.

Purpose: The aims of our study were to investigate the importance and advantages of the audits performed by Evira in the slaughterhouses.

Methods: In May 2015, chief OVs from 11 of the 13 red meat high-capacity slaughterhouses in Finland participated in a standardized, open-ended interview. The interviewees were asked to evaluate the importance of auditing on a scale from 1 (very unimportant) to 10 (very important) and to describe the benefits gained from auditing. Also the preferred frequency of the audits was inquired.

Results: The chief OVs assessed it important that meat inspection (mean 7.9, 95% confidence interval [CI], 7.0–8.8) and food safety inspections (mean 8.2, 95% CI 7.4–8.9) in the red meat slaughterhouses are audited by Evira. During the audits, OVs received feedback that enabled them to improve their inspections and most of the interviewees also stated that the audits hastened the correction of the slaughterhouse's non-compliances. The interviewees considered that auditing of both meat and food safety inspections should be performed at least every second or third year.

Significance: Regular audits of official control in slaughterhouses contribute to the quality of meat and food safety inspections.

P1-51 Food Business Operators' Views on Official Food Control in Approved Establishments

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Introduction: Effective food safety risk management in food production is a basis for preventing food borne

outbreaks in the food supply chain. Food business operators' (FBO) attitudes towards official food control may affect their food premises' hygiene level and food safety. Understanding the factors affecting FBOs' perceptions of food control is essential for enhancing effective food control practices and food safety.

Purpose: The aim of the study was to survey FBOs' opinions about significance and uniformity of official food control.

Methods: An inquiry examining FBOs' views on official food control and food safety inspections was sent to all approved meat, fish and dairy establishments ($n = 634$) under municipal food control in Finland in fall 2015. Statistical analysis was performed using SPSS statistical software.

Results: Only 33.1% (39/118) of the respondents analyzed ($n = 126$) considered official control to be uniform, and 20.2% (24/119) perceived that official control has been arbitrary. However, 84.5% (104/123) of the respondents considered official control of their establishment as significant for food safety. The better the respondents assessed the cooperation with the inspector, the more uniform and the more beneficial for their establishment's hygiene they perceived official control to be (Spearman's ρ $P < 0.000$ and $P < 0.000$, respectively).

Significance: Even though most FBOs perceive official food control as significant for food safety, there is a rather notable group finding deficiencies in its uniformity and consistency. Cooperative approach in the inspecting procedures should be emphasized as it seems to affect positively on FBOs' views on official food control and thus presumably has positive effects on food safety. Further analysis of the data is needed to detect the features behind the negative views on official food control.

P1-52 The Content of Histamine in Fresh and Smoked Fish Commercially Available in Poland

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Introduction: Biogenic amines, including histamine, belong to compounds that can affect human health. Histamine naturally occurs in many species of fish with dark meat, especially of the families: *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, and *Pomatomidae*. A high concentration of histamine in fish and fish products may cause food poisoning in humans (scombrototoxic fish poisoning; SFP). This intoxication causes cardiovascular, gastrointestinal, and neurological symptoms, such as skin rashes, urticaria, oedema, local inflammation, nausea, vomiting, diarrhoea, cramping, hypotension, headache, palpitation, and oral burning

Purpose: The aim of this study was to evaluate the histamine level in fish/fish products available at retail in Poland.

Methods: The high performance liquid chromatography with diode array detection (reference method in accordance with the Commission Regulation No. 2073/2005) was used in the study.

Results: A total of 199 samples of fresh and 35 samples of smoked fish were collected from various local shops in the south-east region of Poland. After purchasing, the samples were immediately delivered to the laboratory at refrigerated conditions and then processed. Histamine was detected in 32 of 199 samples of fresh fish (16.1 %) and in four out of 35 samples of smoked fish. The concentration of histamine in fresh fish ranged between 2.6 and 156.41 mg/kg. The maximum amount of histamine (156.41 mg/kg) was found in the salmon. In smoked fish, histamine was in range of 4.34–24.05 mg/kg. In all examined samples the content of histamine was below the admissible limit (200 mg/kg). The study showed that fresh fish meet the food safety criteria for histamine listed in the Commission Regulation (EC) No. 2073/2005.

Significance: The low content of histamine in fish and fish products is an indicator of freshness and shelf life, which showed that fish were fresh and manufacturing process was carried out correctly. The results obtained demonstrated that fresh fish available on Polish markets are safe for the consumers.

P1-53 Allergen Protein Content Determination of Korean Rice Cultivars in Korea

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Introduction: Rice is the staple food of Asian countries including Korea. Though rice is known to be a relatively low allergenic food and there are few reports of rice allergies, an increase in the number of patients sensitized to rice allergen with or without clinical symptoms has been reported recently. There are some reports about the protein allergens as the major IgE-binding components in rice. However, the rice allergen contents and the immunologic characteristics of rice allergen are unclear. The aim of this study was to identify the rice allergen and differences between Korean cultivars.

Purpose: This study was designed to determine the contents of major allergens in Korean rice cultivars. Further, we would like to analyze correlation between allergen contents and significance.

Methods: Among the known allergens, we selected two major allergens of rice, glyoxalase 1 and α -amylase inhibitor. After the production of major allergens by genetic expression, antibodies were made for each allergen. Twenty Korean cultivars were cultivated and collected. After the extraction of total protein from the grains, we examined the contents of each allergen by ELISA and compared between cultivar. All of these data were reviewed.

Results: The selected allergen contents of Korean rice cultivars were measured. Some rice cultivars had higher allergen content than others. The Nakdong and Sobi cultivars had the largest content of glyoxalase in grain which was about 500 ng/g dry weight. The average glyoxalase contents were 322 ng/g dry

weight. In an amylase inhibitor, Kumoh and Nakdong cultivars had larger contents than others. The average amylase inhibitor contents were 105 ng/g dry weight.

Significance: The contents of major allergens were measured in Korean rice cultivars. These results can be used for food safety assumptions and identifying allergenic reactions.

P1-54 Evaluation of the Thermo Scientific SureTect Real-time PCR Assay Method for Detection of *Cronobacter* species in Powdered Infant Formula (PIF)

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Introduction: ISO/DIS 22964:2015 Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp., does not specify a sample size for analysis, providing flexibility for testing laboratories. Lactic acid bacteria (LAB) used to supplement probiotic PIF reduce the pH of standard enrichment media during incubation causing inhibition of *Cronobacter* growth; with larger samples, the greater ratio of LAB to *Cronobacter* could amplify the pH drop and inhibit growth of *Cronobacter* further.

Purpose: This study aimed to verify performance of an alternative selective enrichment broth for 300g probiotic PIF samples, followed by the Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay against the proposed ISO method with 10 g probiotic PIF samples.

Methods: Eighteen samples from five different probiotic PIF brands were spiked with 0-10 CFU per sample of desiccated *Cronobacter*. Samples were diluted 1/10 in either BPW or the alternative selective enrichment broth and incubated at 37 ± 2°C for 16–19 hours. Post incubation, samples enriched with the alternative broth were tested using the SureTect *Cronobacter* spp. Assay. Culture confirmations were performed for all samples as detailed in the ISO method.

Results: The SureTect Assay method correctly identified an additional 11% of samples as positive compared to the ISO method after 16 hours enrichment, rising to 33% after 19 hours. The pH dropped during enrichment between 16 and 19 hours, likely caused by growth of LAB, which resulted in die-off of *Cronobacter* spp. The pH of ISO samples post enrichment was on average 2 pH units lower than the corresponding enrichment broth used with the SureTect Assay method.

Significance: The proposed new SureTect Assay method for detection of *Cronobacter* spp. with 300g PIF samples outperforms the ISO method when analysing 10g samples. The SureTect Assay method is faster and detects the presence of *Cronobacter* species in a higher number of samples compared with the ISO method.

P2-01 An Italian Micro Point of View, in the Implementation of "Food Safety" in SMEs

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Introduction: Euroservizi Impresa Srl (ESI), an Italian consulting company throughout Italy and northern EU, has proposed its "Global Management System" or "ESI Methods" including the multiple and simultaneous management of food safety, safety on workplace and privacy code, to the Italian and Northern European food companies.

Purpose: With the introduction of HACCP and the inclusion of the concept of self-control, Italian and European food companies have gone from a control on the final product to a preventive control of the entire chain. Italian and European food companies are characterized by a small dimension; in fact 99% have from 2 to less than 10 workers. We will try to explain how in the EU Union, characterized by micro enterprises, it was possible to adapt to a radical regulatory change.

Methods: The new regulations were transformed from simple sheets in black and white to color-rich designs manual that transformed the new concepts in friendly characters. The winning idea was changing the whole communication system; thanks to this changing the companies' managers easily understand the new language of the EU Regulations.

Results: The change of behavior has been made possible by the simple understanding of growth opportunities created by the new food safety system. Due to the commitment and openness to change of the client companies, regulatory compliance has been achieved in just two years in 98% of the total.

Significance: Behaviour change is possible. It was difficult at the starting point but became amazing when we understood the right channels of communication that would facilitate individuals and companies to achieve the proper implementation of laws and standards and the protection of public health.

P2-02 Edible Insects, New Future Food: Threats and Opportunity

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Introduction: Edible insects could be the future source of protein for human consumption in a growing world population. The edible insect resource is a category of non-wood forest products collected from natural resources such as aquatic ecosystems, forests and agricultural fields.

Purpose: Feeding a growing world population with more demanding consumers will necessarily require an increase in food production. This will inevitably place heavy pressure on already limited resources such as land, oceans, fertilizers, water and energy. If agricultural production, deforestation, and environmental degradation continues in its present form, this pressure is set to continue. For these reasons this kind of new food could represent a real substitute for all usual food animal proteins.

Methods: The different possible types of ecosystems, species and livestock rearing systems were analyzed. The health risks related to the consumption of insects and the benefits to the environment and society were considered.

Results: Consuming insects has a number of advantages: they have high feed-conversion efficiency (an animal's capacity to convert feed mass into increased body mass, represented as kg of feed per kg of weight gain); they can be reared on organic side streams, reducing environmental contamination while adding value to waste; they require significantly less water than cattle rearing; they have few animal welfare issues, although the extent to which insects experience pain is largely unknown; and they pose a low risk of transmitting zoonotic infections.

Significance: Edible insects could be a promising alternative for the conventional production of meat, either for direct human consumption or for indirect use as feedstock. Eating insects is not only good for health, it is good for the planet.

P2-03 Organoleptic Characteristics of Camel and Donkey Milk – A New Opportunity for Human People Intolerant to Milk

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Introduction: Milk is one of the most common causes of food allergies among children under one year of age. No specific therapy exists for this allergy, and thus the only feasible response is to avoid consumption of milk and derived products. Camel and donkey milk are alternatives.

Purpose: Analyze the nutritional characteristics of the milk of camel and donkey in appraising their specific properties.

Methods: Milk samples were collected in sterile containers and stored at -40°C until analysis. The samples taken at the same stage of lactation were thawed, pooled, and portions were taken for analyses. *Camelus dromedaries* milk (20 samples) was obtained from healthy camel; *Equus asinus* milk (20 samples) was obtained from healthy donkey. Camel and donkey milk samples were analyzed for pH, fat, total protein (casein, whey protein), lactose, and mineral content.

Results: Camel milk contained higher protein and less lactose as compared to human milk. Camel milk is known for its glycemic control effect, for high content of protein, casein, potassium and Vitamin C. Donkey milk contains protective proteins and also a higher amount of zinc, was shown to be lower in protein and fat and richer in lactose, which is more similar to human milk than to other mammalian milk.

Significance: The donkey's milk is recommended for the containment of allergies to cow's milk proteins in children and adults, convalescent patients, the regularization of the gastrointestinal flora, prevention of cardiovascular disease, inflammatory and autoimmune diseases, certain diseases of geriatric relevance, etc. Milk powder based on both camel and donkey milk could be a good alternative for infants or newborns deprived of mother's milk.

P2-04 Cumulative Kite Project Evaluation: Potential Impact upon Food Technology and Food Safety in Small and Medium-Sized Enterprises (SMEs) in Wales and Alignment with Government Priorities

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Introduction: The Food and Drink Manufacturing and Processing (FDMP) industry is a fundamental element of the food supply chain. In Wales (UK), the majority (98%) of FDMP businesses are SMEs; technical competence, compliance to food safety regulations and obtaining 3rd party accreditations are essential for business sustainability, innovation and growth. In 2009 a Welsh Government/European Union 'Knowledge-Innovation-Technology-Exchange (KITE) feasibility project was launched to facilitate collaborative partnerships between industrial(SME)partners, knowledge-based partners and affiliates(graduate/individuals with industrial-experience), to enable knowledge transfer and improving necessary scientific/technical/food safety skills to deliver required business needs.

Purpose: This study aims to undertake an end-of-project evaluation of the KITE project by assessing impact in FDMP SMEs, particularly related to food safety, technical compliance and accreditation. Cumulative outputs/outcomes will be aligned with 2014-2020 Government food industry strategies/priorities.

Methods: A mixed-method approach was undertaken for a process and output/outcome evaluation of the entire KITE project. Project impact was determined analysing key recorded outputs, in-depth qualitative consultations with KITE partners (n = 36) and administering quantitative evaluation questionnaires to partners in all KITE programmes (n = 92). Project approaches, reports, documentation and media articles (n = >200) relating to SME partners were evaluated using content analysis.

