

PROGRAM & BOOK OF ABSTRACTS

Assuring the integrity of the food chain: FIGHTING FOOD FRAUD

April 6-7, 2016
Prague, Czech Republic

Jana Pulkrabová, Monika Tomaniová, Jana Hajšlová and Paul Brereton
Editors



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the food chain:
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Assuring the integrity of the food chain: **FIGHTING FOOD FRAUD**

FOODINTEGRITY 2016

April 6-7, 2016 • Prague • Czech Republic

Diplomat Hotel Prague

Organized by

University of Chemistry and Technology, Prague, Czech Republic

&

Ensuring the Integrity of the European food chain (FoodIntegrity)



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Conference is held under auspices of the minister of agriculture of the Czech Republic Marian Jurecka.

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newfood



Assuring the integrity of the food chain: Fighting food fraud



6–7 April 2016 • Prague, Czech Republic

FOODINTEGRITY 2016

PROGRAM

Assuring the integrity of the food chain: FIGHTING FOOD FRAUD FOODINTEGRITY 2016

April 6-7, 2016

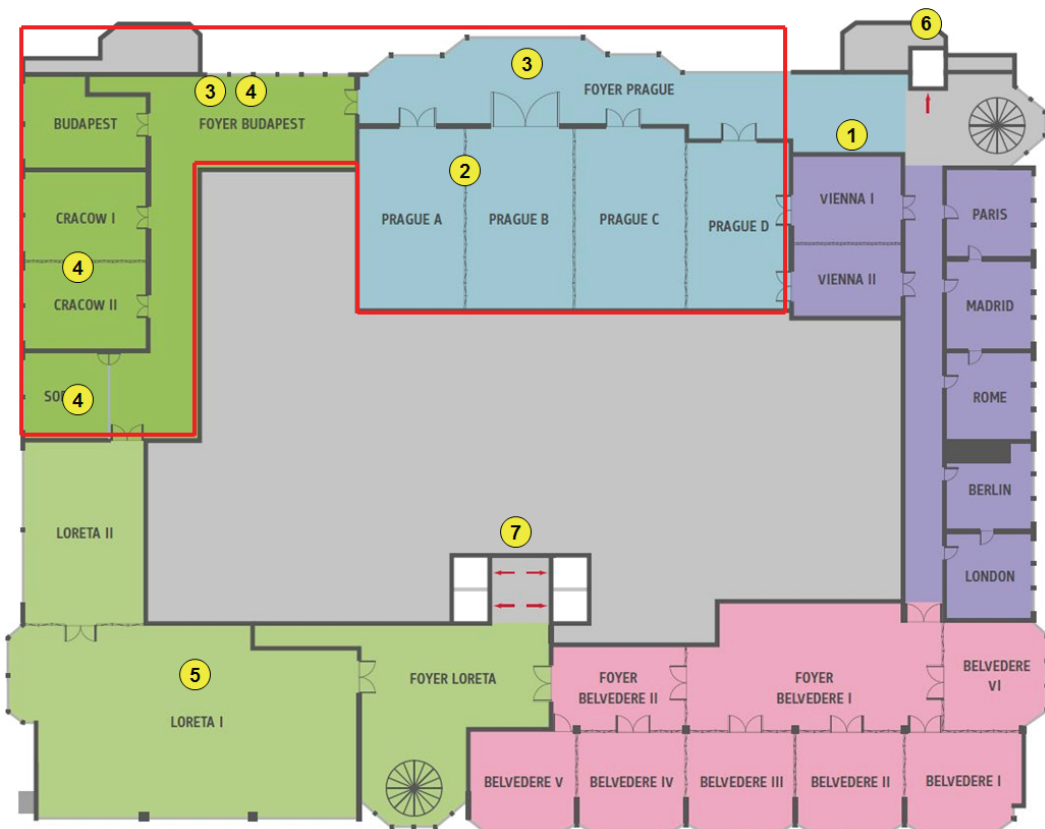
Diplomat Hotel Conference Centre • PRAGUE • CZECH REPUBLIC



Conference is held under auspices of the minister of agriculture of the Czech Republic Marian Jurecka.

FOODINTEGRITY 2016 Venue

Diplomat Hotel Conference Centre



Area bounded by red line is devoted to the FOODINTEGRITY 2016 conference (lecture halls, exhibition and poster area).

- 1: Registration desk
- 2: Conference halls, vendor seminars and parallel workshops
- 3: Exhibition area & Coffee breaks
- 4: Exhibition area & Demonstration activities & Poster area & Coffee breaks
- 5: Conference restaurant (lunches)
- 6: Lift to the exhibition area
- 7: Lifts to the hotel rooms

FOODINTEGRITY 2016 - PROGRAM AT A GLANCE

WEDNESDAY April 6, 2016		THURSDAY April 7, 2016	
Time / Date	8:00-9:00	8:00-9:00	Registration for the FOODINTEGRITY 2016 workshops
	9:00-9:20	9:00-10:30	Session 6: Workshop: Food Crime, occurrence, motivations and mitigations (part I)
	9:20-10:20		Session 8: Workshop: Industrial perspective for strategies applied for assuring food authenticity (part I)
	10:20-10:50	10:30-11:00	Coffee break / Poster session / Exhibition
	10:50-12:45	11:00-13:00	Session 1: Gaps in current research on food authenticity: New projects funded by the FoodIntegrity on topics (i) Non targeted analysis (ii) Complex foods (iii) Transparency along the food chain (iv) Rapid methods
	12:45-14:30		Lunch / Poster session / Exhibition / Vendor seminars / Demonstration of the approaches for food authentication developed by FoodIntegrity project
	14:30-16:00		Session 2: Citizen and consumer science approaches to food integrity
	16:00-16:30		Coffee break / Poster session / Exhibition
	16:30-18:00		Session 4: Analytical tools for food authentication
			Session 5: Authenticity of herbs and spices
	18:00-20:00		
	20:00-23:00	13:00-14:00	Lunch

Coffee breaks will be served in the conference area; lunches will be served in the hotel restaurant Loreta.

Assuring the integrity of the food chain: Fighting food fraud (FOODINTEGRITY 2016)

April 6-7, 2016, Diplomat Hotel Conference Centre, Prague, Czech Republic

TUESDAY, April 5, 2016

16:00-18:00

Registration for the FOODINTEGRITY 2016 conference

WEDNESDAY, April 6, 2016

8:00-9:00

Registration for the FOODINTEGRITY 2016 conference

9:00-9:20

Prague A-D
halls

OPENING of the conference and WELCOME

UCT Prague representative

Paul Brereton & Jana Hajslova, Chairs of the FOODINTEGRITY 2016 conference

L1

INTRODUCTION TO THE FOODINTEGRITY

Announcement of the next FOODINTEGRITY conference in 2017

Paul Brereton, Fera Science Ltd, York, UK

9:20-10:20

Prague A-D
halls

PLENARY SESSION:

Integrity along the food chain: Setting the scene

Chairpersons: Chris Elliott (QUB) & Michele Lees (Eurofins)

9:20-9:40

L2

THE IMPACT OF FOOD FRAUD ON CHINA: RISKS AND PREVENTION STRATEGIES

Wu Yongning, China National Center for Food Safety Risk Assessment (CFSA), Beijing, China

9:40-10:00

L3

FOOD AUTHENTICITY: CONSUMER EXPECTATIONS (AND DISAPPOINTMENTS)

Sue Davies, Which?, London, UK

10:00-10:20

L4

NON-TARGETED METHODS: ADVANCES AND CHALLENGES AHEAD

Jeff Moore, US Pharmacopeia, Rockville, United States of America

10:20-10:50

Conference
area

Coffee Break / Exhibition / Poster session

10:50-12:45

Prague A-D
halls

SESSION 1:

Gaps in current research on food authenticity:

New projects funded by FoodIntegrity

Chairpersons: Paul Brereton (Fera Science Ltd) & Saskia van Ruth (WUR)

10:50-12:45

L5-L12

8 presentations on topics on:

- (i) Non targeted analysis
- (ii) Complex foods
- (iii) Transparency along the food chain
- (iv) Rapid methods

Discussion

10:50-11:20

Topic 3: Information sharing along the food chain

L5

FEASIBILITY STUDY ON INFORMATION SHARING AND ANALYSIS ALONG THE FOOD CHAIN TO IDENTIFY EMERGING FOOD INTEGRITY ISSUES

Hilde Cnossen (TNO)

L6

CHECK X - IMPROVING SUPPLY CHAIN INTEGRITY THROUGH DATA SHARING

Gerald Herrmann (Organic Services)

11:20-12:00	Topic 2: Innovative approaches to assure the integrity of complex foods
L7	CONSUMER AND BRAND PROTECTION IN COMPLEX FOODS FROM PROTEIN SIGNATURES USING MASS SPECTROMETRY <i>Andrew Watson (IFR)</i>
L8	INTEGRITY OF COMPLEX FOODS: INNOVATION IN ANALYSIS AND COMMUNICATION <i>Rolando Lorenzetti (Consorzio Italbiotec)</i>
L9	FROM SEEDS TO COMPLEX FOODS: COMPOSITION AND STABILITY OF BIOACTIVE COMPOUNDS ALONG THE FOOD-PRODUCTION CHAIN <i>Daniel Wunderlin (ICYTAC)</i>
12:00-12:30	Topic 4: Rapid Methods
L10	F.I.S.HUB - FISH IDENTIFICATION SOFTWARE HUB <i>Pier Luigi Acutis (IZSPLVA)</i>
L11	NIRS MICROSENSORS AND ICT PLATFORMS FOR ENSURING ON-SITE AUTHENTICATION OF HIGH ADDED VALUE EUROPEAN FOODS <i>Ana Garrido & Lola Perez (UCO)</i>
12:30-12:45	Topic 1: Standardisation and harmonisation of untargeted food integrity methods
L12	INTELLITRACE <i>Marco Arlorio (UPO)</i>
12:45-14:30 Hotel restaurant Loreta	Lunch / Poster session / Exhibition / Vendor seminars / Demonstration of the approaches developed by FoodIntegrity for food authentication

WEDNESDAY, April 6, 2016

13:30-14:20

VENDOR SEMINARS

Prague A&B
hall

Orbitrap technology: the new frontiers in food profiling

ThermoFisher
SCIENTIFIC

Prague C&D
hall

Ensuring the authenticity of food - New advances in LC-MS/MS workflows bringing routine closer than ever



13:30-14:30

POSTER SESSION

Cracow &
Sophia halls

WEDNESDAY, April 6, 2016

13:30-14:30
Foyer
Budapest

Demonstration of the approaches developed by FoodIntegrity for food authentication

HOW TO GET INVOLVED ?

Do you wish to receive information about news, progress and events related to the FoodIntegrity project?

Please **register for the project COMMUNICATION** on www.foodintegrity.eu/ page **Contact us**

FoodIntegrity is a European five-year project, which will draw from a well of experience consisting of 38 partners in the EU, China and Iceland to tackle issues surround the authenticity of food. The project will provide a focal point for the sharing and exploitation of European research aimed at protecting the integrity of food production in Europe.

The aim of the FoodIntegrity demonstration is to provide you with a brief update on some of the progress on this multi-faceted project and let you know how you can get involved. We hope you find it useful.

You can join us and discuss with FoodIntegrity experts the latest developments and strategies in the field of food integrity - safety, quality, authenticity and traceability.

WHAT CAN YOU LEARN?



FoodIntegrity Knowledge base: an information resource on food authenticity, description of the database and progress achieved so far

To bring together available information on suitable analytical tools and associated reference data for the detection of food fraud in a Knowledge Base, to facilitate access to this information for industry, regulatory authorities and research organisations



Identifying the gaps in current research on food authenticity



Industrial perspective of relevant food chains vulnerabilities vs Current analytical methods and technologies that can be applied

To bring together available data on industrially exploited analytical tools for detection of food fraud, and identify reliable indicators/markers to use for horizon scanning of possible fraud events



Chinese consumer attitudes to food fraud, short description of the survey and its outcomes

To examine Chinese consumers' attitudes and perceptions towards the safety and integrity of imported European foods



Survey of the Olive oil sector, description of the survey and its outcomes

Sensory analysis of olive oils: Do you recognise adulterated product?