Results: Cumulatively, data indicated efficient delivery of 92KITE programmes in a variety of food sectors (40% processed foods/liquids; 39% bakery/confectionary; 21% dairy). Overall, SME partners

reported £103.3million increased sales as a result of strengthened technical performance. KITE facilitated attainment of 83 2nd/3rd party accreditations (e.g., British Retail Consortium) which enabled improved SME technical/food safety knowledge as well as business sustainability, increased market potential and new contracts. Over 1700 jobs have been reportedly safeguarded/created (including increased food technologists employed in Wales) and > 500 new products developed and launched using innovative/novel approaches.

Significance: Critical technical support provided through KITE has considerably benefited the Welsh FDMP sector; outputs delivered not only significantly exceed project targets but also meet Government needs planned for the FDMP industry 2014-2020. KITE is a significant tool to facilitate improved food safety/technical performance in a worldwide market.

P2-05 A Review of Consumer Food Safety Research to Identify Domestic Risk Factors Associated with Listeriosis

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Introduction: Listeriosis is associated with the highest hospitalization and mortality rates of foodborne illnesses; listeriosis incidence has doubled in recent years in Europe. Consumer recommendations to reduce listeriosis risk factors include following 'use-by' dates on unopened ready-to-eat (RTE) food products, avoiding prolonged storage of opened RTE foods and ensuring safe refrigeration temperatures. Currently, data detailing consumer cognition and behaviour associated with listeriosis risk factors are lacking.

Purpose: This study aimed to review consumer food safety studies to consolidate and cumulatively determine consumer cognitive and behavioural risk factors that may be associated with listeriosis in the home.

Methods: Consumer food safety research data (n = 200) were reviewed and analysed using a content analysis approach. Findings were summarized according to assessment of knowledge, attitudes, self-reported practices, and/or actual behaviours of listeriosis risk factors.

Results: Overall, only 43% of studies assessed consumer cognitive or behavioural data associated with listeriosis risk factors; 27% assessed refrigeration practices, 23% determined storage length of opened RTE foods and 21% ascertain adherence of 'use-by' dates. Majority (71%) of studies utilized survey based data collection methods (questionnaires/interviews), consequently, the majority of findings were based on self-report (78%) and knowledge (59%). Observation (21%) and focus groups (8%) were less commonly used. Consequently findings of this study indicate that actual behaviours and attitudinal data relating to listeriosis risk factors are lacking. Although findings suggest consumers may deviate from recommended practices, a lack of observational data suggests a need

to determine the actual behaviours of consumers in domestic kitchens in relation to listeriosis risk factors.

Significance: This review reveals a need for in-depth research to determine food safety attitudes and actual behaviours of consumers in conjunction with self-reported practices and knowledge of listeriosis risk factors. Such data combined with review findings would inform development of targeted food safety education to reduce risks associated with listeriosis in the home.

P2-06 A Review of UK Food Safety Information Provision for Chemotherapy Patients

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Introduction: Chemotherapy patients have an increased risk of foodborne illnesses as a result of immunosuppression, and are reported to have a five-times greater risk of listeriosis. To enable chemotherapy patients/carers to minimise risk of illness by implementation of risk-reducing behaviours is essential. Provision of food safety information prior to and during treatment is needed to raise awareness of the potential risks relating to foodborne illness by informing patients/carers of control measures and responsibilities of reducing critical risk factors.

Purpose: The aim of this study was to review food-related information available to chemotherapy patients/carers in the UK and evaluate the inclusion of risk-reducing food-safety behaviours.

Methods: Food-related information available to chemotherapy patients/carers in the UK were collected from health care providers including UK NHS trusts. Sources were reviewed and analysed using a content analysis approach. Findings were summarized according to key topics critical to food safety and listeriosis, (e.g., refrigeration practices, cross-contamination, consumption of at-risk food products).

Results: Overall, food-related information for cancer patients was obtained from 42 of 141 NHS chemotherapy providers and three UK cancer charities. Although 64% explained why patients are at an increased risk of developing infection during treatment, many failed to highlight the importance of food safety to prevent infection. Recommendations to ensure thorough cooking were most frequently included, although 42% recommended the avoidance of raw meat, poultry and fish, only 9% recommended the use of a thermometer to achieve a core temperature of 75°C. Practices relating to avoiding listeriosis were particularly lacking.

Significance: Although information is available, considerable gaps exist and information provided varies greatly between sources. There is a need to establish the potential impact of such food-related information sources on cancer patient/carer food safety knowledge, attitudes towards reducing the risks of foodborne disease during chemotherapy treatment and implementation of risk-reducing food safety practices in the home during chemotherapy.

Such data will inform the development of food safety education interventions targeting patients/carers.

P2-07 Self-reported Food Safety Perception among Home and Consumer Studies Students in Relation to Knowledge and Trust

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Introduction: Our behavior might be affected by our source of knowledge as well as our trust for that source. Confidence in an unreliable source can mean that a risk behavior in relation to food safety is developed which might imply an increased risk for foodborne infections for the individual consumer. High credibility for a source also means less chance that a behavior will change. Historically the home has been a place for food safety knowledge to be transferred through generations but due to lifestyle changes this has been reported to decrease. If true, what new sources have come instead and how does teaching of Home and Consumer Studies stand as a source of knowledge and trust?

Purpose: To investigate sources of knowledge and trust for different information sources in relation to food safety among students in school Year 9 in the Swedish Compulsory School.

Methods: A national questionnaire survey was conducted by using a Student Response System at the participating schools. A total of 529 students at 16 schools located at different parts of Sweden participated.

Results: Mother was reported to be the most common as well as the most trusted source for knowledge, especially among girls. Home and Consumer Studies teaching was however reported to be of importance in relation to food safety knowledge, especially among boys who seldom reported to cook at home. Boys reported to be more at risk in terms of food safety and to have higher confidence for other more uncertain sources.

Significance: The results indicate that food safety in Home and Consumer Studies teaching needs to be improved in order to be an extensive and reliable source for food safety knowledge communication. This is of importance for all students as future food consumers, but extra important for the boys.

P2-08 The Government's Implication in Accelerating Food Safety Implementation

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Introduction: The food industry is subject to various regulations to insure safety. These rules are often set-up by the industry and the government. Companies like GWR consulting offer technical counselling and guidance to restaurants to ensure the implementation of food safety regulations. Such a task

is, however, costly in time and effort. In October 2014, the Lebanese Ministry of Health (MOH) initiated a strict food safety campaign on practices on the Lebanese food business.

Purpose: To determine if the MOH attention to food safety frameworks in Lebanon facilitated the implementation of safety standards in restaurants.

Methods: Food Safety reports from 56 randomly selected GWR clients were collected over 12 consecutive food safety inspections. Clients were divided into two groups. The first group (Group A, $n = 28$) included restaurants who were clients at least 12 months before the start of the MOH campaign. The second group (Group B, $n = 28$) included restaurants who became clients upon the MOH campaign initiation. Data regarding food safety practice were collected using the GWR Food Safety Questionnaire (FSQ) based on Codex Alimentarius standards. A passing score of 80% on the questionnaire was used as an internal standard for a minimal safe system.

Results: FSQ scores were improved by 13 to 15% in both groups across the 12 audits and both groups reached the 80% threshold score. Group B, however, reached the desired passing (80%) score only after 4 audits and 4 audits (4 month) before Group A. Both groups maintained a score over 80% for the remainder of the year.

Significance: This study demonstrates that enforcement of food safety practices by the Lebanese MOH sped up the correct implementation of food safety standards. National legal frameworks are essential for an effective and timely food safety improvement.

P2-09 Hygiene and Food Safety in Viana do Castelo School Canteens

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Introduction: School canteens are intended for a population and age group in which nutritional balance is critical and must follow strict hygiene and food safety criteria in order to safeguard the potential occurrence of foodborne outbreaks.

Purpose: The objective of this study was to assess the hygiene/sanitation and food safety conditions in canteens of primary schools and kindergartens in the municipality of Viana do Castelo, Portugal in two distinct periods.

Methods: This cross-sectional descriptive study was carried out in 17 school canteens. The sampling instrument used throughout the audits was a checklist, based on Portuguese and European legislation. Following the application of the checklist, the results were transformed into “conformities”, “non-conformities” and “not applicable.”

Results: The application of the checklist in canteens achieved a compliance rate ranging from 47% to 67% in 2007, a rate that increased in 2014 when the compliance percentage recorded ranged from 62% to 80%. While in 2007, the hygiene and food safety

conditions were described as being “acceptable” and “not acceptable,” in 2014 they were considered to be “acceptable” and “good.” It can be concluded that over this 7-year period there was a positive evolution in school canteens regarding hygiene and food safety. However, there are still major non-conformities to be corrected. The increase of hygiene and food safety standards and the elimination of the existing non-conformities can only be achieved with the help and commitment of schools and fund managers.

Significance: The aim of this study was to evaluate the evolution of hygiene and food safety conditions in elementary schools and kindergartens’ canteens in the municipality of Viana do Castelo, Portugal in two distinct time periods, separated by 7 years.

P2-10 Food Consumption, Food Handling, and Diarrheal Illness

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Introduction: Food safety education is designed to teach consumers about risks in foods and how those risks may be avoided or mitigated. Previous research has shown that educational interventions improve food safety knowledge and self-reported behaviors in the home. However, the evidence on effects of behavior on actual risk are mixed, with, for example, Azevedo et. al (2014) finding no link between behavior and presence of microorganisms in Portugal, while Sheth and Obrah (2004) found a link between education, behavioral change, and reduced diarrheal illness in India.

Purpose: This study seeks to evaluate the effect of self-reported food consumption and food handling behavior (at home) on incidence of diarrheal illness in the United States.

Methods: Data is taken from a supplement to the Behavioral Risk Factor Surveillance System and was obtained directly from four states that participated in the supplement. The cleaned data yielded a working sample of 8,870 observations. OLS and Probit regression models were used to assess the effect of cross-contamination and food consumption variables on incidence of diarrheal illnesses, controlling for other risk factors and demographics. Multiple model specifications were examined to assess the robustness of results.

Results: Approximately 11% of the sample had experienced diarrheal illness in the last month, over 60% of respondents engage in optimal food handling practices, and between 1.3% and 32.0% of respondents consume various risky foods. Self-reported optimal food handling behaviors have no significant effect on diarrheal illness, while consumption of some risky foods (pink hamburger, raw eggs) increases the risk of diarrhea ($P < 0.05$).

Significance: These results suggest that we should be cautious in asserting that self-reported reductions in food handling behaviors are equivalent to reductions in foodborne illness.

P2-11* Effect of Adaptation to Acetic Acid and Low pH on the Acid Resistance of *Salmonella enterica* ssp. *enterica* Serovar Enteritidis in Laboratory Medium and Mayonnaise

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Introduction: It is well known that bacteria are able to activate mechanisms that help them survive under adverse conditions, thus rendering the effectiveness of hurdle technology questionable.

Purpose: To investigate the impact of exposure to sublethal acid conditions (low pH in the presence of undissociated acetic acid) on the resistance of *Salmonella* Enteritidis to lethal pH (laboratory medium, mayonnaise).

Methods: Growth and adaptation of *Salmonella* was performed in Tryptone Soy Broth without dextrose (TSB Glu(-)), while acid challenge in TSB adjusted to pH 2.5 with HCl and mayonnaise. For broth experiments, different concentrations of total acetic acid (AA; 15, 25 and 35mM) were used and the pH was adjusted to 4.0, 4.5, 5.0, 5.5 and 6.0 using HCl/NaOH. Non-AA adapted cells (0mM/pH 4.0, 4.5, 5.0, 5.5 and 6.0) were also used. Based on the above experiments, two acetic-adaptation inocula, those showing the highest (15mM/pH 6) and lowest (35mM/pH 5.5) reduction together with non-AA adapted cells (0mM/pH 5.5 and 6.0), were inoculated into commercial packages of mayonnaise (initial pH 3.9). Samples were stored at 5°C and 10°C. Non-adapted cells were grown in non-acidified TSB Glu(-). Experiments were conducted twice in two replicates.

Results: In broth experiments, cells adapted to AA, and especially those adapted within a range of dissociated AA (e.g., 35mM/pH 5.5), were countable for longer time than those pre-exposed to HCl. Non-adapted cells had the fastest reduction. However, in mayonnaise experiments, non-adapted cells together with cells adapted to AA 35mM/pH 5.5 remained countable for longer time compared to the other inoculum preparations. *Salmonella* was detectable in all samples by enrichment at the end of storage period.

Significance: Our findings can provide new insight concerning the acid response of *Salmonella* Enteritidis after exposure to sub lethal acid conditions and may help food industries improve food safety strategies.

P2-12 Intercellular Production of H₂O₂ in Tomatoes as a Prevention to *Salmonella* Proliferation

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Introduction: Due to increases in produce associated gastroenteritis, it is becoming clearer

that we know little about the ecology of human pathogens in vegetables. Previous research has shown that *Salmonella* (14028) can grow in numbers up to 10⁵ compared against the initial inoculum. It was also found that Piccolo (cherry) tomatoes were generally less conducive to *Salmonella* than any of the larger cultivars (up to 2 logs less).

Purpose: We hypothesise that cherry tomatoes naturally produce higher levels of H₂O₂ in their intercellular fluid than larger tomatoes.

Methods: Around 100 cells of *Salmonella* were inoculated in wounds under the pericarp in both the cherry and Alicante (larger) tomatoes. *Salmonella* proliferation was measured after a week of incubation by plating on Xylose lysine deoxycholate agar (XLD agar). To test H₂O₂ levels a novel method was used. Small pieces of the pericarp (from both cultivars) totaling 2g were subjected to a pressured vacuum to infiltrate distilled water into the intercellular space. The pieces were then spun in a centrifuge to extract all fluid from the intercellular space. Samples were de-proteinised using 10kD spin columns. A fluorometric assay was then used to test the concentration of H₂O₂. Four biological and two technical replicas were carried out.

Results: The increase of *Salmonella* from one week of initial incubation was up to 10⁴ CFU/tomato in Alicante cultivar, and 10² CFU/tomato in the cherry cultivar. The difference in *Salmonella* proliferation was significant ($\alpha = 0.05$). The cherry tomatoes showed an average of 0.76nmol/ml of H₂O₂ whereas the Alicante cultivar showed an average of 0.46nmol/ml. The difference in H₂O₂ concentration between the tomato cultivars was significant.

Significance: These findings open opportunities into genetic research of the tomato. The oOxyR gene in *Salmonella* activates the regulon of H₂O₂ inducible genes. Such pathways can be involved in *Salmonella* proliferation in tomatoes.

P2-13 Toxin Produced or Not Produced during Food Manufacturing?

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Introduction: Cereulide is a toxin, provoking nausea and vomiting, produced by *Bacillus cereus* and commonly found in food due to its ubiquity in environment. Cereulide is raising increased concerns in food industry as it is performed in food and cannot be inactivated due to its extreme heat and pH stability properties.

Purpose: While *B. cereus* growth boundaries at various temperatures are well established, less is known regarding cereulide production boundaries.

The gathered data will help industry with food safety decisions and improvement of existing HACCP studies.

Methods: Gene transcription, translation, cereulide production and *B. cereus* growth were therefore studied with the toolbox for toxigenic *Bacillus cereus* in broth medium at temperatures ranging from 12 to 46°C.

Results: The results of this study showed that cereulide production is highly dependent on temperature. Additional experiments performed in food matrices showed that cereulide production is also highly matrix dependent.

Significance: As storage is a common manufacturing step where time and temperature settings are important, these results provide scientific data to assist food safety decisions in case of abusive storage conditions. They challenge as well existing food safety margins based on *B. cereus* growth only.

P2-14* Withdrawn

P2-15* Molecular Identification and *In Vitro* Evaluation of Fungal Growth from Sweet Brioche-like Products

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Introduction: Brioche-like products are considered to be intermediate moisture foods, which are significantly susceptible to fungal spoilage and sensory deterioration.

Purpose: Molecular identification and determination of *in vitro* growth potential of fungi isolated from filled brioche products and their raw materials.

Methods: Freshly produced praline-, biscuit- or strawberry- filled brioche or raw materials (flour, fillings) were supplied daily by the manufacturer and analyzed immediately or during their shelf life (20–37°C, 50–60 days) for fungal presence. A total of 91 fungal isolates were molecularly identified by sequencing the internal transcribed spacer (ITS) region. *In vitro* fungal growth of selected isolates (40 out of 91), representing the major fungal genera identified, was assessed with spot inoculation on Malt Extract Agar of pH 6.1 (adjusted with 5N NaOH) and a_w 0.99 (without glycerol) and 0.83 (adjusted with 38% v/v glycerol). The low a_w was studied to imitate the respective values of brioche and brioche fillings. Fungal growth was monitored at 25°C (recommended temperature by the industry) and 37°C (as temperature abuse) by measuring radial diameter for *max.* 60 days.

Results: More than 65% of fungal isolates were identified as *Penicillium* sp., followed by 20% of *Cladosporium* sp. and 7% of *Aspergillus* sp. *In vitro* growth assessment revealed that high a_w permitted the growth of all isolates ($n = 40$) at 25°C, whereas only 33% had a growth potential at 37°C, most of

which belonged to genus of *Penicillium*. On brioche-like media (a_w 0.83), no growth was observed at all isolates except for an *Aspergillus flavus* isolate at 37°C. Contrary to temperature abuse conditions, more than 80% of the isolates, which included all the identified genera, were able to grow at 25°C and a_w 0.83, highlighting the potential visual deterioration of brioche products during their shelf life.

Significance: Results of the present study could contribute to the identification and minimization of the risk of fungal spoilage in brioche-like products.

P2-16* Cold Atmospheric Pressure Plasma Treatment as an Innovative Approach for the Decontamination of Edible Insects and Insect-based Products

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Introduction: Edible insects represent a valuable alternative protein source but for successful marketing of insect-based food and feed products, food safety needs to be ensured. Cold atmospheric pressure plasma (CAPP) is a promising technology for the gentle surface decontamination of food and feed. Therefore nonthermal CAPP offers an innovative approach for improving microbial safety during post-harvest processing of insects and insect products.

Purpose: Aim of this work was to evaluate the impact of CAPP treatment on the surface and total microbial load of mealworms (*Tenebrio molitor*) and mealworm flour with the long-term goal of developing effective decontamination procedures for high-quality insect-based food and feed products.

Methods: *Tenebrio* larvae and flour were subjected to semi-direct CAPP treatment (dielectric barrier discharge, 3.0 kHz, 8.8 kV, in air) for up to 15 min. Plasma-induced effects on surface and total microbial load as well as on quality parameters of the insect products were investigated.

Results: The initial microbial load on the surface of the larvae was 6.9 log CFU/g_{DM}; the total microbial load was 7.5 log CFU/g_{DM}. Independent of the treatment time, exposure to CAPP led to complete inactivation of the surface contaminants, whereas the total microbial load of the larvae was reduced by 0.3, 1.2, 3.8, 4.0 and 4.5 log CFU/g_{DM} by exposure to CAPP for 2.5, 5, 7.5, 10 and 15 min, respectively. A plasma-induced mass loss of up to 10% was detected accompanied by a decrease in surface pH of the larvae from 6.9 to 5.3. Applying identical exposure times, CAPP treatment of the flour spoiled with initially 7.5 log CFU/g_{DM} led to a reduction of 1.1, 1.7, 2.3, 2.5 and 2.9 log CFU/g_{DM}. Plasma processing induced a slight mass loss (dry matter content of the flour 0.84 g/g) whereas often reported plasma-related pH shift did not occur.

Significance: Regarding safety aspects, results of this study clearly indicate the potential of the CAPP technology in the insect post-harvest chain.

P2-17 Time Temperature Indicators (TTI) Based on Chromogenic Bacterium *Janthinobacterium* sp

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Introduction: The cold chain of perishable food products can often be disrupted by temperature abuse. Time temperature indicators (TTI) or integrators are simple and inexpensive devices that make the continuous monitoring of the time temperature history of chilled products possible throughout the cold chain. *Janthinobacterium* sp. is a chromogenic bacterium which appears purple during its growth. The time that this bacterium colony appears purple depends on the pH value of the medium where it is cultivated, temperature and atmosphere composition.

Purpose: To develop TTI for food industry based on the chromogenic ability of *Janthinobacterium* sp.

Methods: Tryptone soy agar (TSA) with pH (HCl) of: 6, 6.5, 7, 8 or 9 was surface-inoculated with 3, 4, 5 or 6 log CFU/cm² of *Janthinobacterium* sp. and incubated aerobically at 0°C, 5°C, 10°C and 15°C for 17, 14, 7 and 6 days respectively. Microbiological and pH measurements of TSA were performed during incubation and the growth parameters of *Janthinobacterium* sp. at different conditions, were estimated using the Baranyi model. In parallel, image analysis was employed in order to explore its potential on estimating microbiological results. The maximum growth rate (μ_{max}) was used to determine through the Arrhenius equation the activation energy (E_a) of *Janthinobacterium* sp. for different pH.

Results: The optimum growth conditions for *Janthinobacterium* sp. used in the study were 25°C and pH=7 (under aerobic conditions). Results of microbiological analysis, showed that their population reached the highest levels (9-9.5 log CFU/g) about 72-100h at 15°C and 334h at 5°C. Significant pH changes (1.5-2.5 units) were observed in TSA samples with initial pH 6 or 6.5. The E_a ranged between 27.84 Kcal/mol (pH: 9) and 21.59 Kcal/mol (pH: 7). The E_a of the microbial growth rate in food ranges from 7.8 to 28.7 Kcal/mol. The endpoint (the time at which a distinct visual color change to the final purple was observed) of the TTI at these pH (9 and 7) when they incubated at 15°C were 118h and 92h, respectively.

Significance: Such a study could offer a new TTI in the food industry, based on the appearance of purple color of *Janthinobacterium* sp. as a signal of spoiled food product.

P2-18 Adhesion Ability of *Listeria monocytogenes* and Sensitivity to Food-contact Surface Sanitizers

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Introduction: Contamination by *Listeria monocytogenes* in food processing environments is difficult to control due to its wide dissemination and adaptation, which requires constant monitoring and adoption of efficient procedures to eliminate this pathogen.

Purpose: This study evaluated the occurrence of *L. monocytogenes* in the processing environment of a butcher shop, assessed the adhesion potential of obtained isolates, and checked their sensitivity to two sanitizers used during the cleaning in this facility.

Methods: Surface samples were obtained from tables, knives, inside of butcher displays, grinders and meat tenderizers (8 samples per point). All samples were subject to detection of *L. monocytogenes* according to ISO 11.290-1, and the obtained isolates were characterized by their serogroups and subjected to PCR for detection of virulence genes. Finally, the isolates were evaluated for *in vitro* adhesion capacity and sensitivity to two sanitizers: A (Mister MaxDG1™) and B (B-Quart Sept™).

Results: Of the total of 40 samples, 75% were positive for *Listeria* spp. and 22.5% for *L. monocytogenes*. Twenty isolates were characterized as belonging to serogroup 1/2c or 3c, and showed positive results for all checked virulence genes. All isolates showed some adhesion potential, ranging from weak to moderate, and only one isolate presented a strong adhesion. The evaluated sanitizers had the potential to inhibit the multiplication of isolates, being also able to inhibit adhesion and remove previously formed biofilms. After evaluation, the sanitizers were adopted by the butcher shop in its sanitation routine, and after two months surface samples were collected and none presented positive results for *L. monocytogenes*.

Significance: The results allowed us to characterize the *L. monocytogenes* contamination in the butcher shop and to predict their virulence potential. Collected data allowed identification of adhesion potential by *L. monocytogenes* and the effectiveness of the tested sanitizers to control contamination by this pathogen.

P2-19 Biofilm Matrix Composition Affects the Susceptibility of Food Associated Staphylococci to Cleaning and Disinfection Agents

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Introduction: Staphylococci are frequently found in food processing environments. Survival has been explained by tolerance to desiccation, efflux mechanisms leading to tolerance to disinfectants and biofilm formation.

Purpose: To investigate the role of the composition of the biofilm matrix produced by food-associated staphylococci for tolerance to cleaning and disinfection

Methods: Biofilm formation and detachment after exposure to enzymes degrading polysaccharide

(Dispersin B), proteins (Trypsin and Proteinase K) and the cleaning agent hypochlorite were investigated using a microtiter plate-based assay. Susceptibility to the disinfectant benzalkonium chloride was tested in suspension and using a biofilm assay with stainless steel coupons (exposure at 20°C, 5 min). The presence of biofilm associated genes was investigated by whole genome sequencing.

Results: Three out of eight food-associated coagulase negative staphylococci appeared to produce a matrix composed of polysaccharide based on presence of the *ica* operon (known to encode a polysaccharide matrix), and results showing that biofilms were detached by dispersin B but not by proteinase K or trypsin. The remaining five strains studied appeared to produce a matrix mainly composed by protein as biofilms were detached by exposure to proteinase K or trypsin but not Dispersin B. These strains were *ica* negative and three isolates contained genes homologous to the biofilm associated protein *bap*. All biofilms were detached by alkaline hypochlorite. Strains producing protein matrix were more susceptible to the disinfectant benzalkonium chloride both in suspension and biofilm than strains producing a polysaccharide type matrix.

Significance: Failure of sanitation in food and clinical environments may be related to staphylococci producing a polysaccharide-based biofilm matrix. The use of cleaning agents that can degrade the matrix components is crucial to eliminate staphylococcal biofilms.

P2-20 Biological pH Reduction of Wine with the Use of Autochthonous Yeasts as Starter Cultures

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Introduction: Wine preservation is an important necessity in winemaking and sulfur dioxide addition is thus, considered indispensable. However, its use should be limited because of its negative effects on human health.

Purpose: A biological method to reduce the pH and increase the total acidity of wines by the use of autochthonous yeast starters is presented, in order that bacterial spoilage management is reinforced.

Methods: *Saccharomyces cerevisiae* strains 21PW6 and 47PW1 were inoculated in pasteurized musts in mono-cultures (“mono”) or in mixed-cultures along with *Lachancea thermotolerans* strains 27PL4 and 16PL2, respectively. Two modes of inoculation were tested in mixed-cultures, i.e., sequential (“seq”) inoculation of *L. thermotolerans* followed by *S. cerevisiae* and simultaneous inoculation (“sim”) of both species. Total acidity, pH, free and total SO₂, volatile acidity and major volatile compounds were determined and compared.