Investigation of available and potential future technologies for authentication of branded spirits and/or categories

"Hands on" demonstration of authentication of spirit drinks



How to reduce product misdescription in the seafood sector?

Fish sampling in restaurants: help us to recognise whether you eat what you have ordered!



Do you wish to participate in the FoodIntegrity events?

Do you wish to be trained in food authentication strategies?

Join us and learn more on opportunities we offer!

WEDNESDAY, April 6, 2016

SESSIONS 2 & 4, in parallel

14:30-16:00
Prague A&B
hall

SESSION 2: Citizen and consumer science approaches to food integrity

Chairpersons: Sue Davies (Which?) & Moira Dean (QUB)

14:30-15:00 **L13**

EUROPEAN FOOD AUTHENTICITY AND CHINESE CONSUMERS. REDUCING CONCERNS ABOUT FOOD SAFETY

Lynn J. Frewer, Newcastle University, Newcastle Upon Tyne, UK

15:00-15:25 **L14**

AN OVERVIEW OF THE USE OF CITIZEN SCIENCE, AND LESSONS FROM THE ENVIRONMENT SECTOR IN THE UK

Ralph Blaney, WRc, Swindon, UK

15:25-15:50 **L15**

CITIZEN SCIENCE APPROACH TO IDENTIFYING MISLABELLING IN THE FISH SECTOR: STUDY DESIGN AND POTENTIAL IMPACT IN RESTAURANTS

Miguel Angel Pardo, AZTI, Derio, Spain

15:50-16:00

Discussion - implications for the food sector?

16:00-16:30
Conference
area

Coffee Break / Exhibition / Poster session

SESSIONS 3 & 5, in parallel

16:30-18:00
Prague A&B
hall

SESSION 3: Tools for food integrity assessment

Chairpersons: Petter Olsen (Nofima) & Christophe Cavin (Nestle)

16:30-16:50 **L16**

CONCEPTUAL FRAMEWORK FOR AN ONLINE EARLY WARNING SYSTEM FOR FOOD FRAUD DETECTION

Vahid Mojtahed, Fera Science Ltd, York, UK

16:50-17:10 **L17**

DEVELOPMENT OF EARLY WARNING SYSTEMS TO DETECT, PREDICT AND ASSESS FOOD FRAUD

Hans Marvin, RIKILT Wageningen UR, The Netherlands

17:10-17:30 **L18**

TRACEABILITY AND BRAND PROTECTION

Espen Braathe, Tracetracker, Oslo, Norway

17:30-17:50 **L19**

CHECK ORGANIC: ENSURING THE INTEGRITY OF THE ORGANIC FOOD SUPPLY CHAIN

Gerald Herrmann, Organic Services GmbH, Tutzing, Germany

17:50-18:00

Discussion

20:00-23:00

**Conference dinner
Poster award ceremony**
Prague downtown

WEDNESDAY, April 6, 2016

SESSIONS 2 & 4, in parallel

14:30-16:00
Prague C&D
hall

SESSION 4: Analytical tools for food authentication

Chairpersons: Michele Suman (Barilla) & Begoña Perez Villarreal (AZTI)

14:30-14:50 L20

EUROPEAN KNOWLEDGEBASE ON ANALYTICAL METHODOLOGY AND DATABASES FOR FOOD AUTHENTICITY

Michele Lees, Eurofins Analytics France, Nantes, France

14:50-15:10 L21

EXPANDING ANALYTICAL CAPABILITIES WITHIN SPIRIT DRINKS AUTHENTICATION

Ian Goodall, The Scotch Whisky Research Institute, Edinburgh, UK

15:10-15:30 L22

EMERGING PORTABLE SPECTROSCOPICS FOR NON-DESTRUCTIVE FOOD AUTHENTICATION

Yannick Weesepeel, RIKILT - Wageningen UR, The Netherlands

15:30-15:50 L23

AUTHENTICATION OF ORGANIC FRUITS BY THE ANALYSIS OF THEIR MICROBIAL COMMUNITIES: HOW TO PREVENT ORGANIC FRUIT FRAUD? APPLICATION OF A ROBUST APPROACH

Didier Montet, CIRAD-UMR Qualisud, Montpellier Cedex 5, France

15:50-16:00

Discussion

16:00-16:30
Conference
area

Coffee Break / Exhibition / Poster session

SESSIONS 3 & 5, in parallel

16:30-18:00
Prague C&D
hall

SESSION 5: Authenticity of herbs and spices

Chairpersons: Carsten Fauhl-Hassek (BfR) & Rebecca Kokkinofa (SGL)

16:30-16:50 L24

AUTHENTICATION OF SPICES AND HERBS

Carsten Fauhl-Hassek, Federal Institute for Risk Assessment, Berlin, Germany

16:50-17:10 L25

A SENSE OF 'SPICED': PEPPER AND NUTMEG AUTHENTICATION

Saskia van Ruth, Wageningen UR, The Netherlands

17:10-17:30 L26

HIGH RESOLUTION MASS SPECTROMETRY BASED METABOLOMIC FINGERPRINTING, AN EFFICIENT TOOL TO DETECT FRAUD ON HERBS AND SPICES: CASE STUDIES

Jana Hajslova, University of Chemistry and Technology, Prague, Czech Republic

17:30-17:50 L27

ADULTERATION OF HERBS: THE OREGANO STORY

Simon Haughey, Institute for Global Food Security, Queen's University, Belfast, UK

17:50-18:00

Discussion

20:00-23:00

Conference dinner Poster award ceremony

Prague downtown

Session: Authenticity of herbs and spices

Spices and herbs, condiments, are contained in almost every processed food, including ready-to-eat products, and are used in restaurants and by the consumer for flavouring purposes of food. The EU market is one of the largest markets for spices and herbs in the world. The spices most often consumed in the EU are pepper, paprika and pimento; whereas the most often consumed herbs are thyme and oregano. Only a few spices are produced within the EU - mainly paprika of which the main producers are Hungary and Romania.

Herbs and particularly spices have always been highly-priced commodities and are therefore subject to several adulterations. By economic reasons, fraudsters are mixing cheap material with the high-priced spices. Ungrounded spices are rarely falsified, but confusion with similar plants or fruits is possible. Grounded spices can easily be falsified, e.g. by colouring with forbidden dyeing agents such as lead oxide or Sudan red or by stretching the products with filling material like sand or starch. The history of fraud in relation to spices and herbs is long and ongoing, as new examples such as the falsification of oregano with olive leaves most recently observed in UK show.

The authenticity testing of spices and herbs was and still is therefore subject of several European research projects and other studies. Because the classical approaches for assessing authenticity suffer from a number of disadvantages, namely, the increasing number of analytes, which must be introduced in dedicated test procedures, the limited knowledge of the "normal" composition of materials and most importantly their incapacity to detect unforeseen agents, one focus of current research activities points on non-targeted analytical techniques (fingerprinting techniques) to investigate any kind of distinctive features. The workshop will present results related to new authentication approaches to condiments and will provide an insight into future, rapid and cost-effective, approaches for quality and safety control of spices and herbs.

THURSDAY, April 7, 2016

8:00-9:00 Registration for the FOODINTEGRITY 2016 workshops

SESSIONS 6 & 8, in parallel

9:00-10:30 Prague A&B hall
SESSION 6: Workshop on Food Crime, occurrence, motivations and mitigations (part I)
 Moderator: Jon Spencer, University of Manchester, UK

9:00-9:20 **L28** THE MODELING OF FOOD SUPPLY TO ASSIST FRAUD DETECTION
Cecilia Flores & David Allen, University of Manchester, Manchester, UK

9:20-9:40 **L29** CORPORATE CRIME IN THE MEAT SUPPLY CHAIN
Wim Huisman, VU University, Amsterdam, the Netherlands

9:40-10:00 **L30** DUTCH PIG FARMERS: NON-COMPLIANCE IN CONTEXT
Fiore Geelhoed, VU University, Amsterdam, the Netherlands

10:00-10:20 **L31** BRIDGING THE GAP - A CRIMINAL INTELLIGENCE PERSPECTIVE ON FOOD CRIME
Andy Morling, Food Standards Agency: England and Wales, UK

10:20-10:30 Discussion

10:30-11:00 Conference area
 Coffee Break / Exhibition / Poster session

SESSIONS 7 & 9, in parallel

11:00-13:00 Prague A&B hall
SESSION 7: Workshop on Food Crime, occurrence, motivations and mitigations (part II)
 Moderator: Andy Morling, Food Standards Agency, UK

11:00-11:15 **L32** FOOD FRAUD PREVENTION: POLICY, STRATEGY, AND DECISION-MAKING - IMPLEMENTATION STEPS FOR A GOVERNMENT AGENCY OR INDUSTRY - INCLUDING TRANSLATION TO CHINESE
John Spink, Michigan State University, USA

11:15-11:30 **L33** CRIMES AND HARMS IN THE MEAT INDUSTRY
Loes Kersten, KU Leuven, Leuven, Belgium

11:30-11:45 **L34** CRIMINOLOGICAL APPROACHES TO FOOD FRAUD
Jon Spencer, University of Manchester, Manchester, UK

11:45-12:00 **L35** THE ROLE OF THE REGULATOR
Peter Whelan, Food Safety Authority: Ireland, Ireland

12:00-13:00 Discussion

13:00-14:00 Hotel restaurant Loreta
 Lunch

Workshop: Food Crime, occurrence, motivations and mitigations

Workshop Aims and Objectives

There has been a significant increase in the social science research into Food Crime over the past four or five years and it brings together diverse disciplines. The science of fraud detection has relied on new methods of testing and increased levels of audit. This scientific work has a social dimension and the development of a theoretical criminological approach to understanding food crime is innovative and exciting. However, the detection food fraud happens 'after the event' of fraud or adulteration, testing identifies the adulterated product.

The aim of this workshop is to bring together criminologists and social scientists working in the area of food crime with those working in the food industry to explore the activity of food crime from different perspectives.

The objectives of the workshop are to:

- i. Provide a number of different papers that discuss the issue of food crime from a range of perspectives and contribute to the developing theoretical framework to aid our understanding of food crime
- ii. To explore the different perspectives of the Social Sciences and how they enhance our understanding of food crime
- iii. Encourage and aid discussion and identify the emerging issues in the area

Workshop Format

The workshop will be structured around two elements. First, there will be a series of short presentations that will consider the 'state of the art' of what we know about food crime. These papers will draw on current research and practice. There will be time for discussion of each presentation. There will be a discussant for the session that will assist in identifying the knowledge gaps and stimulate discussion as to how these gaps might be addressed.

The second part of the workshop will focus on developing scenarios of future food crime issues. This will be structured using a roundtable of experts drawn from industry, academia and regulation to consider future food crime scenarios. This approach begins to address one of the key issues in the food crime area, which is the ubiquity of food frauds and adulteration. Scenario planning may assist us in further understanding the interaction between a numbers of competing dynamics within the food crime area that will aid prevention.