Results: Ferments produced through “seq” fermentations showed the lowest pH values (3.24

± 0.00) compared to “sim” (3.41 ± 0.03) or “mono” (3.51 ± 0.13). Total acidity was significantly increased in “sim” (8.39 ± g of tartaric acid /L), “mono” (7.06 ± g of tartaric acid /L), and at much higher levels in “seq” (15.24 ± g of tartaric acid /L). Differences were also detected in the major volatile compounds of the ferments. The presence of *L. thermotolerans* strains in fermentations altered the pH, total acidity, and volatile acidity of ferments, while its dominance in “seq” fermentations revealed its ability to strongly influence these parameters.

Significance: Here we present an inoculation protocol with *L. thermotolerans* strains which can significantly lower the pH value and raise the total acidity of wines. We anticipate its use as an alternative preservation tool in winemakers’ hands, towards the production of safer wines with fewer additives.

P2-21* Chemical and Microbiological Safety of the Street Vended Lego Drink in Abeokuta Metropolis

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Introduction: The study assessed the chemical and microbial safety of street vended Lego drink collected from three different vendors in Abeokuta metropolis.

Purpose: There is a high demand for these flavoured drinks especially among the school children and in the roadside shops, recreational areas (parks), and in the busy market places. The microbiological quality of the supplied juices remains questionable.

Methods: A control sample was prepared in the laboratory and nine samples of three different flavours (orange, pineapple and blackcurrant) were collected. Total *Vibrio* count, total faecal coliform and total Staphylococcus count were estimated in all the samples.

Results: Total *Vibrio cholera* was completely absent while five samples were found to harbour total staphylococcal counts of bacteria within the range of 0.7–1.5×10⁴CFU/ml. All the samples exhibited the presence of coliform counts within the range of 1.0–9.5×10⁴CFU/ml and *Staphylococcus aureus*, *Micrococcus acidophilus* and *Klebsiella aerogenes* species were bacteria isolated from the samples collected. Heavy metals were also estimated in all the collected samples. Chromium and lead were absent but cadmium was present and is within 0.01–0.025 mg/g. The lowest was from orange flavour with 0.01 mg/g while the highest was in pineapple flavour with 0.025 mg/g. Total titratable acidity ranges from 0.175 – 0.219 and the pH ranges from 4.21 ± 0.14 and 5.40 ± 0.00. Hence, this implies that Lego flavoured drink is bacteriologically and chemically unsafe for consumption.

Significance: Foodborne diseases may be associated with the consumption of these flavoured drinks due to poor processing and handling. This underscores the need for safety assessment of this emerging beverage mostly consumed by school children.

P2-22 Inhibition of *Clostridium sporogenes* by *Streptococcus thermophilus* ACA-DC 0040 under Conditions Simulating Gruyere Cheese Production and Ripening

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Introduction: The growth of several *Clostridium* spp. in hard cheeses is the main cause of spoilage. Additionally some *Clostridium* species, i.e., *Clostridium botulinum*, also produce toxins dangerous for the public health. Moreover, toxins can be produced without overt spoilage of hard cheeses during the ripening process.

Purpose: The objective of this study was to evaluate the ability of bacteriocin-producing strain *Streptococcus thermophilus* ACA-DC 0040 to inhibit *Clostridium sporogenes* C22/10 spores outgrowth under conditions prevailing during Gruyere cheese production and ripening. This species was used as a model for *C. botulinum*, since the fermentation products of its toxigenic strains are indistinguishable from those of *C. sporogenes* strains cultured in the same medium.

Methods: Two fermentations in skim milk were used under conditions mimicking cheese production and ripening. *S. thermophilus* ACA-DC 0040 (*bac*⁺) and *S. thermophilus* ACA-DC 0004 (*bac*) were used in fermentations A and B respectively, and 10³ cfu ml⁻¹. *C. sporogenes* spores were used to inoculate the fermentor for both fermentations. Fermentations were carried out in a 2.5-L glass fermentor with temperature and O₂ control. Samples were drawn every hour during fermentation and after 1, 5, 15, 30, and 60 days of ripening at 18°C for bacteriocin activity determination, microbial population enumeration and organic acid profile detection by HPLC chromatography.

Results: In fermentation A with the *bac*⁺ strain, Thermophilin T was produced during the initial stages of fermentation reaching up to 2560 AU ml⁻¹, which was reduced up to 320 AU ml⁻¹ during the ripening period. Neither vegetation nor outgrowth of *Clostridium* spores were detected during the whole ripening time. On the contrary, in fermentation B high outgrowth of *Clostridium* spores was counted after 15 days of ripening and high amounts of acetate (2.93 mmol) was detected from the 15th day and afterwards.

Significance: The quality and safety of Gruyere cheese was ensured in a biopreservation model.

P2-23 Assessment of Feta Cheese Adulteration in the Region of Thessaly, Greece – Implications for Consumer Protection

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Introduction: The threat of food adulteration in the field of milk and dairy products has reached a concerning level during the last years. The most common “fraud” in dairy products is the blending of different kinds of milk. These food adulterations have impact on both human health (allergies) and also on rural economy. In that sense, Feta cheese is considered a valuable product, where cow’s milk addition is not permitted, taking into account the PDO (Protected Designation of Origin) specifications.

Purpose: The aim of this study was the assessment of Feta cheese adulteration in the region of Thessaly, in order to evaluate the relevant risk for consumers.

Methods: Following a market research in dairy stores, restaurants and supermarkets in Thessaly, a total of 34 samples were collected and analyzed by Enzyme Linked Immunosorbent Assay (ELISA; BIO-SHIELD COW CHEESE-B1748).

Results: Adulterated Feta cheese samples were found in a percentage of 41.17% (14/34). Feta cheese adulteration levels ranged from 0.1% to 5%, while 80% of the adulterated samples were related to foodservice places.

Significance: Feta cheese adulteration rates in the region of Thessaly were relatively low but not zero, a fact that enhances the need for stricter controls, in order to ensure consumer’s protection. Interestingly, most adulterated samples originated from restaurants where the consumer has no access to the food labels.

P2-24 Selection of Potential Probiotic Lactic Acid Bacteria Isolated from Traditional Dairy Products by *In Vitro* Tests

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Introduction: Probiotics are food microorganisms able to survive in the gastrointestinal (GI) tract in populations sufficient to exert their beneficial effects to the host, such as antipathogenic, antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive and immunomodulatory properties.

Purpose: This study evaluated the probiotic potential of indigenous lactic acid bacteria (LAB) isolated from raw milk and traditional Greek PDO Galotyri and Graviera cheeses.

Methods: Thirty representative strains of various taxonomic groups of LAB isolates were subjected to *in vitro* assays simulating the conditions prevailing in the human GI tract. All strains were screened for resistance to lysozyme, acid pH, bile salts, proteolytic enzymes (pepsin, pancreatin) and bile salt hydrolysis (BSH) activity.

Results: Responses were strongly strain- rather than species-specific, with only seven strains, *Enterococcus faecium* KE82, *E. faecium* GL31, *Enterococcus durans* KE100, *E. durans* GL70, *Lactococcus lactis*

subsp. *lactis* KE109, *Lc. lactis* subsp. *cremoris* M104 and *Lactobacillus plantarum* H25, revealing a high probiotic potential. All strains were highly resistant to lysozyme, bile salts and pancreatin. Strains KE82 and H25 exhibited strong BSH activity towards taurodeoxycholate (TDC) and glycodeoxycholate (GDC), strains KE100, GL70 and GL31 hydrolyzed TDC strongly, whereas both lactococcal strains KE109 and M104 showed no BSH activity. Strains GL70 and M104 retained the highest viability (6 log CFU/ml) at pH 2.5 while the rest of them remained viable at levels of 4 to 5 log CFU/ml. However, only strains KE109, KE100, GL70, M104 and GL31 retained viability upon challenging at pH 2.0 in the presence of pepsin (3 mg/ml).

Significance: In conclusion, all seven strains above constitute good probiotic candidates for use in dairy foods. However, additional studies are required to validate their actual ability to colonize the human GI tract to express their potential probiotic capabilities *in vivo*.

P2-25 Analysis of Chemical Composition and Characterization of Bacterial Population of Kefir from Different Regions of Chile

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Introduction: Kefir is a beverage produced from the fermentation of milk by a mixture of lactic and acetic acid bacteria and yeast embedded in a natural polysaccharide-protein matrix. In Chile, kefir is typically made at home where it is known as “yogurt de pajaritos”, or “yogurt made by the little beings,” highlighting the lack of awareness of the wider public on how kefir is produced. Its consumption has been associated with health benefits, but it is scarcely consumed and not widely available in the market.

Purpose: To investigate potential differences in the chemical composition of Chilean kefir and the microbiological composition of kefir grains from diverse geographical areas of Chile.

Methods: Whole UHT milk was inoculated (10% w/v) with kefir grains obtained from three different geographical areas of Chile. Samples were incubated at 26°C until pH 4.5 was reached and then analyzed for total dry matter, fat, protein and ash contents (AOAC). Microbiological characterization of the kefir grains was carried out by nested PCR targeting the 16S rDNA V3 region. Denaturing gradient gel

electrophoresis (DGGE) of the PCR products was performed. Finally, purification and sequencing of the gel bands was completed by a commercial service.

Results: Differences ($P < 0.05$) were found between the three kefir samples for total solids (10.55 - 9.99 g/100^g), protein (2.86 - 2.71 g/100^g), ash (0.67 - 0.55 g/100^g) and carbohydrates (4.2-3.53 - g/100^g); no difference was found between the fat contents. Sequencing of the selected bands showed that *Lactobacillus kefirianofaciens*, *Lactobacillus helveticus* and *Lactobacillus crispatus* were present in all the samples.

Significance: The potential antimicrobial and probiotic activity of the kefir grain microbiota will be studied. By characterizing the kefir produced in Chile and the microorganisms forming part of the kefir grains, a commercial starter culture to produce a standardized kefir drink providing potential health benefits could be developed.

P2-26 Production of Bioactive Peptides and Probiotic Potential of Lactic Acid Bacteria Isolated from Traditional Greek Dairy Products

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Introduction: Fermented dairy products containing lactic acid bacteria (LAB) with bio-functional features are considered to be beneficial for human health. Probiotic potential and production of bioactive peptides are considered among the most promising features of LAB to be exploited as starter cultures in the industrial production of functional foods.

Purpose: In the present study, 106 LAB strains isolated from traditional Greek products have been studied regarding their probiotic potential as well as their ability to produce, when grown in cow, sheep and goat milk, bioactive peptides with inhibitory activity against the angiotensin-converting enzyme (ACE-I).

Methods: Strain typing and identification at the species level were performed by rep-PCR and 16S rDNA sequencing, respectively. The ACE-I activity was evaluated using a spectrophotometric assay. Semi-preparative HPLC, MS and MS/MS analyses were performed for ACE-I peptide purification and identification. Adhesion was studied using collagen-

coated microtiter plates and the human enteric cell line HT-29. Immunomodulatory properties were evaluated by an *in vitro* co-culture model with human THP1 cells, while expression of COX2, iNOS, IL-10 and IL-12 genes was estimated by qPCR.

Results: Among the 106 tested LAB strains, 77 exhibited ACE-I activity while MS/MS analysis revealed the production of ACE-I and immunomodulatory peptides deriving from α s1-, β - and κ -caseins. Ten and two strains were able to adhere to collagen plates or HT-29 cells, respectively. COX2 and iNOS were equivalently expressed at low levels, while three strains exhibited high ratios of IL-10/IL-12, an indicator of anti-inflammatory potential.

Significance: These results indicate the potential use of the strains under investigation in novel probiotic dairy products.

P2-27* **Kaimaki Type Ice Cream as a Food Carrier of the Probiotic Strain *Lactobacillus fermentum* ACA-DC 179**

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Introduction: Ice cream is an ideal matrix for delivery of probiotic organisms to the human body compared to fermented dairy products. The pH of ice cream is almost neutral, whereas that of fermented dairy products could be much lower, and low pH may affect the survival and metabolic activity of probiotic bacteria. Nevertheless, freezing and thawing may seriously damage the cells, causing death or growth inhibition and thus diminishing the potential advantages of probiotics.

Purpose: In the present study the probiotic strain *Lactobacillus fermentum* ACA-DC 179 was used for the production of probiotic Kaimaki type ice cream. The survival of the strain throughout production as well as the physicochemical and sensorial features of the final product were determined.

Methods: Ice cream was produced using full fat high temperature pasteurized milk. Incorporation (10% v/v) of *Lactobacillus fermentum* ACA-DC 179 milk cultures (10^7 CFU/ml) was performed either before or after ice cream mix ripening, while both fermented (16 h) and not fermented mixtures were considered. Microbiological analysis was performed on day 0, 7, 14 and 28. The final product was subjected to physicochemical analysis, i.e. acidity, pH, fat and protein content, while melting rate, overrun and sensorial characteristics were also examined.

Results: After 28 days of storage, levels of *Lactobacillus fermentum* ACA-DC 179 remained high (10^6 - 10^7 CFU/g), especially in the case of the fermented products. Although fermented ice cream samples showed higher acidity in comparison to the non-fermented ones, their physicochemical and sensorial properties were overall acceptable.

Significance: This is the first study on the application of the probiotic *Lactobacillus fermentum* ACA-DC 179 in ice cream production, with the final product being organoleptic appreciated.

P2-28* **Study on Coagulase Positive Staphylococci and Staphylococcal Enterotoxins Distributions in Naturally Contaminated Cheeses**

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Introduction: Small dairy productions have a high value in terms of cultural and quality of products and represent a significant economic resource but are often conditioned by the territories of production and the technologies applied. One of the hazards for dairy products is the presence of Staphylococcal Enterotoxins (SEs) in raw milk cheeses that typically have a heterogeneous distribution.

Purpose: In this work we studied the distribution of Coagulase Positive Staphylococci (CPS) and SEs in naturally contaminated cheeses, in order to establish the impact of the sampling sites.

Methods: Seven cheeses were analyzed for CPS enumeration (ISO 6888-2) and detection of SEs (European Screening Method). Each cheese was sampled on four different areas: peripheral and central rind, peripheral and central core. From each sample, up to two CPS isolates were identified and characterized for the presence of SE genes (multiplex PCR) and biotyped. In parallel, SEs were quantified (in house ELISA).

Results: The presence of CPS was observed in 23 (82%) samples; CPS amount ranged from <10 to 5.6×10^3 CFU/g for the core portions and from 2.0×10^3 to 8.6×10^6 CFU/g for the rind portions. Forty-eight isolates were identified as *S. aureus*; of these, 33 presented Human biotype (69%) and 15 Not-Specific Host (NSH) biotypes. In addition, we identified six different SE gene profiles that, combined with the biotype, permitted the identification of 10 profiles. SEA and SED were quantified in the seven cheeses with concentrations ranging from 0.011 to 2.71 ng/g and from 0.061 to 8.980 ng/g, respectively. These concentrations varied depending on the cheese and the area sampled.

Significance: This work underlined the heterogeneous distribution of CPS and SE in naturally contaminated cheeses highlighting potential sampling issue for CPS enumeration as well as SEs detection.

P2-29 Exploring the Microbial Consortia of Greek Table Olives Using Culture-Dependent and -Independent Approaches

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Introduction: Table olives are considered to be among the most significant fermented vegetables, mostly in Southern European countries. The microbial ecology of the table olive fermentation process and specifically the lactic acid bacteria (LAB) and yeast populations is of fundamental importance to obtain high quality products.

Purpose: Identification of LAB and yeast populations in the Kalamon, Conservolea and Amfissa olive cultivars using both culture-dependent and -independent techniques.

Methods: The olive samples were initially subjected to the classical microbiological analysis using selective growth media. The genomic DNA of the bacterial and yeast isolates was extracted as described previously. The isolates were grouped using the genotyping technique of rep-PCR and representative bacterial and yeast isolates of each group were identified at the species level by sequencing of the 16S rDNA gene and ITS DNA region, respectively. Concerning the metagenomics analysis, total DNA was extracted from the olive samples using a novel protocol developed in our laboratory and the results obtained from the diversity assay were analyzed with the MG-RAST server.

Results: Using culture-dependent approaches, two main species of LAB were identified, namely *Lactobacillus plantarum* and *Pediococcus ethanolidurans*, while *Pichia* was the most frequently isolated yeast genus. On the contrary, metagenomics analysis revealed a vast diversity of bacterial and yeast genera, i.e., 83 and 67, respectively.

Significance: This study is among the first reports on the metagenomics analysis of the microbial diversity of Greek olive cultivars.

P2-30 Complete Genome Sequence of the Dairy Isolate *Lactobacillus acidipiscis* ACA-DC 1533

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Introduction: Lactic acid bacteria constitute a significant group of microorganisms for the food industry, as they play a key role in food fermentation and consequently in human health. *Lactobacillus acidipiscis* is a recently described species and *L. acidipiscis* ACA-DC 1533 is a strain isolated from traditional Greek Kopanisti cheese.

Purpose: The *in silico* analysis of the first complete genome sequence of *L. acidipiscis* ACA-DC 1533.

Methods: The genomic DNA was extracted as described previously. Sequencing of *L. acidipiscis* genome was performed using the HiSeq 2000 and PacBio RSII sequencing platform technologies. SOAPdenovo software was used to assemble the reads after filtering, while SOAPsnv and SOAPindel were applied for error correction. Furthermore, the genome assembly was validated against an *NbeI* optical map of the *L. acidipiscis* genome. Protein-coding sequences were predicted by Glimmer, rRNA genes by RNAmmer, tRNA genes by the tRNAscan-SE server, tandem repeats by Tandem Repeat Finder and the reconstruction of metabolic pathways was performed using the KEGG pathway database.

Results: The sequencing analysis resulted in one continuous genomic scaffold of 2,678,726 bp with a G+C content of 39.75% and two plasmids of 3,554 (37.65% G+C content) and 13,512 (34.99% G+C content) bp. The genome contains 2,525 protein-coding genes on the chromosome covering up to 82.09% of the genome sequence. We also found 63 tRNA, 18 rRNA and 14 tandem repeat sequences. Finally, the reconstruction of metabolic pathways provided interesting information about the basic biological functions of the genome.

Significance: The *in silico* analysis of the first complete genome sequence of *L. acidipiscis*.

P2-31 Production of Traditional Greek PDO Galotyri Cheese Using *Lactococcus lactis* subsp. *cremoris* M104 and *Enterococcus faecium* GL31, Two Indigenous Bacteriocin-producing Strains with Probiotic Potential

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Introduction: Several indigenous strains of lactic acid bacteria (LAB) isolated from raw milk and traditional cheeses possess desirable combinations of enzymatic, antimicrobial and/or probiotic properties, and thus, are of high biotechnological interest for use as adjunct cultures to increase cheese quality and safety.

Purpose: This study evaluated *Lactococcus lactis* subsp. *cremoris* M104 and *Enterococcus faecium* GL31 as co-starter adjuncts in the production of PDO Galotyri cheese by a traditional method. Strains M104

and GL31 possess probiotic properties and produce nisin A and enterocin A, respectively, in milk.

Methods: Both strains were challenged against *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) in co-culture with a commercial thermophilic starter culture (CTSC) in pre-sterilized ewes' milk incubated at 37°C for 6 h and then shifted to 22°C for additional 42 h. Afterwards, combinations of the CTSC+M104 or the CTSC+M104+GL31 were used to produce Galotyri cheeses, which were evaluated microbiologically and for pH and sensory quality.

Results: Both pathogens increased by ca. 2 log units in all milk cultures after 6 h at 37°C. Following that, however, LM growth was inhibited completely by strain GL31 while strain M104 caused a 2-log reduction in LM viability. Growth and survival kinetics of SA were similar to LM after 24 h. At 48 h, however, SA was minimized in the presence of strains M104 and GL31. Both bioprotective LAB strains grew at levels higher than 7 to 8 log CFU/g in the presence of the CTSC in Galotyri cheeses, which had a pH below 4.4 and were of high microbiological and sensory quality.

Significance: *Lactococcus lactis* subsp. *cremoris* M104 and *E. faecium* GL31 are suitable for use as co-starter adjunct cultures to produce traditional PDO Galotyri cheese of high nutritional value and quality.

P2-32 High-throughput, Sequence-based Analysis of Kefir Microbiota

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Introduction: Kefir grains constitute complex microbial communities composed primarily of lactic acid bacteria and yeast. The kefir grain microbiota may vary depending on grain origin. Hence, the present study used a metagenomic approach to identify bacteria and yeast present in kefir grains of different origin.

Purpose: The purpose of the study was to elucidate the microbial composition of kefir grains of different origin using a high-throughput, sequence-based approach.

Methods: DNA was extracted from kefir grains originating from Athens (kefir A) and Crete (kefir B) using a commercial kit. Identification of bacteria and fungi was based on amplification and sequencing of the V4 variable region of the 16S rRNA gene and of the Internal Transcribed Spacer region, respectively. Sequencing was performed on an Illumina MiSeq instrument following the manufacturer's guidelines. Sequence data were processed to remove barcodes and primer sequences, denoised, and clustered at 3% divergence to generate Operational Taxonomic Units (OTUs) followed by removal of chimeric sequences. Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes, RDP II, and NCBI.

Results: A total of 730,517 raw sequences were generated. Firmicutes and Ascomycota were the predominant phyla for bacteria and fungi, respectively. Lactobacillaceae was the dominant family in both kefirs (ca. 98%) followed by Ruminococcaceae (0.6%), Lachnospiraceae (0.4%), Bacteroidaceae (0.2%) and Syntrophomonadaceae (0.2%). The microbiota of kefir A was more diverse, consisting of 124 different bacterial genera compared to 114 genera in kefir B. Fungal OTUs belonged to the family Dipodascaceae (99.9%) and the predominant genus was *Galactomyces*.

Significance: The detailed composition of the kefir microbiota from different sources obtained in this study will be valuable in order to screen for beneficial strains from this traditional fermented probiotic dairy product. Also, this study provides novel data on the microbial ecology of Greek kefir.

P2-33 Metagenomics Analysis of the Feta Cheese Microbial Ecosystem

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Introduction: Feta cheese is the flagship of the Greek cheeses with a complicated microbiota, which is well characterized mainly by classical microbiological methods or molecular techniques. However, so far no full fingerprinting has been performed using metagenomics analysis.

Purpose: A comprehensive investigation of the Feta cheese microbial ecosystem using metagenomics analysis.

Methods: In the present study, 12 artisanal Feta cheese samples from geographically dispersed regions in Greece along with five industrially produced Feta cheese samples were considered. After sensorial evaluation, 4 artisanal and 2 industrial samples were selected for further analysis. Samples were subjected to classical microbiological analysis, using selective growth media. Moreover, after total DNA extraction performed using a novel protocol developed in our laboratory, metagenomics analysis was performed by employing the 16S (bacteria) and ITS (yeasts) diversity assays. The data of the metagenomics analysis were analyzed using the MG-RAST server.

Results: The results of the classical microbiological analysis showed the presence of both bacteria and yeasts in all Feta cheeses, with bacteria being the dominant microbial community. Metagenomics analysis revealed the high variation among the numbers of bacterial and yeast species present in the ecosystem of the Feta cheese samples, with the number of the yeast species being almost twofold compared to the respective number of bacteria. Concerning bacteria, the dominant species were *Streptococcus thermophilus* and *Lactobacillus delbrueckii* (industrial samples) and *Lactococcus*

lactis (artisanal samples). Furthermore, in all cheese samples, 4 yeast species were found to be the most abundant, namely *Kluyveromyces lactis*, *Debaromyces hansenii*, *Pichia membranifaciens* and *Cryptococcus cyanovorans*.

Significance: This study is the first report on the metagenomics analysis of the microbial diversity of Feta cheese.

P2-34 *In silico* Assessment of the Technological Potential of the Dairy *Streptococcus thermophilus* ACA-DC 2 through Genome Analysis and Comparative Genomics

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Introduction: Although the *Streptococcus* genus includes mainly pathogenic species, *Streptococcus thermophilus* is a widely used dairy starter culture in the food industry. *S. thermophilus* has been adapted to milk probably through a degenerative evolution process that has led to the loss of typical streptococcal pathogenic traits.

Purpose: The genome sequence of the yogurt isolate *S. thermophilus* ACA-DC 2 was analyzed in order to shed light on its technological potential. Comparative genomics analysis among the existing complete genome sequences of *S. thermophilus* was also performed.

Methods: The genome of *S. thermophilus* ACA-DC 2 was sequenced with a combined approach of Illumina HiSeq and PacBio sequencing technologies. The Illumina FASTQ sequences were assembled into contigs (ABySS 1.5.1). The latter were linked and placed into scaffolds based on the alignment of the PacBio continuous long read data. Finally, gap closure within the scaffolds was performed (GapFiller 1.10). The genome sequence was annotated using the RAST pipeline. Full chromosome alignments were calculated with Progressive Mauve, providing information for the pangenome, the core genome and the singletons of the examined sequences. The GIs, CRISPRs and the antimicrobial peptides were predicted with IslandViewer, CRISPRcompar and BAGEL3, respectively.

Results: The genome analysis of *S. thermophilus* ACA-DC 2 revealed the absence of pathogenic features. Full chromosome alignments showed a high degree of synteny among the different strains. The pangenome of the 12 strains sequenced so far comprised about 2,300 genes. Concerning *S. thermophilus* ACA-DC 2, approximately 110 unique genes involved in various biological processes were identified. Four potential antimicrobial peptides but no CRISPR system were predicted.

Significance: The extensive use of *S. thermophilus* strains as starter cultures in dairy industry and the demand for qualitative and safe products render

the *in silico* analysis of the genome sequence of *S. thermophilus* ACA-DC 2 a useful tool for the exploitation of its technological potential.

P2-35* Establishment of Pretreatment Method to Quantify Added Agar in Shrimp

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Introduction: Since the color of shrimp extract interferes with the absorbance measurement, it is difficult to quantify the agar added into shrimp intentionally.

Purpose: The purpose of this study was to establish a pretreatment method of shrimp extract applicable to quantify the agar which is used factitiously to increase the weight of shrimp.

Methods: Agar gel (0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4 and 5%) was injected into the head, body and tail part of shrimp from 1 to 5 ml, and the shrimp was frozen and thawed to measure the weight change. The shrimp was minced and extracted by heating under pressure. The added agar in shrimp was quantified by 3,5-dinitrosalicylic acid (DNS) method with pretreatment method applied (33% of a trichloroacetic acid solution; TCA) to eliminate the color of shrimp extract. The sample precipitate was separated at 3000 rpm for 20 min by centrifuge and was heated in a steam bath by adding 3 ml of hot distilled water. The boiling solution was emptied into 50 ml vial, and then was analyzed by DNS method with acid hydrolysis.