THURSDAY, April 7, 2016

8:00-9:00 Registration for the FOODINTEGRITY 2016 workshops

SESSIONS 6 & 8, in parallel

9:00-10:30
Prague C&D
hall

**SESSION 8: Workshop on
Industrial perspective for strategies applied for assuring food
authenticity (part I)**

How to improve protection from food frauds and adulterations
Moderator: Michele Suman, Barilla, Parma, Italy

9:00-9:10 **L36** **OVERVIEW OF CURRENT RESEARCH AND PRACTICAL OUTCOMES
OF FOODINTEGRITY PROJECT**

Michele Suman, Barilla SpA - Advanced Laboratory Research, Parma, Italy

9:10-9:20 **L37** **USING THE FOODINTEGRITY NETWORK & THE FOOD INTEGRITY
KNOWLEDGE BASE**

Michele Lees, Eurofins Analytics France, Nantes, France

9:20-9:30 **L38** **RAPID METHODS PERSPECTIVES (FOCUS ON THE SPIRIT DRINK
SECTOR)**

Shona Glancy, The Scotch Whisky Research Institute, Edinburgh, UK

9:30-9:40 **L39** **Cancelled**

9:40-9:50 **L40** **1H-NMR NON-TARGETED DETECTION OF ADULTERANTS IN
VEGETABLE OIL**

James Donarski, Fera Science Ltd, York, UK

9:50-10:00 **L41** **SPECTROSCOPIC TECHNOLOGIES AND APPLICATIONS FOR
AUTHENTICATION & ANTI-COUNTERFEITING**

Neville Davies, Ocean Optics, Duiven, the Netherlands

10:00-10:10 **L42** **INDUSTRIAL SELF-CONTROL IN THE FRUIT JUICE INDUSTRY: A
MODEL FOR OTHER FOOD INDUSTRY SECTORS**

Aintzane Esturo, SGF International, Nieder-Olm, Germany

10:10-10:20 **L43** **HONEY IDENTITY: NEW APPROACHES TO THE BOTANICAL ORIGIN
OF HONEY BY NEXT GENERATION SEQUENCING**

Maria Teresa Barreto Crespo, iBET, Oeiras, Portugal

10:20-10:30 **L44** **THE USE OF STABLE ISOTOPES FOR MONITORING OF PRODUCTS
CLAIMING REGIONAL ORIGIN. A PROOF OF CONCEPT**

Markus Boner, Agroisotop GmbH, Jülich, Germany

10:30-11:00
Conference
area

Coffee Break / Exhibition / Poster session

SESSIONS 7 & 9, in parallel

11:00-13:00
Prague C&D
hall

**SESSION 9: Workshop on
Industrial perspective for strategies applied for assuring food
authenticity (part II)**

How to improve protection from food frauds and adulterations
Moderator: Michele Suman, Barilla, Parma, Italy

11:00-13:00

An interactive team working exercise among the participants to address future scenarios related to:

- (i) Fragmentation of the chains,
- (ii) Effective rapid screening approaches,
- (iii) Confirmatory analytical strategies,
- (iv) Assessments and prevention models

13:00-14:00

Hotel restaurant Loreta

Lunch

Workshop: Industrial Applications for Assuring Food Authenticity (HOW TO IMPROVE PROTECTION FROM FOOD FRAUDS AND ADULTERATIONS)

Workshop Aims and Objectives

European food is of prime importance to the European Agri-food economy.

The authenticity of European food and the integrity of supply chains is under constant threat from fraudulent activities.

The topic of assuring the integrity of the food chain brings together producers, distributors, processors, retailers, regulators, researchers, enforcers and consumers.

The present workshop will be an opportunity for all these stakeholders to raise their awareness of food authenticity issues and get together in order to achieve the following objectives:

- i. Share common relevant issues combined with best practices, enlarging the existing network and boosting exchange and collaboration
- ii. Allow open and constructive discussions on how current research is expected to develop practical mitigation solutions for companies to use to protect themselves from food fraud
- iii. Review all the relevant activities and tools that FoodIntegrity project has already made available and/or is developing for the industry (e.g. FI Network, FI Knowledge Base,...)

Workshop Format

The first part of the workshop will be devoted to have a series of short talks that will consider the 'state of the art' achieved from the FoodIntegrity project on the industrial side, together with stimulus and good practices provided directly from industrial player presenters from different points of the food chain.

At the end of each presentation, a moderator of the session will assist in identifying the knowledge gaps and stimulate discussion as to how these gaps might be addressed.

The second part of the workshop will be dedicated to an interactive team working exercise among the participants to address future scenarios related to: (i) fragmentation of the chains, (ii) effective rapid screening approaches, (iii) confirmatory analytical strategies, (iv) assessments and prevention models.

POSTER SESSION

WEDNESDAY - THURSDAY, April 6-7, 2016

13:00-14:30 **POSTER SESSION (Wednesday, April 6, 2016)**

Posters are displayed during the whole conference.

- P1** DETERMINATION OF PRIORITIES BY THE MANUFACTURER FOR FRAUD PROCESSED MEAT PRODUCTS, IN TURKEY
Alev Akpınar Borazan
- P2** METROFOOD-RI: A NEW PAN-EU RESEARCH INFRASTRUCTURE TO SUPPORT FOOD INTEGRITY
Giovanna Zappa, Claudia Zoani, Isabel Castanheira
- P3** ADVANCES IN TOOLS TO SUPPORT FOOD FRAUD VULNERABILITY ASSESSMENT AND RISK MITIGATION
Karen Everstine, Jeffrey Moore, Henry Chin, Shaun Kennedy
- P4** FOOD FRAUD PREVENTION GUIDE FOR AGRIFOOD SECTOR. AN INTEGRITY ASSESSMENT TOOL FOR APPLIED STRATEGIES IN ORDER TO ASSURE FOOD AUTHENTICITY
Catherine Vidal, Gloria Cugat, Adriana Fernandez, Montserrat Sibera, Rosa Maria Biel
- P5** MULTIDISCIPLINARY APPROACH FOR FOOD FRAUD DETECTION
Leo van Raamsdonk
- P6** THE VIRTUAL FOOD AUTHENTICITY NETWORK
Selvarani Elahi, Stephen Ellison, Mark Woolfe, Michelle McQuillan, Lucy Foster, Sophie Rollinson
- P7** ARE THEY AT RISK AND DO THEY KNOW? FOOD SAFETY KNOWLEDGE OF POULTRY MEAT CONSUMERS IN SLOVENIA
Sonja Smole Možina, Meta Sterniša, Špela Zorko, Sonja Levstek, Andreja Kukec, Mojca Jevšnik, Peter Raspor
- P8** BIG DATA FROM A SMALL LAB: COMPLETE HOLISTIC/NON TARGETED/FINGERPRINTING OVERVIEW USING ALTERNATIVE INSTRUMENTAL APPROACH FOR FOOD AUTHENTICATION AND FRAUD PREVENTION
Roberto Piro
- P9** MAPPING THE BEEF SUPPLY CHAIN FROM FARM TO FORK FOR TRANSPARENCY
Stephanie Brooks, Christine Walsh, Michelle Spence, Christopher Elliott, Moira Dean
- P10** REAL-TIME PCR FOR SALMON IDENTIFICATION
Amanda Naaum, Robert Hanner
- P11** APPLICATIONS OF VIBRATIONAL SPECTROSCOPY FOR FEED SAFETY CONTROL: DETECTION OF ANIMAL ORIGIN MATERIAL BY NIR AND RAMAN SPECTROSCOPY
Luisa Mandrile, Giuseppina Amato, Daniela Marchis, Gianmario Martra, Andrea Mario Rossi
- P12** PEPTIDE PROFILES AS NOVEL AND HIGHLY SENSITIVE MARKERS FOR THE HEAT TREATMENT OF MILK
Sevim Dalabasmaz, Monika Pischetsrieder
- P13** GC-MS DETERMINATION OF CYCLOPROPANE FATTY ACIDS: A NEW TOOL AGAINST PARMIGIANO REGGIANO COUNTERFEIT
Angela Marseglia, Marco Nocetti, Veronica Lolli, Gerardo Palla, Augusta Caligiani
- P14** MINIATURIZED NIRS FOR NON-DESTRUCTIVE AUTHENTICATION OF PACKAGED CHICKEN FILLETS
Yannick Weesepeoel, Saskia van Ruth
- P15** VALIDATION CRITERIA FOR SIMULTANEOUS MULTI COMPONENT QUANTITATIVE NMR ANALYSIS AND NMR FINGERPRINTING METHODS
Vito Gallo, Piero Mastrotrilli, Mario Latronico, Pasquale Scapicchio, Nicola Intini, Antonino Rizzuti

- P16** **NEW IMMUNOASSAYS FOR MAJOR MILK FRAUD**
Willem Haasnoot, Lucia Streppel, Ana Frangolho, Claus Schafer-Nielsen, Sotirios Kakabakos
- P17** **FREE RANGE FRAUD: HOW MODERN ANALYTICAL TECHNIQUES CAN BE USED IN CONJUNCTION WITH AGRICULTURAL EXPERTISE TO AUTHENTICATE FREE RANGE AND ORGANIC EGG PRODUCTION**
 Alison Johnson, Robert Posey
- P18** **SCREENING AND IDENTIFICATION OF FOOD SUPPLEMENT ADULTERANTS USING LIQUID CHROMATOGRAPHY WITH HIGH-RESOLUTION MASS SPECTROMETRY**
Katerina Mastovska, Lukas Vaclavik, John R. Schmitz, Jean-Francois Halbardier
- P19** **FRAUD DETECTION IN MARINE PRODUCTS WITH MOLECULAR ANALYSIS TECHNIQUES. A CASE STUDY IN ELASMOBRANCHII**
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- P20** **TACKLING FISH FRAUDS: STRATEGIES TO DISTINGUISH FRESH FROM FROZEN FISHERY PRODUCTS**
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- P21** **C-SNIF-NMR - A COMPLEMENTARY TOOL IN FOOD AUTHENTICITY CONTROL**
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- P22** **SENSITIVE DETECTION OF ECONOMICALLY MOTIVATED ADULTERATION OF HONEY BY BULK AND COMPOUND SPECIFIC C ISOTOPE RATIO MASS SPECTROMETRY USING LIQUID CHROMATOGRAPHY AND ELEMENTAL ANALYSIS INLET DEVICES**
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- P25** **FATTY ACID COMPOSITION AND δ C ISOTOPIC RATIO CHARACTERIZATION OF PUMPKIN SEED OIL**
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- P34** DETERMINATION OF CARBON ISOTOPE RATIO OF ETHANOL IN CHINESE SPIRIT BY LIQUID CHROMATOGRAPHY COUPLED TO ISOTOPE RATIO MASS SPECTROMETRY
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Vendor seminars

APRIL 6, 2016 (13:30-14:20)

ThermoFisher S C I E N T I F I C

VENDOR SEMINAR: ORBITRAP TECHNOLOGY: THE NEW FRONTIERS IN FOOD PROFILING

Introduction to Thermo Fisher Scientific Solutions for Food Profiling

Cesare Rosini, Thermo Fisher Scientific, Italy

Orbitrap technology: the new frontiers in food profiling

Prof. Jana Hajslova**Chyba! Záložka není definována.**, Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic

Quality, safety and authenticity represent the key pillars of food integrity. A wide range of laboratory techniques has been employed to control these attributes, aiming at protection of consumers' health and fraud prevention. As regards food authenticity, while combination of various targeted analyses was used in past time for this purpose, nowadays, non-targeted fingerprinting /profiling approaches have become the 'gold standard'. In this way, a more comprehensive information on respective sample may be obtained within a single analytical procedure.

In our presentation, two case studies will be shown. The high resolution, accurate mass spectrometry (HRAM) enabled by hybrid quadrupole-Orbitrap mass spectrometer as a challenging tool for classification of olive geographical origin. The U-HPLC-HRMS/MS fingerprinting of polar metabolome fraction followed by sophisticated data processing were employed for. The strategy to identify specific authenticity markers will be discussed. The second study was focused on cannabinoids profiling in various cannabis products. With regards to fairly different concentration of cannabinoids present in various cultivars of Cannabis sativa plants, the quantification strategy had to be optimized to prevent inaccurate results due to the measurements outside the linearity range.

APRIL 6, 2016 (13:30-14:20)



**VENDOR SEMINAR:
ENSURING THE AUTHENTICITY OF FOOD - NEW ADVANCES IN
LC-MS/MS WORKFLOWS BRINGING ROUTINE CLOSER THAN
EVER**

**Ensuring the authenticity of food - New advances in LC-MS/MS workflows
bringing routine closer than ever**

Dr Ashley Sage, SCIEX, Warrington, Cheshire, UK
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Food testing can be a challenging and complex job. From sample preparation (so many different matrices!) to residue detection (so many different compounds from pesticides and mycotoxins, and not to mention the mysterious unknowns!), going from the raw sample to the final result of "What is in this food sample?" is no trivial task. And how do you know that the food is authentic?

Luckily, a number of analytical tools and workflows are available to ease the pain and help you to answer the question above, quickly and efficiently, but also with the confidence that you arrived at the right result, every time.

In this presentation, we will describe new LC-MS/MS technology and software tools that will make your food testing workflows more routine than ever. We will highlight new High Resolution LC-MS/MS instrumentation that can allow you to screen large samples sets for hundreds of contaminants and residues, whilst reducing the risk of reporting a positive results and lowering the likelihood of missing a result (fewer false positives). We will also show new routines for bringing together both quantitation and identification data into a single, intuitive to use platform, for streamlined data interrogation, and touch upon novel ways to reduce troublesome matrix interferences. And we will also show how the authenticity of food can be confirmed in a routine way using high resolution LC-MS/MS.

**A novel approach for authentication of durum/common wheat based on
liquid chromatography high-resolution tandem mass spectrometry merged
with chemometrics**

Josep Rubert¹, Laura Righetti², Kamila Hurkova¹, Milena Stranska-Zachariasova¹, Gianni Galaverna², Jana Hajslova¹, Chiara Dall'Asta²

¹ Department of Food Analysis and Nutrition, Technická 3, 166 28 Prague, Czech Republic

² Department of Food Science, University of Parma, Parma, Italy

Control of authenticity and safety of milk thistle based food supplements

Milena Stranska-Zachariasova, Alena Zachariasova, Petra Slavikova, Jana Hajslova
Department of Food Analysis and Nutrition, Technická 3, 166 28 Prague, Czech Republic

Distinguishing botanical origin of berries based on newly identified markers

Kamila Hurkova, Josp Rubert, Milena Stranska-Zachariasova, Jana Hajslova
Department of Food Analysis and Nutrition, Technická 3, 166 28 Prague, Czech Republic

Oral sessions

L1 INTRODUCTION TO THE FOODINTEGRITY

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Food Integrity “the state of being whole, entire, or undiminished or in perfect condition”. Providing assurance to consumers and other stakeholders about the safety, authenticity and quality of European food (integrity) is of prime importance in adding value to the European Agri-food economy. The integrity of European foods is under constant threat from fraudulently labelled imitations that try to exploit that added value. The FOODINTEGRITY project will directly address this issue and will be an international focal point for harmonisation and exploitation of research and technology for insuring the integrity of European food. Comprising an inner core of project participants from industry, academia, research institutes, technology providers and a global network of stakeholders, FOODINTEGRITY will rationalise and harmonise capability to provide a coherent structure and process for assuring the food supply. FOODINTEGRITY will: facilitate the sharing of information between stakeholder groups regarding European food integrity; establish processes for harmonising and exploiting existing databases; establish fit for purpose methodology to address stakeholder needs; identify and address research gaps by procuring and delivering €3M of commissioned projects; establish a self-sustaining Food-fraud early warning system for identifying emerging food fraud risks; establish a self-sustaining worldwide network of stakeholders to ensure maximum uptake of the project legacy. Improved verification procedures will be developed for food control and industry stakeholders using 3 key commodities as exemplars: olive oil, spirit drinks and seafood. In addition a consumer study in China will assess their consumer attitudes in the face of substantial counterfeiting of European food. Finally it will establish expert food authenticity platforms that will supply independent expert opinion on food authenticity/food fraud to the European Commission, Codex and other national/international bodies.

Keywords: food integrity, food safety, food quality, food authenticity, food traceability

Acknowledgement: The project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L2 THE IMPACT OF FOOD FRAUD ON CHINA: RISKS AND PREVENTION STRATEGIES

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The lecture includes two topics, one is Economically Motivated Food Adulteration in China, and another is Strategies to Address Economically Motivated Food Adulteration in China. The main content will include in the relationship between China's food industry and food safety, the economically motivated (EMA) food adulteration, major EMA cases in China, challenges from EMA food adulteration that China should face, introduction of The List of Non-edible Substances Might be Adulterated in Food (The Black List), the background of developing The Negative List for Non-edible Food Ingredients, the revision principle, and working mechanism, the establishment of Expert Panel of Non-edible Ingredients Might be Adulterated in Food and the responsibilities and role of the expert panel.

Keywords: food fraud, China; risks, prevention strategies, list of non-edible substances might be adulterated in food

Acknowledgement: Supported by the Special Public Welfare Project (No 2012104003)

L3

FOOD AUTHENTICITY - CONSUMER EXPECTATIONS (AND DISAPPOINTMENTS)

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Food authenticity has long been an issue for consumers and consumer organisations. From large scale product recalls as a result of Sudan dye contamination of chilli powder to use of pork and beef proteins to allow water to be added to chicken, fraud has been an issue that can leave consumers out of pocket, but also present health risks and raise issues of ethical acceptability. The horsemeat scare brought issues of fraud to the fore – and highlighted just how complex the food supply chain has become, as well as how vulnerable. Testing by Which? and our sister consumer organisations has found issues across a range of meat products, including lamb takeaways, burgers and kebabs. But it has also highlighted how other products such as goat's cheese and honey can also be at risk of substitution with cheaper ingredients where there is an opportunity for profit to be made and supply chains are often a lot more complex than consumers would expect. Following the horsemeat scare, controls over fraud received a lot of scrutiny at UK and EU level. Professor Elliott's report, setting out a consumers first, zero tolerance approach was strongly supported by consumer groups and accepted by the Government. The EU action plan highlighted the importance of effective exchange of information about food fraud, traceability and dissuasive penalties. Three years on and at a time when official controls are under increased pressure and food standards work, in particular, has been in decline, it is essential to ensure that early identification to prevent fraud and effective enforcement action when it does occur remain a priority. Authorities need to keep one step ahead. Consumers should be able to trust that their food is what it says it is and our consumer research suggests that there can be a lasting impact on consumer confidence when it is not.

L4 NON-TARGETED METHODS: ADVANCES AND CHALLENGES AHEAD

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Food fraud costs the global food industry several billion dollars every year, negatively impacts public confidence in food producers and regulators, and can result in unfortunate public health consequences. Non-targeted methods have gained recent interest due to their potential to detect new unexpected adulterants and deter adulteration in general from entering supply chains. A multinational collaborative team led by a US Pharmacopeia expert panel is researching and developing a tool-box of non-targeted analytical methods and supporting reference materials for detecting adulteration in milk ingredients. This presentation will give an update on the outcomes to date of this collaborative effort, including studies conducted to evaluate and develop NIR, Raman, NMR, MALDI-TOF-MS, UPLC and wet-chemistry technologies. An update will be provided on a USP Guidance being developed on how to develop and validate non-targeted methods, as well as a discussion on the importance of reference materials to support non-targeted methods. Lastly the presentation will highlight the challenges ahead to advance the development and widespread utility of non-targeted methods.

Keywords: non-targeted analysis, rapid adulteration detection methods, food authenticity

L13

EUROPEAN FOOD AUTHENTICITY AND CHINESE CONSUMERS. REDUCING CONCERNS ABOUT FOOD SAFETY

**Lynn J. Frewer^{1*}, Helen Kendall², Mei-Yen Chan³, Beth Clark⁴, Moira Dean⁵,
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Authenticity assurances associated with food and drink may reduce consumer concerns about food safety and fraudulent production. This research reports the results of a mixed methods study, which aimed to understand the relationship between the concerns of Chinese consumers regarding food safety, and the role that authenticity assurance may play in relieving those concerns. Initially, qualitative research explored concerns held by Chinese consumers in relation to food safety and authenticity. The results, together with insights from the relevant literature, were used to inform the design of a quantitative survey instrument which was used to test hypotheses about the relationship between perceptions, attitudes, and behavioural intentions regarding the purchase of “authentic” European products. The results of 6 focus groups in 3 Chinese cities indicated that study participants perceived that many locally produced foods were fraudulent and raised food safety issues. Serious concerns among Chinese consumers about the quality and safety standards of the Chinese domestic food supply chain when compared to other developed global food markets were revealed. Against this, evidence of authenticity and traceability were associated with improved food safety. Three main barriers were identified by participants as preventing consumers from assessing food as being authentic and safe; the regulatory environment; the complexity of the food supply chain and intentional fraudulent activity made possible by system complexity. Given the perceived lack of regulation and consumer protection offered by government and industry, a number of “risk relieving” strategies were used by consumers to manage the perceived prevalence of inauthentic food, including the use of heuristics and judgments based upon tangible product cues. Mistrust was perpetuated by numerous food scandals with participants resigned to the fact that fraudulent activity was widespread within the domestic supply chain, and hence unavoidable. The results of the focus groups were used to inform a survey (N=850 participants, 3 Chinese cities). The results of this quantitative survey indicate a high level of concern about adulterated foods, counterfeit foods and (mis)description of contents. The application of Structural Equation Modelling confirmed that the greater the level of concern about food safety, the greater the perceived *risks* associated with food safety, and the perceived *benefits* of authenticity, and authenticity cues, including association with European products. A more positive attitude towards, and intention to purchase, foods and drinks that have been traced for authenticity was observed. Differences in the strength of these relationships across different product types will be discussed. In conclusion, demonstrating authenticity improves the trust of Chinese consumers in the food system, and authenticity cues (e.g. product labels) direct consumer food choices to relieve food safety concerns.

Keywords: food safety, food authenticity, Chinese consumers, European products

Acknowledgement: The research has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L14 AN OVERVIEW OF THE USE OF CITIZEN SCIENCE, AND LESSONS FROM THE ENVIRONMENT SECTOR IN THE UK

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Science is our most reliable system of gaining new knowledge. Citizen science, also known as ‘crowd-sourced science’, ‘volunteer monitoring’, ‘amateur science’ and ‘public participation in scientific research’, is the public involvement in research and the discovery of new scientific knowledge. A citizen science project can involve just a couple of people, or millions collaborating towards a common goal across the globe. A citizen scientist is an individual who voluntarily contributes his or her time, usually on an issue which interests or concerns them. This involvement mainly relates to either data collection or data analysis, but is not limited to this. Citizen science has grown massively over the last decade, in parallel with home computing/smart phone ownership and the use of social media. Examples of the fields that citizen science works in include astronomy, environment and ecology, medicine and public health, genetics, psychology and many more. Citizen science can deliver large data sets as well as real-time monitoring. In addition human brains can be harnessed for problem solving alongside distributed-computing power to deliver ‘distributed thinking’, a higher-order citizen science. The ultimate direction citizen science may take is unknown, but it presents many opportunities. This excitement should be tempered by the realities of developing and running a citizen science project. The issues will vary according to the type of citizen science envisaged. A recent analysis of citizen science in the UK environment sector can provide some relevant lessons.