Results: There was no loss of agar in pretreatment step, but the agar was not recovered fully through the extraction process. The absorbance at 570 nm of agar by DNS reaction after pretreatment was decreased to 59 to 45% depending on the amount of agar in shrimp, but still it was able to quantify the injected agar at least 10 mg per 500 g of shrimp.

Significance: These results imply that the established pretreatment step for eliminating the color interference prior to the DNS method would be used effectively to detect the fraud of imported seafood and quantify the amount of agar added into them.

P2-36 The Effect of Nisin on the Quality of Shrimp during Refrigerated Storage

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Introduction: Shelf life of shrimp during refrigerated storage is deeply influenced by both endogenous and microbiological enzymatic activity, for example melanosis. Melanosis in crustaceans is normally controlled by sulphite derivatives. However these derivatives are known to produce allergic reactions and severe disorders in asthmatic patients. Therefore, finding new substitutes for sulphites is an important issue.

Purpose: The goal of this study was to investigate the effect of nisin on the enzymatic oxidation and quality of white shrimp during refrigerated storage of seven days.

Methods: Five experiment groups were designed and compared: control, flake ice, sodium metabisulphite, nisin and mix. In each experiment group, pH, total viable count (TVC), mesophilic, psychrophilic, lactic acid bacteria (LAB) and Enterobacteriaceae, total volatile base nitrogen (TVBN), and color changes were measured

Results: Preliminary results indicated control group contributed no inhibition of melanosis and total viable count. Sodium metabisulphite showed better control for melanosis than flake ice group. The inhibitory activity of nisin seemed to follow a dose-dependent manner. With storage, treating the shrimp with either 75 µg/g could appreciably retard the total viable count and prevent melanosis.

Significance: The findings could be used by the food industry to substitute for sodium metabisulfite as a melanosis inhibitor of shrimp.

P2-37 Applicability of Hazard Analysis and Critical Control Points (HACCP) System in Beef Processing Factories in Khartoum State

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Introduction: Complying with HACCP system in food manufacture is vital for both food safety and food trade. In the last two decades there are tremendous increments in processed meat industry in Khartoum State/Sudan which require a unique food safety method to ensure products integrity.

Purpose: The goal of this cross-sectional, descriptive and analytical study is to evaluate the capabilities of meat factories in Khartoum State, so as to implement HACCP principles.

Methods: The current prerequisite programs (PRPs) of meat factories were assessed towards their adequacy for applying HACCP by constructed, standardized and scored checklist. The most significant food safety parameters for each categorized element were determined during inspections of the meat processing facilities. The risk assessment was made based on the most significant food safety parameters. Meat hygiene and safety competencies and HACCP awareness of meat processing workers were evaluated and 157 designed questionnaires were completed. Microbiological investigation was conducted for eighty-six randomly collected samples of processed beef end product including; Mortadella, Burger, Sausage, Kofta and Minced meat to identify the most associated species of bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella* spp. and *Shigella* spp. In addition, forty samples of these products were analyzed to determine the levels of added sodium nitrite salt (NaNO₂)

Results: Apart from *Listeria monocytogenes*, all other targeted bacterial species were isolated from all

types of processed meat. The quantitative bacterial species of total viable count showed high and critical levels. Sodium nitrites levels in 11% of beef sausage, 16.7% of beef kofta, and 22.2% minced beef and 27.8% of beef burger exceeded FAO acceptable limits. However, NaNO₂ was also found exceeded the FAO acceptable limits in many samples. Furthermore; the final scores of assessment revealed that: the lowest mark was 45% and highest was 78%. Therefore, the microbiological and chemical analysis, current hazards were identified. Eleven major critical control points (CCPs) were recognized; control measures and corrective actions for each factory were suggested.

Significance: To produce safe and wholesome processed beef in Khartoum State and in light of the conducted investigations and findings obtained. HACCP system could be applied at six meat-processing factories out of nine (67%), however adequate controls need to be done in each factory in order to comply with HACCP system. The main problem appeared from this study is that most of the risk factors and shortfalls were associated with the HACCP prerequisites programmes since they are the base line of HACCP applications. This made the possibility of applying the HACCP plan is difficult. Although the HACCP and related food safety management systems application theoretically appear possible, as proved by the scoring system used, such application however could not be effective in controlling food safety hazards from processed meat in Khartoum State without correct fulfillment of prerequisites to HACCP system. The situation of applying HACCP system needs the full involvement and commitment of the food control authorities in the State as such prerequisites are fundamentals in food control regulations to facilitate proper and sustainable implementation of the HACCP system in meat product.

P2-38 Effect of Marination and High Pressure Processing against *Listeria monocytogenes* in Cooked Pork Meat during Storage

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Introduction: Natural marination ingredients and High Pressure Processing (HPP) during manufacturing of meat products may extend their shelf life and improve their microbiological safety.

Purpose: To investigate the antimicrobial effect of HPP, in combination with natural marinades, against *Listeria monocytogenes* in a ready-to-eat, cooked pork meat product, during chilled storage.

Methods: Lemon juice (1:1v/v), pomegranate juice (1:1), white wine (1:1) and oreganum oil (0.1 or 0.2%), were screened against *L. monocytogenes* (4-5 log CFU/ml; 5-strain composite), in a sterile meat homogenate (10% w/v), within 30 min at 4°C. The two most effective ingredients were selected and further evaluated for their antimicrobial activity in combination with 400 or 600 MPa/2 min of HPP, in a cooked (70°C, 10 min) pork meat product inoculated

with 3–4 log CFU/g *L. monocytogenes*. Marinade- and HPP-treated samples were stored aerobically at 4 or 10°C and were analyzed for pathogen populations and Total Viable Counts (TVC), at appropriate time intervals.

Results: Results showed a 0.5 (pomegranate juice, white wine) to 1.6 (lemon juice) log CFU/ml reduction of *L. monocytogenes* counts. Marinades combined with 600 Mpa/2min of pressure reduced ($P < 0.5$) the pathogen below the detection limit (< 0.3 log CFU/g) until the end of storage (40 days). Similar trends were obtained for TVC. Effective antimicrobial treatments and combinations, from the most to the least effective were: Lemon juice (L)+600 MPa, pomegranate juice (P)+600 MPa, L+400 MPa, P+400 MPa, 600 MPa, 400 MPa, P, L.

Significance: These data showed that marination combined with HPP may be used by the meat industry as alternative preservation technologies.

P2-39 Application of Edible Films Supplemented with Probiotic Bacteria in Ham Slices Packaged after High Pressure Processing

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Introduction: Probiotic foods receive market interest as health promoting functional foods. Traditionally, probiotic bacteria have been added to fermented products such as cheese, sour milk, yoghurt, table olives and sausages, but there is an increasing demand for non-fermented probiotic products.

Purpose: The aim of the work was to deliver probiotic bacteria in a high pressure processed meat product through the application of Na-alginate based edible films and further evaluation of the efficacy of such carriers in ham slices packaging.

Methods: Probiotic bacteria (4 strains of *Lactobacillus plantarum* L125, T571, B282, *Lactobacillus pentosus* L33) were incorporated in Na-alginate forming solution in levels of 9 log CFU/ml to form edible films. Ham slices (treated or not with HPP -500MPa/2 minutes) were packaged under vacuum in contact with the alginate films containing probiotic bacteria and were stored at 3 temperatures (4, 8 and 12°C) for 66, 47 and 40 days, respectively. Samples (ham slices and edible films) were microbiologically tested and pH measurements were recorded. The presence of the potential probiotic strains and their levels at the end of the shelf life were verified using Pulsed Field Gel Electrophoresis.

Results: The probiotic bacteria were enumerated above 6 log CFU/g (as it is accepted for the probiotic claim of a food) in all ham samples during their shelf

life at all temperatures. The HPP treated samples were less acidic according to pH measurements and sensorial testing.

Significance: The results of this work are promising since edible films proved to be successful carriers of probiotic bacteria in packaged foods after thermal or pressure treatment.

P2-40 Occurrence of Patulin in Fruit Products Produced or Imported in Greece (Preliminary Results)

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Introduction: Patulin is a mycotoxin produced by a variety of molds, in particular, *Aspergillus*, *Penicillium* and *Byssoschlamys*. Patulin exhibits a number of toxic effects in animals and its presence in food is undesirable. Most commonly found in rotting apples, thermal processing of apple juice appears to cause only moderate reductions in patulin levels. The food safety authorities (EFSA, FDA, Codex) have set a maximum limit for patulin of 50 µg/kg in fruit juices and in drinks containing apple juice or derived from apples. For solid apple products, such as apple puree, the limit is 25 µg/kg.

Purpose: To test the patulin content in fruit products in the frame of compliance with the food safety regulations.

Methods: A total of 105 commercial fruit products, including 83 juices (67 concentrated and 16 ready to drink juices), and 22 solid fruit products (20 puree and 2 jam samples), either produced by Greek companies (36 samples) or imported (48 from Turkey and 21 from other countries) during the period 2010-2015, have been analyzed for patulin occurrence. The accredited HPLC method was used with liquid/liquid partition clean-up (based on EN 14177 European Standard).

Results: Fifty (50)% of the samples had patulin concentrations higher than 5 µg kg⁻¹, which is the limit of quantification. However, the level of contamination was relatively low with 34% of the samples having patulin concentrations < 25 µg kg⁻¹, and only three samples exceeded the EU permitted level (50 µg kg⁻¹). There were no significant differences based on sample origin but there was a significant reduction in patulin concentration during the five year period.

Significance: Data of patulin occurrence in final fruit products contribute to risk assessment studies. The preliminary results showed that there is a trend of patulin reduction in recent years indicating the adaptation of GAP for the prevention and reduction of patulin content in fruit products to finally meet the maximum permitted levels.

P2-41* Eggshell Sterilization Methods

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Introduction: Countless microbes are found on egg shells, and these microbes often signify health risks for any organisms that produce faecal matter, including human beings. In order to destroy these bacteria, the industry has discovered numerous eggshell sterilization methods, including UV light treatment, "cold fog," ozone, and washing. Ozone sterilization has drawbacks: small capacity and questions of safety regarding the environment and workers. Washing, on the other hand, removes the egg's layer of natural defense against bacteria.

Purpose: Thus the studies reported here represent a comparison of the two most widespread treatment technologies: UV light treatment and "cold fog" technology.

Methods: Freshly laid eggs were artificially inoculated separately with fresh cultures of either *Listeria monocytogenes*, *Salmonella* Enteritidis or *Escherichia coli* and incubated for 24 hours to produce on the egg surface a viable cell count of approximately 10^8 - 10^9 CFU/ml. Then attempts were made to model both UV light and "cold fog" treatments in the way they are applied industrially.

Results: One result of the trials was that the time period of UV light as applied by the industry was insufficient in every case of bacteria we examined. Five minutes of treatment failed to approach the 10^5 - 10^6 order of magnitude viable cell count reduction that was expected. The "cold fog" process brought the microbe count within the level of observability. Using this treatment, eggshells were sterilized according to current and frequently used parameters, besides which the shells contained no traces of preservatives.

Significance: It may be said, in summary, that as far as possible, it is advisable to choose eggs whose shells have been sterilized with "cold fog" process disinfectant material. Further, production plants where UV light sterilization is in use would be advised to change their technology such that the longest possible treatment time is applied.

P2-42 A Case of Eosinophilic Myositis due to *Sarcocystis hominis* Confirmed by Multiplex PCR

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Introduction: Cattle are common intermediate hosts of sarcocysts. The prevalence of *Sarcocystis* in adult bovine muscle is close to 100% in most regions of the world where it has been studied. Bovine muscle can harbour three species, namely *Sarcocystis cruzi* with canids as definitive hosts, *Sarcocystis hirsuta* with felids as definitive hosts and *Sarcocystis hominis* with primates as definitive hosts. *Sarcocystis* species have been suggested to play a role in bovine eosinophilic myositis (BEM), a specific inflammatory myopathy characterized by multifocal grey-green lesions in striated muscle of cattle.

Purpose: In this study, a case of BEM in an 18-month-old cow, born and raised in north-west Italy, was investigated; the objective was to determine if BEM was associated with a particular *Sarcocystis* species, by assessing with a multiplex PCR, the presence of different species inside the lesions (intralesional) and outside the lesions, in normal muscle tissue (extralesional).

Methods: Up to 10mg of material from 17 typical BEM lesions (only the necrotic center) was scalped from different points of the carcass; between 25 and 50 mg of normal muscular tissue was sampled 37 times from the same areas. The DNA extraction and the multiplex PCR were performed following a formerly described protocol.

Results: The presence of *S. hominis* DNA has been detected in 14 (82%) of samples from lesions, against 4 (10%) from normal muscle. The analysis demonstrated therefore that the presence of *S. hominis* DNA in the lesions was significantly higher than that in the normal muscle (Fisher test: $P = 10^{-6}$); *S. cruzi* DNA, on the other hand, has been detected in 4 (25%) and 12 (32%) samples from lesions and normal muscle, respectively. None of the samples was found positive for the presence of *S. hirsuta* DNA.

Significance: The evidence indicates a human source of infection, supporting a causal relationship between *S. hominis* infection and BEM in cattle.

P2-43 Effect of Temperature and Undissociated Acetic Acid Concentration on the Inactivation Boundaries of *Salmonella* spp. in Chicken Fillets Treated with Apple Cider Vinegar and Validation with Various Vinegar Marinades

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Introduction: Marination with vinegar is a common practice for safer meat products. Thus, investigation of the inactivation areas of *Salmonella* spp. in vinegar marinated chicken fillets is of great importance.