Keywords: citizen science, UK environment sector

L15 CITIZEN SCIENCE APPROACH TO IDENTIFYING MISLABELLING IN THE FISH SECTOR: STUDY DESIGN AND POTENTIAL IMPACT IN RESTAURANTS

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Seafood is the most traded food commodity in the world and its production has been steadily growing over the last decades. In fish trading, it has been repeatedly recognized that the use of common names or commercial designations to describe various fish types can hamper consumer choice, since this groups together species for sale that have markedly different prices. In this global chaos, the utilization of both locally and internationally recognizable names in fish product labeling must be officially taken into consideration to ensure traceability in the fish chain. In addition, mislabeling and erroneous identification of fish catches, or their geographical origin, is one of the factors involved in underreported catches from specific stocks and could threaten the sustainability of fisheries, therefore contributing to the depletion of fishery resources, or even the eventual extinction of the overexploited species. The identification of fish is mandatory in the European Union, as stated in the Council Regulation (EC) No 1379/2013 of 11 December 2013 on the common organization of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000. These regulations require that fish labels indicate the complete scientific and commercial name of the species without inducing errors. Recent studies have stated that the average percentage of reported misdescription is 30%. In general, incidents in restaurants and takeaways are much more common than in supermarkets and retailers. However, specific studies should be conducted to confirm it because about 10% of samples were obtained from restaurants. Such an undertaking requires an enormous effort that can be faced with the support, in whole or in part, by amateur or nonprofessional scientists as collectors and that is exactly what we have done in the present study which is the first of its kind for Europe. To date, we have collected approximately 400 fish samples from 250 restaurants in 17 European countries taking advantage of the participation of citizens. The first part of this study was to establish a representative sample size from different regions to obtain a statistically significant study with the less margin of error. Later on, samples will be analyzed by sequencing (PCR-FINS) to identify the fish species and to evaluate the percentage of misdescription in this important sector.

Keywords: fish species, misdescription, restaurants, PCR-FINS

Acknowledgement: The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L16 CONCEPTUAL FRAMEWORK FOR AN ONLINE EARLY WARNING SYSTEM FOR FOOD FRAUD DETECTION

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In this talk, we discuss the challenges in building an online Early Warning System for food fraud detection that is the main aim of FoodIntegrity. A conceptual framework has been developed and has been refined to tackle such challenges. Within this framework, we describe the process of estimating the likelihood of fraud for a certain commodity based on a set of triggers that have been proposed in the literature or by the stakeholders. This probability notion helps the end-users, e.g. producers and retailers, to direct their resources for testing products on the basis of emerging risk. The online property of the model means that it will not rely on the past events only, but rather tries to identify emerging risks by analysing available data and can be updated using Bayes rule to incorporate new information that becomes available through time. Within this process, we also provide taxonomy of the potential triggers of the food fraud with a special focus on the triggers linked to Economically Motivated Adulteration, as opposed to other types of fraud, based on commodity's characteristics and socio-economic characteristics of the countries including prices of the commodities, trade, climate, change in regulations, etc. Using the estimated probability of the fraud and the data that describes the exposure (damages to business, number of customers affected, etc.), we aim to monetize the direct and indirect costs of fraud in some case studies.

Keywords: food fraud detection, early warning system, conceptual framework

Acknowledgement: The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L17 DEVELOPMENT OF EARLY WARNING SYSTEMS TO DETECT, PREDICT AND ASSESS FOOD FRAUD

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Within the work pack 8 of the EU FP 7 project FoodIntegrity tools and approaches are developed to help direct and prioritise industry monitoring and regulatory enforcement activities against food fraud. To this end, a new filter has been constructed within the European Media Monitor (EMM) portal "Medlsys" that collects media publications on food fraud globally in eight different languages. In the period September 2014 to December 2015, 1201 media reports on food fraud were detected of which the majority was about meat followed by seafood, milk and alcohol. At present, the efficiency of this filter is being assessed. In addition, we have explored the application of Bayesian network (BN) modelling to predict the type of fraud reported in Rapid Alert for Food and Feed (RASFF) as to aid risk manager/ controller in deciding which fraud type to check when importing products from outside the EU. A BN model was constructed from food fraud alerts reported in RAS FF in the period 2000-2013 and used to predict the type of food fraud reported in RASFF in 2014. The constructed model could predict 80 % of food fraud types correctly when the country of origin and food category had been reported previously in RASFF. We further explored the usefulness of BNs in connecting different drivers, data sources, expert knowledge and their interactions in a holistic approach in order to determine their interdependency in relation to the food fraud reports in RASFF and EMA (Economically Motivated Adulteration Incidents Database).

Keywords: early warning system, food fraud

Acknowledgement: The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L18 TRACEABILITY AND BRAND PROTECTION

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Most traceability solutions have had food safety and recall as their main focus. Now we are seeing a shift towards active use of traceability as an integral part of the brand message. There has also been a shift in power from the brand owner to the consumer, which through Social Media can make or break a brand in a matter of hours. The global supply chains of today, demands a new level of control. A major threat to any brand today is fraud in their own supply chain. Tracetracker and partners are focused on helping brand owners take steps to strengthen brands and fight fraud. One of the techniques in this quest is to make it difficult to copy a brand, like this exciting example from Sweden describes. Last year Hälsingestintan launched a world first fully traceable meat product range. The key innovation lies in the unique mobile butchery they have developed. Cows no longer travel long distance from their home farm. This resulting in better animal welfare, tighter control of the supply chain and presumably better tasting meat. A QR code on the package gives access to the full history of the meat. This has been very well received by consumers. In light of the current large scale fraud being exposed in Sweden the timing could not have been better. In Sweden all cattle are tagged and registered at birth or import into a nation database. In order to ensure authenticity at farm level the RFID ear tag is scanned several times throughout the animal's life, recording weight, time and location. During slaughter the ID is read and stored combined with a location and timestamp. The carcass is split into four parts, given new unique serialized IDs. These IDs are used in the critical cutting process where the carcass parts are divided into several individual pieces, each then packaged and labelled with new unique QR-codes. The key to ensuring a trustworthy story about origin is to capture and verify the most critical information in the chain with a reference to the origin. This also includes creating new information, like label printing. If the reference to the origin is not verified, the system will just supply the ordinary traceability information. All the critical traceability events are captured into the central traceability engine. When codes are scanned by the consumer, all relevant information are merged and presented to the user dynamically. In conclusion we have found the current labeling methods and certification programs lacking in their ability to fight food fraud. Spot checking and annual inspections fail to detect deliberate fraud, and can in many cases actually make it easier as their "stickers" are easily available and easy to copy. The only logical next step is to introduce serialization of the label. If one wish to secure the value of the brands and obtain consumer trust, one has to take control over the information flow, including what is printed on the labels.

Keywords: traceability, brand, fraud

L19

CHECK ORGANIC: ENSURING THE INTEGRITY OF THE ORGANIC FOOD SUPPLY CHAIN**Gerald Herrmann^{1*}, Frank Gerriets²**^{1,2} Organic Services GmbH, Tutzing, Germany

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The organic food sector has been rocked by a number of scandals that have tested the strength of the control system put in place to safeguard its integrity. Unfortunately, major weaknesses of this system are the use of certificates, which can be easily forged, and the sale of conventional food and feed as organic, which can be easily mislabelled. These weaknesses are evidenced by the growing number of fraudulent organic certificates and mislabelled conventional food and feed uncovered in the last 5 years. With the rapid growth of the food sector and the introduction of criteria based audit systems, the need to combat fraud is extremely important. The reality of the situation though is that audit data is stored in a variety of locations, making the verification process lengthy and cumbersome. To tackle this issue, Organic Services has developed Check X, a global, real-time certification database that offers verifications of audit data, notifications of certificate status changes, supplier lists, supply chain mapping and volume monitoring as cloud based solutions. Check X is a tool designed to increase the level of transparency in food supply chains through improved management techniques. This feature enables operators and traders to mitigate risk within their supply chains and to save time and money by streamlining the verification process. Furthermore, operators and traders can bring this transparency to their individual supply chains and know immediately when there are potential integrity issues, better preparing them for inspections through enhanced documentation. Check X simplifies supply chain management by bringing audit data together on one platform, and by providing the tools necessary to efficiently and effectively use this data. What makes Check X unique is the technology on which it is based – the industry leading certification and supply chain management software from Intact Consult GmbH, Austria. The first application developed from Check X is Check Organic for the organic and fair trade sector. Check Organic is a service for this sector; therefore, the costs for such a solution should fall on the operators and traders who use its services, not on the certifiers who help make this solution possible. Moreover, Organic Services has realised that it cannot go down this path alone and has partnered with leading organic sector organisations to ensure that Check Organic is able to fill the voids found in the current approach to integrity. Through one of these partnerships, a nationwide pilot project of the volume monitoring service has already gotten underway in Italy with FEDERBIO. Check Organic's supply chain mapping service has already been tested and proven as a helpful solution as FAIRMONITOR, which is used on a global scale for Fairtrade supply chains. Organic Services will present its solution Check Organic, findings from its experiences with its ongoing projects and the way forward for its continued success.

Keywords: supply chain integrity, traceability, real-time verification, organic food fraud, industry based solution

L20

EUROPEAN KNOWLEDGE BASE ON ANALYTICAL METHODOLOGY AND DATABASES FOR FOOD AUTHENTICITY

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There is a wealth of information available on suitable analytical tools and associated reference data for the detection of food fraud or for assessing the authenticity of a food product. A substantial proportion of this information is already in the public domain or can be obtained through scientific publications, but it not always easily accessible for enforcement agencies, food operators or even research organisations. In the period 1989 to 2014, the European Commission funded 95 projects on food authenticity and traceability through its research programmes in the various Framework Programmes. The majority of these have involved the development of new analytical techniques. A suitable tool is required to make it easier to reach the results of these projects. A European Knowledge Base is being developed as part of the FoodIntegrity project to bring together the available information on, on the one hand, the methods used to ensure the integrity of food products and ingredients and, on the other, the data generated by those methods. It is designed as a Web-based tool for use by both industry and regulatory authorities and will provide intelligence on the type, frequency, and impact of various food fraud practices together with recommended analytical strategies to deal with the issues. A specially-designed search engine will enable the stakeholder to select a food category from a drop-down list, enter a CN code and/or specify a type of fraud (dilution, substitution, etc.) or food integrity issue (geographical origin, product composition, etc.). The results will feature existing analytical solutions, links to scientific publications and sets of analytical data when available. It will also be possible to link up with the FoodIntegrity Network for advice from the relevant expert.

Keywords: analytical methodology, food fraud, knowledge, analytical data, database

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L21 EXPANDING ANALYTICAL CAPABILITIES WITHIN SPIRIT DRINKS AUTHENTICATION

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The spirit drinks sector is important for consumers, producers and the agricultural sector within the European Community. It is the most valuable European agri-food export sector (€10bn in exports, representing a trade surplus close to €9bn). Analytical methods that can ensure the safety and authenticity of European spirit drinks have been developed to meet sector requirements. However, the spirit drinks industry recognises that improvements in the assurance of its products can still be made using available and developing analytical tools. The Spirit Drinks Works Package of the FoodIntegrity project has targeted some of the areas where the industry believes that analytical techniques can be progressed to the benefit of spirit drinks authentication. The use of "in-field" technologies that will allow counterfeit samples to be quickly identified at point of sale or distribution is one target area. The development of laboratory techniques to provide more comprehensive, authoritative and rapid detection of counterfeits is another. Training in appropriate analytical methods and interpretation of analytical data is also an area where improvements are desired. This presentation will consider some of the analytical authentication challenges posed by the FoodIntegrity Spirit Drinks Work Package. Selected analytical solutions being evaluated within the Work Package will be used to help illustrate the problems posed, and the types of techniques that are being developed to address them.