Purpose: Investigation of the effect of undissociated acetic acid (ACV) concentration and temperature on the inactivation of *Salmonella* spp. in vinegar marinated chicken fillets stored under aerobic conditions.

Methods: Chicken breast fillets, inoculated with 5 strains of *Salmonella* spp., were immersed in 4 concentrations of ACV ranging from 0.5 to 2% in acetic acid, for 1 hour at 4°C. The molar concentration of the undissociated acid (UAC) was 24-204 mmol/L. After marination, samples were stored aerobically at 4, 8, 12, and 16°C for 9 days. Changes in pH, Total Viable Counts, and *Salmonella* spp. were determined, while data were fitted to a second order logistic regression model. For model validation, several vinegar marinades containing oil and various herbs were tested. Pulsed Field Gel Electrophoresis (PFGE) was conducted to monitor *Salmonella* strains after marination and at the end of storage.

Results: No inactivation was observed in samples with 24 mmol/L UAC at any temperature, whereas at 16°C, inactivation was observed in samples with 204 mmol/L UAC (2% acetic acid) only, and after 5 days of storage. The degree of agreement between predictions and observations was 93.7% concordant whereas 4.8 and 9% of the misclassified predictions were false positive and false negative, respectively. Validation on various vinegar marinades data was not effective in 31 (27 fail-safe and 4 fail-dangerous) out of 170 cases. PFGE results revealed that at the end of the storage the strain *S. enteritidis* P167807 phage type 4 was detected at a proportion greater than 80% after treatments with ACV.

Significance: The developed model could be used to control *Salmonella* spp. in vinegar marinated poultry products.

P2-44 Integrated Quality Management in Meat Plant through HS-SPME GC/MS

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Introduction: There is an increasing need for meat managers to apply rapid analytical techniques to control daily production and provide information for the origin and the quality of meat.

Purpose: The aim of this work was to evaluate the efficiency of Headspace Solid Phase Microextraction Gas Chromatography–Mass Spectrometry in tandem with bioinformatics to discriminate between beef and pork minced meat samples and quantify the microbial load.

Methods: Beef and pork minced meat samples were provided by a meat plant in Athens, analyzed microbiologically (Total Viable Counts, *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria, and *Enterobacteriaceae*), and subjected to HS-SPME-GC/MS. In total, 400 chromatograms corresponding to microbiological counts were collected. The dataset was divided 70/30 for model calibration and validation, respectively. The variables (compounds) that were considered important, through Partial Least Squares Discriminant Analysis (PLS-DA) using the b_w regression coefficients, were further used to build PLS-Regression (PLS-R) models for each meat category independently. PLS-DA models were evaluated in terms of sensitivity and overall correct classification, while the performance of PLS-R models was assessed using the bias and accuracy factors, RMSE, and relative error (%).

Results: Meat volatolome for the two types of mince showed both qualitative and quantitative differences. Specifically, PLS-DA showed 100% correct classification for both meat categories. Moreover, for PLS-R models, bias and accuracy factors in both cases

were close to 1 for both calibration and validation datasets. Considering RMSE, it was calculated close to 0.5 for both meat categories for the training dataset, whereas for validation it was 0.74 and 0.61 for beef and pork mince, respectively. Lastly, 92% of the predictions fell within +/-20% of relative error.

Significance: In conclusion, this study showed the ability of Gas Chromatography-Mass Spectrometry to manage off-line the daily meat production in qualitative and quantitative terms.

P2-45 Assessment of Minced Beef Microbiological Quality Based on Multiple Sensor Data and Validation with Independent Datasets

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Introduction: In recent years, rapid sensor technologies based on hyper/multi-spectral imaging and vibrational spectroscopy coupled with data analysis have been used for the assessment of meat spoilage.

Purpose: The aim of this work was to: (a) develop advanced modelling methodologies based on either one or both multispectral imaging and FTIR spectroscopy, in order to assess minced meat spoilage in various packaging and storage conditions; and (b) validate the results using independent data.

Methods: For this study, fresh minced beef was purchased and divided in 75g-portions in four occasions. They were then placed on styrofoam trays, packaged aerobically and under modified atmosphere packaging (80% O₂-20% CO₂). In the first two occasions, samples were stored in isothermal conditions at 4°C and 10°C until spoilage was pronounced. Four samples were analysed beginning with multispectral image acquisition, followed by the microbiological analysis of total viable counts (TVC) and FTIR spectroscopy per temperature and packaging at appropriate time intervals. Prediction models were developed, such as Partial Least-Squares Regression, Artificial Neural Networks and Support Vector Machines. Models were validated using (a) a percentage of the original data and (b) external validation data consisting of two batches in isothermal (4°C) and dynamic storage conditions (at 4 and 10° iteratively). In total, approximately 340 TVC measurements were collected, along with multispectral images and FTIR spectra.

Results: Results varied depending on the model, instrument and validation scheme. Successful models yielded a Mean Square Error (MSE) close to 0.2 (log CFU/g)², whereas external validation yielded a higher but acceptable MSE and in some cases failed.

Significance: In conclusion, while this study showed the applicability of rapid methods for the assessment of spoilage, it also proved the necessity of external validation for the determination of the

best models, as it avoids overfitting data leading to overoptimistic results, and takes into account the variability found among different meat batches.

P2-46 Safe Food-Handling Knowledge, Perceptions and Self-reported Practices of Greek Consumers

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Introduction: A substantial proportion of foodborne illness is associated with foods prepared in households. Primary understanding of how foods are handled in private homes comes mostly from questionnaire based studies and observational studies.

Purpose: The purpose of this study was to examine Greek consumers' perceptions and knowledge of safe food handling practices. More specifically, their attitudes, opinions, and self-reported practices were studied.

Methods: Data were collected from a total of 400 consumers living in Greece, through the use of a self-administered online survey. The questionnaire consisted of 26 items, 4 positive and 5 negative statements, scored from 1 to 5 according to Likert scale, grouped into 3 subscales for perceptions of "separate", "chill" and "clean". Furthermore 2 closed-ended questions (type yes/no), 4 questions that are related to self-reported food handling practices and 11 demographic questions.

Results: The most commonly known bacteria that cause food related illness according to respondents' knowledge is *Salmonella* (99.7%), followed by *E. coli* (73.9%) and *Listeria* (58.4%). Knowledge and awareness of safe food-handling practices increases at higher education level, while no significant differences were found of those who had experienced foodborne illness in the past 12 months versus those who had not. The prevalence of behaviors, perceptions and practices did not vary by age, sex and working status.

Significance: Understanding consumers' food safety practices is likely to reduce the risk and incidence of foodborne illness. Moreover, information obtained from consumers can be used to shape educational programs, determine where food safety educational efforts would be most effective and the needed content of the messages.

P2-47 Effect of Initial Inoculum and Substrate Composition on Growth and Biofilm Formation of *Aspergillus carbonarius* in Microtiter Plates

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Introduction: Filamentous fungi can easily colonize surfaces and are therefore excellent candidates for biofilm formation, but the aspect is still poorly understood.

Purpose: To develop a new screening method for rapid assessment of growth and biofilm formation by *Aspergillus carbonarius*, a well-known mycotoxigenic fungus, in microtiter plates, in relation to the initial spore inoculum and medium composition.

Methods: 96-well microtiter plates with synthetic grape juice medium (SGM) or yeast extract sucrose (YES) broth and 3 spore concentrations of *A. carbonarius* (10^3 , 10^4 , and 10^5 spores/ml) were incubated for 7 days at 25°C. Fungal growth/biofilm formation was assessed by optical density (OD) at 575 nm after staining the wells with 0.5% crystal violet.

Results: Both fungal growth rate and lag phase duration depended on initial spore concentration, while biofilm formation was correlated negatively with lag phase and positively with OD at the 5th day of incubation. Biofilm formation was dependent on initial spore concentration for both substrates ($P < 0.05$). When SGM was used, 10^4 and 10^5 spores resulted in comparatively higher and significantly different biofilm formation compared to control samples (non-inoculated broth). On the contrary, for YES broth only 10^3 spores led to higher biofilm formation over controls. Statistically higher biofilm formation was observed on SGM in comparison to YES broth for specific inocula (10^3 and 10^5 spores).

Significance: The proposed technique could serve as a rapid detection system for both fungal growth and biofilm formation by filamentous fungi.

P2-48 Biofilm Formation of *Streptococcus macedonicus* under Monospecies and Dual-species (with Foodborne Pathogens) Conditions

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Introduction: *Streptococcus macedonicus* strains are food-grade lactic streptococci, well known for their antimicrobial activity, primarily through the production of bacteriocins. Given the high exploitation potential of *S. macedonicus* in biopreservation approaches, evaluation of its biofilm formation behavior under various culture conditions appears to be interesting.

Purpose: The objective of this study was the characterization of the biofilm formation of *S. macedonicus* under monospecies and dual-species (with foodborne pathogens) conditions. The foodborne pathogens studied were *Staphylococcus aureus* and *Salmonella enterica* serotype Typhimurium.

Methods: Biofilm formation was studied on stainless steel coupons, which were initially incubated (15°C, 3 h) in mono- or dual-species bacterial suspensions (in Ringer's solution) to allow for bacterial attachment. Then, coupons carrying strongly attached bacteria were transferred in brain heart infusion broth, and were further incubated at 37°C for 72 h or at 15°C for 144 h. Biofilm bacterial populations were determined

at 3 h (attachment), and at 24-h and 48-h intervals during incubation at 37°C and 15°C, respectively.

Results: Co-culture with *S. aureus* (15°C) and *S. Typhimurium* (15, 37°C) significantly increased the biofilm-forming ability of *S. macedonicus* compared to its monoculture counterparts, while its biofilm populations in the mixed cultures at the end of the incubation periods were also considerably higher ($P < 0.05$) than those of the pathogens. However, the presence of *S. macedonicus* in dual-species cultures did not appear to affect ($P \geq 0.05$) the biofilm formation behavior of either of the studied foodborne pathogens (relative to that observed under monospecies conditions).

Significance: Research data on the behavior of lactic acid bacteria within multispecies biofilm communities, along with corresponding data on the *in situ* production of antimicrobial and/or signaling compounds, are expected to be of great value from a microbial food ecology perspective.

P2-49 How Can Consumers Differentiate between Fresh and Thawed Beef Liver?

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Introduction: The Egyptian government imports thousands of tons of frozen beef liver as part of a food subsidy program. In the absence of meat inspection in Al-Buhairah province- Egypt, some butchers may mislead consumers into buying thawed, imported liver for the higher price that fresh liver demands. Consumers need ways to distinguish between fresh and previously frozen liver.

Purpose: The objective of the current research was to make consumers aware of some marks that help them to differentiate between fresh and thawed imported livers.

Methods: A total of 50 liver samples were examined to evaluate sensory properties. Samples were examined visually before and after applying Ghazalah test in which the cut surface of liver is exposed to a continuous flow of water for 10 seconds. Thawed livers undergo a rapid change in color (the cut surface becomes faint in color). An obvious livery odor may appear in the case of thawed liver. By palpation, fresh liver is firm.

Results: Eighteen samples (36% of samples) were thawed samples, while 64% were fresh. Some sensory differences were difficult to distinguish by consumers and need a specialist to identify changes in odor and consistency. Results of Ghazalah test were clear in thawed samples in which the cut surface of thawed liver loses its dark brown color and appears faint in color after exposure to a continuous flow of water for 10 seconds.

Significance: Thawed livers are lower quality than fresh livers and so consumers should be able to differentiate between them; this can be done easily by help of Ghazalah test. Also, more governmental efforts are still needed for periodical examination of beef livers in butcher shops by qualified veterinarians.

P2-50 Screening of Biocontrol Agents against *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 and Antimicrobial Efficacy on Iceberg Lettuce

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Introduction: Foodborne disease outbreaks continue to plague the produce industry, indicating a need for alternative or additional microbial control methods. The use of biological control agents postharvest could provide an additional hurdle to challenge pathogen growth. Many bacterial species, including Lactic Acid Bacteria (LAB) and *Bacillus* species, have demonstrated antimicrobial activity and thus are candidates for postharvest biocontrol agents.

Purpose: The purpose of this study was to screen 22 bacterial isolates for antimicrobial activity against *Listeria monocytogenes*, *Salmonella* species, and *Escherichia coli* O157:H7 *in vitro*, then to assess antimicrobial efficacy of select isolates against *L. monocytogenes* on iceberg lettuce.

Methods: The antimicrobial activity of the LAB isolates was determined using a seeded-overlay method and all other isolates were evaluated by spot-inoculating the isolate on pathogen-seeded TSA; antimicrobial activity was determined by the size of the clearing around the isolate. Antimicrobial efficacy on iceberg lettuce was assessed by spraying a cocktail of the three LAB isolates (10^7 - 10^8 CFU/g) onto lettuce spot-inoculated with *L. monocytogenes* (10^2 - 10^3 CFU/g), then incubating at 10°C for 14 days.

Results: Three LAB isolates and six *Bacillus* isolates suppressed *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 *in vitro*. LAB isolates *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* were chosen for use in the iceberg lettuce challenge study. *L. monocytogenes* levels were 1.84 logs lower on lettuce treated with LAB cocktail than untreated lettuce after 14 days incubation at 10°C.

Significance: This study has identified nine bacterial isolates capable of inhibiting *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 *in vitro*. Three LAB isolates suppressed *L. monocytogenes* on iceberg lettuce and merit additional testing to determine commercial applicability.

P2-51 Microbial Consortia in Meat Processing Environment

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Introduction: Microbial contamination in food processing plants can play a fundamental role in food quality and safety. Spoilage organisms can be

transferred from the environment to intermediates of production and may negatively affect the production process and the quality of the final products.

Purpose: The purpose of this study was to characterize the type of microbiota in the environment of meat processing plants and gain insights regarding potential microbial contamination risks for the final products.

Methods: The microbiota of three different meat plants were studied by both traditional and molecular methods (PCR-DGGE) in two different periods. Environmental samples from surfaces and tools were analyzed and the occurrence of pathogens (*Listeria monocytogenes* and *Salmonella* spp.) was also investigated.

Results: Different levels of contamination emerged between the three plants as well as between the two sampling periods. The pathogens investigated were detected in two of the three plants under analysis. Meat mixer, saw bones and conveyor belts resulted in the most commonly contaminated surfaces. DGGE analysis showed the coexistence of *Staphylococcus* sp. and spoilage-associated bacteria, including *Pseudomonas* and *Acinetobacter*, in the analyzed surfaces.

Significance: The description of the microbial consortia in the meat processing environment is important since it is a first step in understanding possible routes of product contamination. Furthermore it may contribute in the development of sanitation programs for effective pathogen removal.

P2-52 Impact of Food Safety Climate on Food Safety and Hygiene Output in Two Vegetable Processing Companies

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Introduction: In our previous research a definition was already set for food safety climate and culture and a conceptual model was established. Also a self-assessment tool was developed to measure the food safety climate in food companies.

Purpose: The objective of this study was to compare the food safety climate in two vegetable processing companies with similar activities, technology and level of the food safety management system. Also the relation between the food safety climate, the food safety management system and the actual output of the company (hygiene and food safety) was investigated (= food safety culture).

Methods: The two vegetable processing companies were screened on their food safety climate and level of implemented food safety management system by application of self-assessment questionnaires. Also objective data of food safety/hygiene output of the companies were collected by means of microbiological product sampling and hand swabbing.

Results: The food safety climate score was significantly higher in company 1 compared to company 2. This difference was further investigated by looking at the correlation of food safety climate

with certain variables. Food safety climate was positively correlated with seniority in the current job, seniority in the food industry and conscientiousness. Also, a permanent contract tends to give higher food safety climate scores than temporary contracts. The similar technology, food safety management system and company characteristics resulted in a high output level for both companies, although food safety climate scores of company 1 were higher than those of company 2.

Significance: It was not possible to see a clear effect of the food safety climate on the output as the good output level could be a consequence of the good technology and elaborated food safety management system ('ceiling effect'). However, the study showed some interesting relations between the different variables measured.

P2-53 Application of UV-C Light Processing on Fresh and Frozen Strawberries, Raspberries and Blueberries to Compare the Inactivation of Bacterial and Viral Pathogens and Their Surrogates and Evaluate the Sensory Aspects of This Technology

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Introduction: Several foodborne outbreaks associated with strawberries have raised safety concerns about various fresh and frozen berry products in recent years. UV-C is applied for the disinfection of drinking water and is considered to be a promising technology for a wider range of beverages and food products. The berry industry needs novel approaches to address the current microbiological issues, especially viruses.

Purpose: The objective of this study was to evaluate the sensory effects of UV-C technology and to compare the inactivation of bacterial and viral pathogens and their surrogates on fresh and frozen strawberries, raspberries and blueberries using UV-C light to critically assess the potential of this technology for the berry supply chain.

Methods: Fresh and frozen strawberries, raspberries and blueberries were spot-inoculated with *Listeria*, *Salmonella*, STEC, Hepatitis A virus (HAV) and their surrogates (*L. innocua*, *E. faecium*, *E. coli* and MS2 bacteriophage) and treated with UV-C using a 95W high output UV-C emitter. Samples were exposed to UV-C for up to 2 minutes. After treatment, microorganisms were extracted and recovered from the samples and quantified using selective media for the bacteria and infectivity assays for the viruses.

Results: Results show a similar reduction on fresh and frozen berries with a significant difference between berry types for the different microorganisms tested. UV-C treatment did not show an obvious impact on the sensory aspects of berries.

Significance: The present study demonstrates that UV-C treatment has the potential to be applied at different steps along the berry supply chain to improve the microbial safety of fresh and frozen berries.

P2-54 Effect of Feeding a Combination of Organic Acids, Mono-glycerides and a Probiotic on *Campylobacter* Colonization in Broilers

PEDRO MEDEL

Imasde Agroalimentaria, Madrid, Spain

Introduction: *In vitro* studies have demonstrated that some probiotics, organic acids, medium chain fatty acids, or their mono-glycerides have a strong bactericidal effect on *Campylobacter* spp. However, inconsistent results have been reported *in vivo*.

Purpose: An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic (*B. subtilis* DSM17299, P) added to the feed on *Campylobacter* counts in broilers.

Methods: There were two treatments applied from 1-42d of age, T1: Positive controls (*Campylobacter*, no additives) and T2:T1+MGOA at 1.5% from 1-10d and 2.5% thereafter +P at 0.1%. A total of 108 one-day-old Ross 308 broilers (male and female, 50%) were divided into the experimental treatments. At

14 d, all broilers were orally gavaged with 100 µl of a solution containing 1×10^5 CFU/ml of ST-45 and ST-21 *C. jejuni* strains. On days 21, 35 and 42, ceca from 14 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as \log_{10} CFU/g ceca content were first tested for normality and then analysed by ANOVA or the nonparametric test of Kruskal-Wallis (SPSS v.19.0).

Results: No significant differences in the *Campylobacter* counts were observed between treatments at 21d (4.06 vs 3.77 \log_{10} CFU/g, for T1 vs T2; $P = 0.53$). However, *Campylobacter* counts were reduced by MGOA+P supplementation at 35 d (6.57 vs 3.89 \log_{10} CFU/g, for T1 vs T2; $P = 0.005$) and the trend was maintained at 42d (7.44 vs 7.01 \log_{10} CFU/g, for T1 vs T2; $P = 0.097$). The reduction in *Campylobacter* counts at 35d was mainly due to a higher number of non-infected birds in the MGOA+P treatment (14.3 vs 57.1% for T1 vs T2; $P = 0.018$).

Significance: It is concluded that supplementation of broiler diets with a blend of monoglycerides with organic acids together with a probiotic can effectively decrease *Campylobacter* in broilers.

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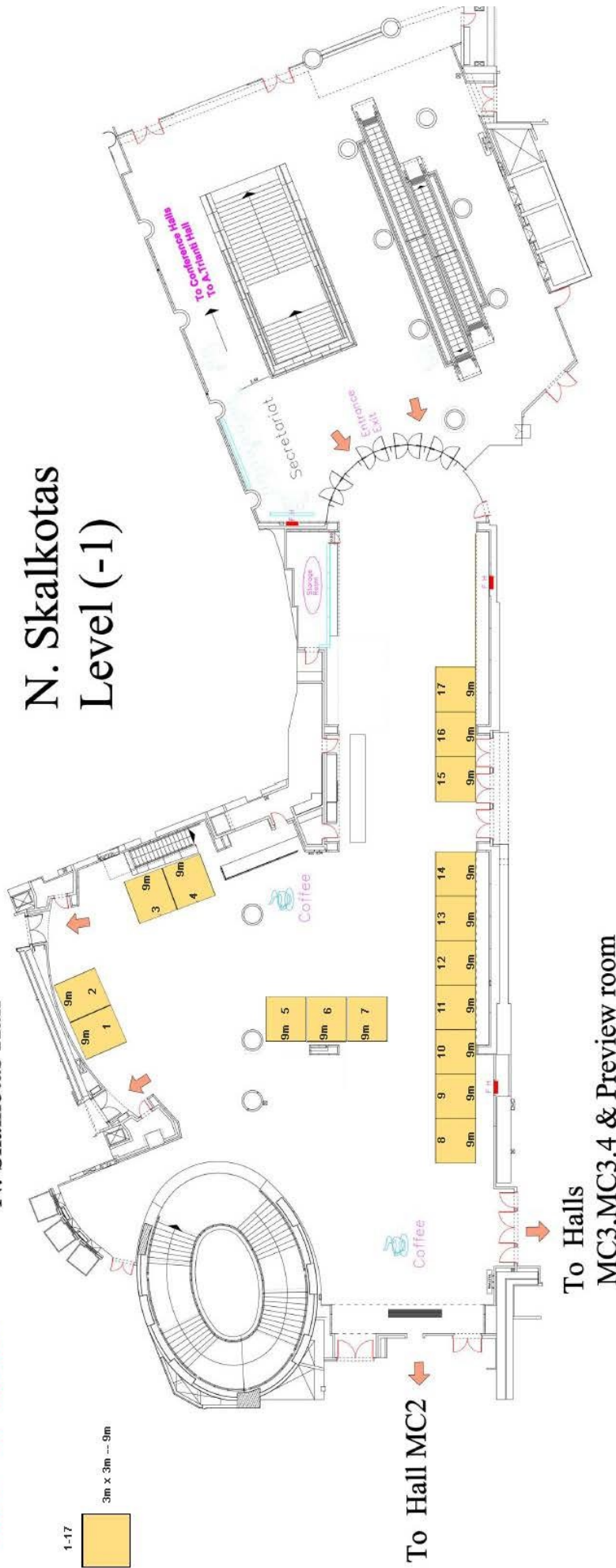
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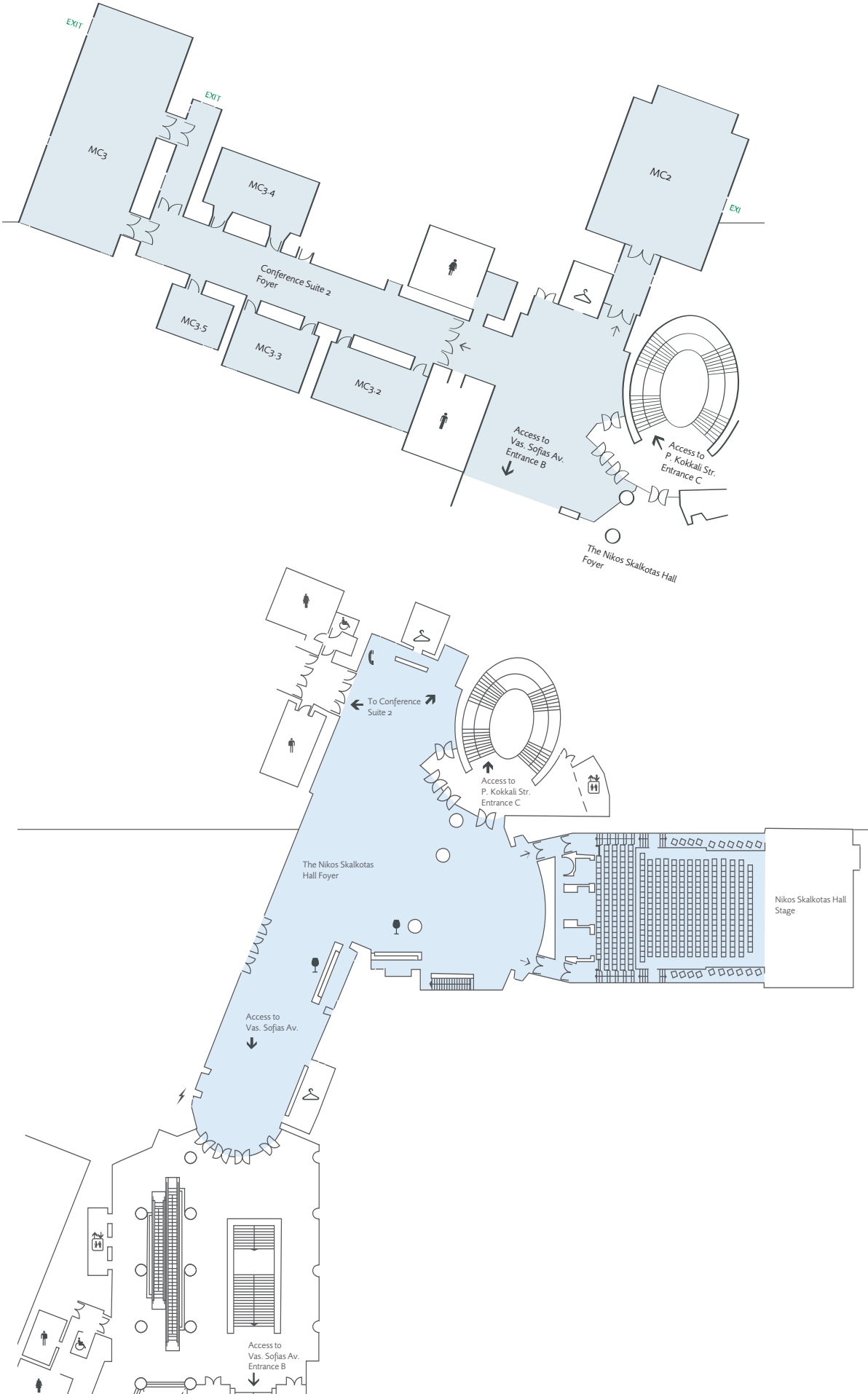


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