Keywords: authentication, counterfeit, spirit drinks, denaturants

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L22

EMERGING PORTABLE SPECTROSCOPICS FOR NON-DESTRUCTIVE FOOD AUTHENTICATION

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Food fraud is a significant and growing problem, driven by globalization, economic opportunity, and the low probability and severity of punishment. On-site identification of food products suspect to adulteration is complicated for food safety inspectors, because products visually do not deviate from their authentic counterparts. Furthermore, laboratory analysis are costly, restricting the amount of samples which can be analysed. Classical ways to pre-screen samples are near-infrared (NIR) and Raman. These techniques rapidly provide information on macro-component level, and can be linked to a chemical parameter for which legal limits are established. A disadvantage is that inhomogeneous samples (meat, bakery goods, fruits, vegetables, etc.) need to be homogenized prior to analysis. In order to circumvent these problems, we investigated the application of miniaturized NIR, Raman and UV-Vis equipment for on-site and non-destructive authentication using a number of food matrices. As an example, the freezing history (fresh-thawed) and the legal moisture – protein ratio of chicken fillets (approximately 150 packages resulting in 1500 spectra) were investigated by portable NIR without opening the packaging material. Sampling was performed over a time-span of 6 weeks, for two retailers, including fillets from different growth systems. Multiple multi-variate data analysis algorithms were tested and support vector machine (SVM) classification and regression algorithms were found to give the most promising results. This probably due to the inhomogeneity of the samples resulting in non-linear data. For classification of chicken fillets in the classes fresh and thawed, false negatives and false positives were in the 1% to 15% range depending on preferred model settings after repetitive validation. Furthermore, for moisture-protein quantification, small deviations from the actual values obtained using the corresponding ISO methods were found (1 - 5%). As a prospectus, the ongoing size- and price reduction of these types of vibrational spectroscopic devices will result in more consumer and professional users. The resulting data streams and multivariate models may lead to new approaches towards assurance of the authenticity of foods.

Keywords: NIR, Raman, food authenticity, chemometrics, machine learning

L23

AUTHENTICATION OF ORGANIC FRUITS BY THE ANALYSIS OF THEIR MICROBIAL COMMUNITIES: HOW TO PREVENT ORGANIC FRUIT FRAUD? APPLICATION OF A ROBUST APPROACH**Céline Bigot¹, Jean-Christophe Meile², Didier Montet^{3*}**^{1,2,3} CIRAD-UMR Qualisud, TA B-95/16, 73, Jean-François Breton, 34398 Montpellier Cedex 5, France

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Following the various food crises, such as the "mad cow" disease or the recent horsemeat episode, the consumer becomes more and more suspicious regarding food products. Thus, the need to ensure food traceability and food authenticity (UE Regulation 178/2002) is becoming increasingly important and became an emerging topic in the food sector. But existing systems are mainly administrative, and food frauds are becoming increasingly sophisticated to bypass controls: they are therefore more difficult to detect by classical analysis. So, it is necessary to resort to advanced analytical tools for detecting non-compliant food products, notably organic foods (European commission, 2013). The aim of our study was to compare microbial communities originating from organic and conventional fruits by using a methodology that can provide the basis for the development of a robust and inexpensive tool to authenticate organic foods in a quick and effective way. To achieve this objective, our hypothesis was based on the fact that agricultural practices would have a different impact on microorganisms present on the surface of fruits. The application of molecular tools allowed us to evaluate the structure of bacterial and fungal microflora detected on the surface of apples from different farming types. The application of PCR–DGGE combined to HRM methods, to analyze the global microbial flora of apples from organic and conventional farming, demonstrated that there was a specific molecular signature of the farming type. The robustness of our methodology was demonstrated by comparing results obtained on two successive harvest years and by estimating the "intra-plot" variability. The observed differences showed that organic apples could be discriminated from conventional ones independently of their position in the field (center or border) or their sampling year. The study of the composition of microbial communities on apples, whether by sequencing analysis of DNA bands obtained by PCR–DGGE or by high-throughput sequencing technologies (Illumina), allowed to have a better knowledge of the natural microbial flora present on the surface of apples as well as to identify the most discriminant microbial groups. The results were conclusive and demonstrated that the differences observed in the microflora of apples were sufficiently important to conclude that they primarily, or exclusively, originate from treatments applied to the field. This study could serve as the basis for establishing an analytical tool that could meet the needs of consumers in terms of authenticity of organic fruits.

Keywords: authenticity, fraud, organic, microbes, fruits**Acknowledgement:** This Project was funded by CIRAD, department Persyst.

L24 AUTHENTICATION OF SPICES AND HERBS

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Spices and herbs are on the first places in current attempts assessing the extent of food fraud for different matrices. As spices and herbs are typically expensive commodities, fraudulent practices promise high economic profit. An increasing consumption in production areas such as Asia contributes to the vulnerability of this commodity against fraud. Research on the supply chain of spices and herbs is conducted for example in the EU project SPICED - Securing the spices and herbs commodity chains (FP 7-Project, grant agreement no. 312631), that will be finished in 2016. The basic ideas and results of this research project will be presented. One major aim was the development of new analytical approaches for authentication of condiments particularly in view of the detection of exogenous materials. The classical analytical method for the identification of adulterants - microscopy - is very powerful but requires a tremendous training and continuous practices including the detection and learning of new adulterants. Probably due to the required high level of specialization microscopic practice appears to be less common in industry and also food surveillance. Therefore, new techniques such as non-targeted analysis (food fingerprinting) are under development and are up-coming in future. Some results on paprika authentication using Fourier transform infrared (FTIR) and Nuclear magnetic resonance spectroscopy (NMR) and further general challenges of non-targeted chemical analysis will be discussed and presented.

Keywords: spices, herbs, authenticity, food fingerprinting

L25 A SENSE OF 'SPICED': PEPPER AND NUTMEG AUTHENTICATION

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Nearly each and every processed food, including ready-to-eat products, comprises spices and herbs. These spices are mostly imported into the EU in dried or crude form from producing regions outside the EU. A considerable part of the imported spices and herbs are used in the industrial sector, especially in the processing of meat and production of convenience foods. However, they end up, ground and packaged, in retail and the catering sector as well. Many alerts from European Countries communicated through the Rapid Alert System for Food and Feed over the past years included spices and herbs. The main objective of the EU project Spiced is: 'Securing the spices and herbs commodity chain against deliberate, accidental or natural biological and chemical contamination'. One of the sub-objectives focuses on the reduction of adulterations to ensure the authenticity of spices and herbs by evaluation and improvement of non-targeted fingerprinting methods. The techniques involve spectrometry and spectroscopy methods with pepper and nutmeg being spices in the centre of the project. Black pepper may be processed to white pepper. Fermentation is the common approach in Indonesia and decortication in Vietnam. The different kind of processing results in unique fingerprints of volatile and non-volatile compounds. For both pepper and nutmeg addition of lower quality product own material is common, which should be declared if added on purpose. This group includes products such as peel, spent, light berries, spiral rejects, etc. Results from the spectroscopy and spectrometry analysis applied in the project shows that these products can be distinguished from the premium pepper and nutmeg products, also when present in mixtures. A survey shows the presence of the lower quality products in spices on the market.

Keywords: pepper, nutmeg, non-targeted fingerprinting methods, authentication

L26 HIGH RESOLUTION MASS SPECTROMETRY BASED METABOLOMIC FINGERPRINTING, AN EFFICIENT TOOL TO DETECT FRAUD ON HERBS AND SPICES: CASE STUDIES

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Alike in case of other food commodities, consumers' interest in spices authenticity has become more and more important. Being aware of various fraud scandals in the past time (synthetic days added, dilution or even replacement with contaminated / toxic plant material) consumers are willing to pay even higher cost as far as the authenticity is guaranteed. This is specifically the case of saffron (*Crocus sativus*), which one of the oldest and most expensive spice in the world – very popular for its unique aroma, taste and color. Another spice which is often be subject of fraud is ground black pepper (*Piper nigrum*). The cases of adulteration with a cheaper plant materials including spent (residue left after essential oil extraction), or mislabeling have been many times documented. In this research, high resolution mass spectrometry (HRMS) based platforms have been used for fingerprinting of a broad spectrum of compounds occurring in tested species. Volatile metabolites extracted from headspace by solid phase microextraction (SPME) were separated by gas chromatography (GC), for separation of nonvolatile and semi-volatile metabolites extracted by aqueous ethanol ultra-high resolution liquid chromatography (U-HPLC) was used. Principal component analysis (PCA) of metabolomic fingerprinting was employed for samples classification. Characteristic 'markers' enabling rapid screening aimed at distinguishing of respective spice origin and/ or processing practice have been identified.

Keywords: saffron, pepper, non-targeted fingerprinting methods, high resolution mass spectrometry, authentication

L27

ADULTERATION OF HERBS: THE OREGANO STORY

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Fraud in the global food supply chain is becoming increasingly common due to the huge profits associated with this type of criminal activity as highlighted by the recent horse meat scandal. Food commodities and ingredients that are expensive and/or are part of complex supply chains are particularly vulnerable. Herbs and spices fit these criteria perfectly and yet strategies to detect fraudulent adulteration are still far from robust. Oregano is a culinary herb most commonly associated with pizzas and other Mediterranean dishes. The main producers of oregano reside in the USA, Mexico, Greece and Turkey. The industry gold standard for testing for adulteration is a microscopy based method. The aim of this study was to develop and fully validate a two-tier approach utilising Fourier-Transform Infrared spectroscopy (FTIR) and Liquid Chromatography High Resolution Mass spectrometry (LC–HRMS) to screen for and confirm oregano adulteration with common adulterants e. g. olive leave, myrtle leaves. When these two techniques are combined with multivariate data analysis software they have the ability to screen and process a large number of samples. The two tier testing strategy was applied to a 78 sample survey obtained from a variety of retail and on-line sources. There was 100% agreement between the two tests that over 24% of all samples tested had some form of adulterants present. The innovative strategy devised could be used as a basis for testing the global supply chains for fraud in many different forms of herbs.

Keywords: adulteration, oregano, FT–IR, LC–HRMS, chemometrics

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L28 THE MODELLING OF FOOD SUPPLY TO ASSIST FRAUD DETECTION

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The aim of the paper is to explore modelling as an instrument for the prediction of food fraud. By focusing on the olive oil industry, we assess aggregate data as well as market and business conditions in order to identify pressures or distress within the industry which are conducive to the creation of opportunities for the adulteration of olive oil for business or economic advantage by supply chain actors. By using Agent-Based modelling we aim to shed light on the identification of such vulnerable nodes within supply chains. In such a scheme network nodes are represented as autonomous agents whose individual behaviour can be readily captured in a flexible and adaptive manner. The collective behaviour of agents acting independently within an environment can give rise to emergent global phenomena not observable at the local level and allows for the testing of multiple 'what-if' scenarios. We conclude by highlighting the problems faced when attempting to model supply chains with an emphasis on olive oil and addressing strategies to assist food fraud detection.

Keywords: fraud, prediction, model, agent, olive oil

L29 CORPORATE CRIME IN THE MEAT SUPPLY CHAIN

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Corporate crime in the meat supply chain Various European countries have recently been struck with cases of food fraud in the meat supply chain. Several company managers are criminally prosecuted in the so-called 'Horsemeat scandal'. In criminology, food fraud is understudied. Europol and Interpol are suggesting that organized crime is moving into the business of food fraud. The global food industry suggests to 'think like a criminal' to prevent food fraud. This paper offers a criminological explanation of fraud in the meat supply chain and the involvement of meat processing companies. As the suspects in the recent criminal cases are regular actors in the meat supply chain, the paper argues that analyzing these cases from a corporate crime perspective is most fruitful. In this paper, lessons from criminological studies on corporate crime will be used to study and understand the motivations and opportunities of food fraud in the meat supply chain. From this analysis, options for prevention and intervention will be deducted.

Keywords: corporate crime, food fraud, meat sector

L30 DUTCH PIG FARMERS: NON-COMPLIANCE IN CONTEXT

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This paper addresses the reasons and motives for compliance and non-compliance to rules by Dutch pig farmers. This paper is a work-in-progress and is based on 46 in-depth qualitative interviews that have been conducted between July 2015 and January 2016 with Dutch pig farmers, pig farming organisations, regulators and law enforcement officers. The central assertion of this paper is that compliance and food integrity issues can only be understood in its wider context. This context involves on a macro level the global market, public images of the industry, international and national regulation, and law enforcement. Yet, also social norms within the sector and the situation and personal norms of pig farmers are relevant to compliance and non-compliance. Although this project has been concerned with various regulations – including regulation concerning manure, taxes and the environment – and is thus not limited to food integrity issues, the link to food integrity will be the central focus of this paper.

Keywords: food integrity, social sciences, explanations, compliance

L31 BRIDGING THE GAP - A CRIMINAL INTELLIGENCE PERSPECTIVE ON FOOD CRIME

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As consumers of food we are disconnected by multiple processes and often thousands of miles from the origins of the food we eat. For this reason it is vital that we can be confident in the honesty and integrity of each and every person involved in bringing food from the farm to our fork. The potential for criminal activity to impact detrimentally on the safety or authenticity of food supply is something that affects us all. We may unwittingly pay over the odds for substandard food products that can sometimes be injurious to our health. Food crime can also damage a nation's economy and tarnish its international reputation. Although with us for hundreds of years, food crime has only very recently been recognised as a discrete criminal typology demanding its own bespoke law enforcement response. The public clamour is understandably for a quick fix to a problem it only recently realised it had. As with insider dealing and international bribery before it, the gap between threat and response may take somewhat longer successfully to bridge. Estimating the scale of food crime and the threat to individual commodities will always be challenging. Fraud is designed to be concealed and the very best fraudsters will also be very skilled concealing their offending. Consumers and food industry victims will seldom be aware that they have purchased or consumed criminally substandard food. It is likely, therefore, that most food criminality does not come to light and is never reported. Whilst the consequences of other forms of serious crime are immediately visible, this is not often the case with food crime which can seem an abstract phenomenon. But absence of evidence is not evidence of absence. Where there is honesty, there is inevitably dishonesty and where there are profits to be made lawfully there are generally larger profits to be made unlawfully. Where there is money, there is fraud and where there is serious money, there is serious fraud. In an industry worth almost £200 billion annually in the UK alone, the rewards available for the food criminal are significant. This presentation looks at what criminal intelligence can tell us about the dynamics and characteristics of food crime and examines some of the unique challenges it presents to the established counter crime response. It explores how lessons learned from other forms of serious crime can be applied, highlighting the value of less conventional intelligence collection techniques. When set against scientific sampling, the default and comparatively indiscriminate method of food fraud detection, the application of serious crime thinking can sometimes offer greater precision. If we really are what we eat, there can simply be no place for criminality of any kind within food and drink supply chains.

Keywords: food, crime, intelligence, NFCU

L32

FOOD FRAUD PREVENTION: POLICY, STRATEGY, AND DECISION- MAKING - IMPLEMENTATION STEPS FOR A GOVERNMENT AGENCY OR INDUSTRY - INCLUDING TRANSLATION TO CHINESE

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This paper addresses the role of governments, industry, academics, and non-governmental organizations in Food Fraud prevention. Before providing strategic concepts for governments and authorities, definitions of Food Fraud are reviewed and discussed. Next there is a review of Food Fraud activities by the Global Food Safety Initiative (GFSI), the Elliott Review in the United Kingdom, the European Commission resolution on Food Fraud, and the US Food Safety Modernization Act including the Preventative Controls Rule. Two key concepts for governments or a company are: (1) formally, and specifically, mention food fraud as a food issue and (2) create an enterprise-wide Food Fraud prevention plan. The research includes a case study of the implementation of the concepts by a state or provincial agency. This analysis provides a foundation to review the role of science and technology in detection, deterrence and then contributing to prevention. This article includes a translation to Mandarin/ Chinese.

Keywords: food fraud, food crime, economically motivated adulteration, authenticity, policy

L33 CRIMES AND HARMS IN THE MEAT INDUSTRY

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The meat industry has experienced a series of crises resulting in a breakdown of consumer confidence in the industry and the controlling authorities, such as the bovine spongiform encephalopathy (BSE) crisis in the UK in the early 1990s, outbreaks of animal diseases (e.g., Food and Mouth Disease), the contamination of their feed as in the dioxin crises in Belgium and the Netherlands – and scandals over fraudulent practices. These fraudulent practices include the discharge of waste in animal feed, the extensive employment of growth hormones, and even the involvement of organized crime figures and methods in the industry. Under the influence of these crises, the European General Food Law (GFL) prioritized the policy goal of protecting ‘human life and health, ... consumers’ interests, including fair practices in food trade, [and], where appropriate, animal health and welfare, plant health and the environment’ (Art. 5). The main policy goal is to minimize harm in this case, above all, to human health. Such harms are rarely framed in terms of criminal offences. Governments and supra-national agencies employ mainly administrative and civil provisions to prevent and reduce the harms associated with the food industry, as in the case of GFL. In this paper both the criminal activities and non-criminalized harmful activities are identified that occurred in the meat industry in the Netherlands and Belgium. By drawing attention to the laws and regulations applicable to these activities, the ambiguity of the concept of ‘crime’ will be discussed.

Keywords: crime, harm, meat supply chain, law and regulation

L34 CRIMINOLOGICAL APPROACHES TO FOOD FRAUD: THE APPLICATION OF A SCRIPTS ANALYSIS

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This presentation develops the criminological approach of understanding the organisation of crime by the use of a script analysis. This form of analysis aids an understanding of how serious crimes are organised and enables the development of a social network analysis. This form of interpretation develops a more complex picture of the interactions of certain actors, their locations within the network and the resources required for a crime to take place. The presentation will establish the theoretical framework of script analysis and using a case study, drawn from current research, apply a script analysis in order to demonstrate the vulnerability points of certain production processes and transaction points.

Keywords: Script analysis, serious and organised crime, food fraud, Social Network Analysis

Acknowledgement: The research is funded by the Economic and Social Research Council (UK).

L35 THE ROLE OF THE REGULATOR

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The presentation will explore the issues that regulators are faced with when investigating cases of food fraud. It will examine the use of open source surveillance and will track the steps taken to-date in an on going food fraud investigation. The investigation will demonstrate links to organised crime and serious criminals. It will also show the international nature of the crime and the need for all enforcement agencies to have a collaborative and united approach.

Keywords: fraud, crime, food

L36

OVERVIEW OF CURRENT RESEARCH AND PRACTICAL OUTCOMES OF FOODINTEGRITY PROJECT

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European foods is under constant threat of frauds & adulterations and the consumer does expect to buy products of which safety, quality and authenticity are assured: these parallel aspects represent a big challenge for the industry. FoodIntegrity project (FI) key activities are to share data and knowledge, evaluate mislabeling, develop both rapid and confirmatory methods and systems for industry, develop early warning systems, understand consumer behavior for export. FI have the ambition to make a fundamental step in advance for assuring the added value of the integrity in the European food chain with a positive impact on EU citizens well-being, guaranteeing at the same time EU food reputation in front of NON EU countries. The industrial partnership and collaboration in the project permits to fully understand the opportunities that this multi-faceted project presents to food businesses, contributing also to compare the perspective of food chain vulnerabilities vs current analytical methods and technologies.

Keywords: food integrity, industry, rapid and confirmatory methods, food business, added value

Acknowledgement: The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L37 USING THE FOODINTEGRITY NETWORK & THE FOODINTEGRITY KNOWLEDGE BASE

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The latest GFSI Guidance Document, due to be published in 2016, will include recommendations on how industry should tackle the problem of food fraud. The main food safety management schemes such as BRC (British Retail Consortium) and IFS (International Food Standard) are already one step ahead and have included specific food fraud requirements in their certification schemes. The two recommendations of the GFSI are (1) that companies should carry out a 'food fraud vulnerability assessment' in order to identify potential weaknesses in the supply chain and (2) that they then set up a 'food fraud mitigation plan' describing measures, including an analytical testing strategy, to target food fraud risk. This means that all food operators will have to put in place a documented procedure clearly indicating when, where and how the integrity of food products entering or leaving the factory is verified. They will need to show that they are aware of historical or developing food fraud risks and that their systems are protected by the most relevant and up-to-date scientific measures. The tools developed in the FoodIntegrity project, including the Expert Network and the Knowledge base will help food operators establish this information. Both are available through the FoodIntegrity website (www.foodintegrity.eu). The FI Stakeholder/Expert Database is a searchable tool to identify people/organisations with a particular skill set in the area of food integrity and can be searched by product type, analytical skill or location. The FI Knowledge base is Web-based tool providing information on various food fraud practices together with recommended analytical strategies. It is designed for use by industry and regulatory authorities to identify, easily and rapidly, potential threats to a given food product or ingredient and the existing solutions. Work is ongoing in the FoodIntegrity project to populate both databases and develop appropriate search engines to facilitate finding the information.

Keywords: food safety management schemes, food fraud, analytical tools, expert database

Acknowledgement: The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L38 RAPID METHODS PERSPECTIVES (FOCUS ON THE SPIRIT DRINK SECTOR)

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In 2011 the European spirit drinks industry produced 37.5 million hectolitres of spirit drinks valued at over €23 billion, approximately two-thirds of which was exported. It is important to protect the reputation of such a large and valuable market from the harmful impact of illegally produced spirits. Counterfeit activities can not only be dangerous to health but extremely damaging to brand reputation. Laboratory methods to identify counterfeit spirit products are often complex and require sophisticated analytical instrumentation. However, some in-field technologies are currently in use and there are more in the development stage. In-field devices enable suspect counterfeit spirits to be identified rapidly at the point of sale or distribution by relatively unskilled personnel. Such a requirement for rapid analysis also extends back to the laboratory where quicker authoritative tests are desired. The Spirit Drinks Work Package of the EU FoodIntegrity Project has targeted the development of rapid analysis methods for spirit drink authentication as one of its primary aims. Initial feedback on such technologies from industry was provided at a FoodIntegrity Spirit Drinks Authentication Seminar held at The Scotch Whisky Research Institute in May 2015. This short presentation will introduce some of the rapid analytical methods evaluated by the FoodIntegrity Project, and perspectives of the spirit drinks industry on their associated benefits and challenges.

Keywords: authentication, counterfeit, spirit drinks, denaturants

Acknowledgement: The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

L40 **^1H NMR SPECTROSCOPY FOR THE NON-TARGETED DETECTION OF ADULTERANTS IN VEGETABLE OIL****James Donarski¹, Adrian Charlton², Giampaolo Venditti³, Roman Romero^{4*}**^{1,2,3} Fera Science Ltd, York, United Kingdom of Great Britain and Northern Ireland⁴ Nestlé Research Centre, Lausanne, Switzerland

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The presentation will cover work carried out to develop non-targeted methods of detection of adulterants in vegetable oil using high field ^1H NMR spectroscopy and will include: Development of matrix-specific extraction strategies and NMR spectral acquisition-processing parameters; Generation of NMR spectroscopic data of representative vegetable oil; and testing of the database with adulterated vegetable oil.

Keywords: authenticity, non-targeted, vegetable oil**Acknowledgement:** Nestlé Research Centre

L41

SPECTROSCOPIC TECHNOLOGIES AND APPLICATIONS FOR AUTHENTICATION & ANTI-COUNTERFEITING

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This talk will give a brief overview of optical spectroscopy measurements across the food sector and how this technology is applied to authentication and anti-counterfeit applications, from an instrument providers perspective.

Keywords: optical spectroscopy, authentication

L42 INDUSTRIAL SELF-CONTROL IN THE FRUIT JUICE INDUSTRY: A MODEL FOR OTHER FOOD INDUSTRY SECTORS.

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SGF is a pioneer of industrial self-control that fights for safeguarding the compliance with legal and industrial quality and safety standards and for a safe and fair worldwide fruit juice market on behalf of members and consumers. Objectives of the association are to promote free and fair competition by increasing the safety and quality of the products, protecting the members against unfair competition as well as supporting members in averting unjust attempts. Since it was founded, in 1974, the task of the SGF has been to monitor the products of the fruit juice branch that are to be found on the market. SGF carries out market analysis and plant audits at the member production plants to monitor compliance with the food and labelling regulations. The aim is to ascertain irregularities, anomalies and adulterations of products already during the initial production phases of processing raw material and finished goods and not waiting until these are already on the market. Since 1986, the SGF's controls include the manufacturers of raw materials and semi-finished products from companies all around the world, with more than 600 members from about 60 countries. Nowadays more than 80% of the raw material coming to Europe is controlled. The control system that permits the traceability of a juice „from the tree to the bottle“ is based on voluntary participants who open the doors of their semi- and finished goods facilities for the SGF auditors and allow samples to be taken of the semi- or finished goods from on-going production and from the warehouse for corresponding testing, together with hygiene audits of the plant facilities. The „complete control chain“ from processing the fruit through to the finished product can provide verification of quality, even if natural changes resulting from origin, growth or variety characteristics cause deviations from normal expectations. At the same time it is easy to detect, localize and prove illicit product manipulation. Any infringements against the food regulations or against the rules of the system trigger corrective action by the SGF with corresponding follow-up inspections. The voluntary control system gives its participants greater security in purchasing semi-finished products and protects the sector from dishonest competitors. It also helps to safeguard the constantly growing quality expectations of retailers and consumers. As a result of the activity of SGF, in the last years adulteration is reduced to isolated cases, detected problems were solved before they became public and, luckily, fruit juice scandals up to now have been avoided. On top of the routine control activities, SGF contributes in implementing new analytical methods applied to fruit juices, such as the Proton – NMR-Spin Generated Fingerprint Profiling (SGF-Profilng TM) and developing projects that contribute to the improving of the quality and assuring the authenticity of the fruit juices.

Keywords: authenticity, quality, self-control, food chain

L43

HONEY IDENTITY: NEW APPROACHES TO THE BOTANICAL ORIGIN OF HONEY BY NEXT GENERATION SEQUENCING

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Food identity is very important for consumers due to their freedom of choice and also to their right to food authenticity and integrity. In turn, food integrity is also highly important to the authorities since traceability (the ability to follow the movement of a food through its production and processing) is mandatory in EU distribution (Regulations 178/2002 and 931/2011). Food can, nevertheless, be very diverse. Its complexity demands for new methodologies and analytical tools that can be used to trace their composition, origin, production, and transportation. Considering authenticity, the ideal sample analysis should present some important features: several targets detected in a single test, reliable results in complex and processed samples, high specificity, high sensitivity, and quick answers at fair price. Next generation sequencing (NGS) is probably the methodology that better fulfils these features, especially if applied to amplicon sequencing, as it ties the ability to produce a huge amount of data with high specificity, ability to detect a high number of organisms in one single analysis and sensitivity of PCR method. It can be applied to complex and processed food products targeting all species in a mixture. If using sample barcoding, it can also be applied to the analysis of several samples at the same time, becoming affordable for routine use. Honey is one of the EU TOP 10 targets most at-risk of food fraud and thus it was the selected matrix for this work. The origin of honey can be perceived by the geographical origin and/or by floral or vegetable origin. The honey matrix includes numerous pollen grains and honeydew elements that altogether provide a good fingerprint of the environment where the honey was produced. Pollen analysis has been therefore used to determine and control the geographical and botanical origin of honey. The objective of this work was to compare the current method of labelling botanical origin of honey, melissopalynology, (counting and morphologically identifying pollen under microscope) with DNA extraction, taxonomic discrimination and relative quantification by SGS. 50 different honey samples collected from different regions of Portugal and having different floral composition were classified by classical pollen analysis of honey. Simultaneously, an extensive DNA extraction optimization was performed as a pre-requisite to the subsequent steps of the analysis. Then, the DNA amplification of the extracted nucleic acids followed by NGS was performed using genomic regions suitable to produce a reliable plant species molecular identification. The primers used were tested for their universality. Finally, the high amount of results was analysed with dedicated software, designed to easily perform the correct identification of plant species. The profile of plant species associated with each honey sample was correlated with their origin and compared with the results obtained with the melissopalynology analysis.

Keywords: food fraud, honey, botanical origin, next generation sequencing

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L44**THE USE OF STABLE ISOTOPES FOR MONITORING OF PRODUCTS CLAIMING REGIONAL ORIGIN. A PROOF OF CONCEPT****Markus Boner¹, Sabine Hofem², Robert Hermanowski³, Rolf Maeder^{4*}**^{1,2} Agroisolab GmbH, Jülich, Germany³ FiBL Germany, Frankfurt, Germany⁴ FiBL, Frankfurt, Germany

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Products from a defined region are getting more and more popular in the European market. At the moment various certificates are available to ensure the origin. Nevertheless the demand of an independent analytical method is requested from the market and authorities as well. In the “watermark” project (funded by the Federal Office for Agriculture and Food) the stable isotope method was tested in the federal state of Hessen (Germany) in order to evaluate the origin of four agricultural products (wheat, potatoes, carrots and apples) and 4 animal products (pork, beef, eggs and milk). All eight products were firstly analysed on the stable isotopes of the water in the tissue water and the organic (D/H, $^{18}\text{O}/^{16}\text{O}$) and secondly on the further stable isotopes of the bio elements as carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$) and sulphur ($^{34}\text{S}/^{32}\text{S}$). To expand the possibilities various parts of the products were analysed separately as the fat (e.g. wheat, meat) or extracted proteins (e.g. potatoes). An essential part of the stable isotope method is always a reliable database. In the project more than 1298 reference samples from these eight products were sampled in the whole area of Hessen. Furthermore the field variation was checked as well. Therefore from 169 fields always four samples from different field location were sampled and analysed. The natural distribution of the field has finally an important relevance to build up an evaluation system to predict the origin of samples. Regarding the enormous data it was firstly possible to compare the stable isotopic signatures of the different products of a region. One result was the high similarity of the D/H ratios of apples (n=49) and potatoes (n=67) in their average value and standard deviation. The developed database was tested with 264 blind test samples to check the robustness of the stable isotope method to predict the origin of the country, regional and field level. A result was that the stable isotope method has a high performance, especially on the evaluation of the field level. Finally the conclusion of the project was integrated in an online database to make the stable isotope method accessible for the market as well.

Keywords: stable isotopes, origin check, regional products, online database, agricultural products**Acknowledgement:** Project was funded from Federal Office for Agriculture and Food.

Poster session

P1 DETERMINATION OF PRIORITIES BY THE MANUFACTURER FOR FRAUD PROCESSED MEAT PRODUCTS, IN TURKEY

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In recent years, awareness of food safety and quality has been increased. In this regard, meat adulterations have become a very important issue from health, economic, religious and regulatory aspects. The meat products fraud can be done by substitution or by adding offal, blood, water, eggs, gluten or products of vegetable origin. In addition of this, the consumption of pork meats is proscribed for Muslims depend on religious reasons. The aim of this work was to investigate priorities of alternatives and the potential of criteria which to detect processed meat product adulteration and counterfeit with different fraud. Analytical Hierarchy Process (AHP, a Multi Criteria Decision Making method) was applied for getting the relative rankings of the processed meat manufacturer fraud parameters, were selected as criteria. Six processed meat were identified as alternatives; meatball, sausage, dried meat flesh (pastırma, turkish), smoked meat, fermented sausage (sucuk, turkish) and deep fried meat (kavurma, turkish). These processed meats are widely used for appetizer or a meal by different groups as working people, students. Both conceptual and operational difficulties were made the calculations complex and cumbersome. The proposed approach enables the transfer of data and information, between the different frauds related to processed meat products. As a result of this study, consumers are becoming much more aware about the processed meat product adulteration and counterfeit.

Keywords: fraud adulteration and counterfeit, processed meat product, ahp, priorities

P2

METROFOOD-RI: A NEW PAN-EU RESEARCH INFRASTRUCTURE TO SUPPORT FOOD INTEGRITY

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METROFOOD-RI “Infrastructure for promoting Metrology in Food and Nutrition” is a new pan-EU Research Infrastructure of global interest included as emerging-RI in the 2016 ESFRI Roadmap (Domain “Health and Food”). By means of METROFOOD-RI it will be possible to carry out different activities supporting data collection and measurement reliability, as well as basic and frontier research in food and nutrition. The general objective is to enhance scientific excellence in the field of food quality & safety, by promoting metrology in food and nutrition, allowing coordination on a European, and increasingly on a global scale. METROFOOD-RI will strengthen scientific knowledge, promoting scientific cooperation and encouraging the interaction between the various stakeholders and the creation of a common and shared base of data, information and knowledge. With this aim, a network of plants, laboratories and experimental fields/farms will be realized (Physical-RI) and an e-RI will be developed. The Physical-RI will enable to carry out different research activities supporting data collection and measurement reliability; quality & safety and traceability of food production, as well as basic and frontier research in food and nutrition. The e-RI will make available a new useful, free access web platform, for sharing and integrating information and data on availability of metrological tools for food analysis and will deal with integration of existing database on food, focusing on emerging needs and collection of data on food composition, nutritional contents and levels of contaminants in foods produced in different geographic regions and by applying different technologies. Thanks to its broad multidisciplinary approach, METROFOOD-RI will be able to greatly support the Scientific Community working on food integrity, leading to important relapses on different application fields (agrofood; sustainable development; food quality, safety, traceability and authenticity; environmental safety; human health). Organised as a distributed RI structured on the basis of a hub and nodes model, METROFOOD-RI is supported by the economic endorsement of 3 Member States (Italy, Portugal and Romania) and the political endorsement of 12 Countries. Current Partnership includes 30 Partners from 15 different Countries (13 Member States and 2 Associated Countries) and an International Partner (FAO). Moreover, many other Institutes and Organizations arising from different Countries (Member States, Associated Countries and non-EU Countries) have already signed Letters of Interest in a future cooperation and/or inclusion in the Partnership.

Keywords: Pan-EU research infrastructure, metrology in food and nutrition, food safety, quality, authenticity and traceability, harmonisation, data integration and sharing

P3**ADVANCES IN TOOLS TO SUPPORT FOOD FRAUD VULNERABILITY ASSESSMENT AND RISK MITIGATION****Karen Everstine^{1*}, Jeffrey Moore², Henry Chin³, Shaun Kennedy⁴**^{1,2} United States Pharmacopeia, Rockville, MD, United States of America³ Henry Chin and Associates, Moraga, CA, United States of America⁴ Food System Institute, St. Paul, MN, United States of America

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Food fraud affects the safety and integrity of food products, reduces consumer confidence, and can result in consumer illnesses and even deaths. The U.S. Pharmacopeia (USP) has developed two tools for use by industry for food fraud mitigation. The USP Guidance on Food Fraud Mitigation was authored by a panel of volunteer scientific experts from industry, regulatory agencies, and academic institutions worldwide. The Guidance provides a framework for the development of a fraud management system that prioritizes and focuses mitigation resources on those ingredients that carry the most vulnerability and would result in the most impact if fraud occurred. The Guidance provides practical tips, examples, and information resources to guide and support vulnerability assessments and implementation of effective measures to mitigate fraud risk in food ingredients. In 2012, USP released the Food Fraud Database, the first public database compiling records related to food fraud. The next generation USP Food Fraud Database, scheduled for release in 2016, will include enhanced features for advanced searching and automated analytics to directly support vulnerability and impact assessments. The enhanced database will include four food fraud record types: incident, inference, surveillance, and method records. Each record type allows users to customize outputs to the information most relevant to their organization. It will allow users to quickly identify food fraud trends in ingredients of interest as well as those adulterants with the potential to cause severe adverse health effects. Outputs from the database can be incorporated into any vulnerability assessment framework and to support regulatory compliance.

Keywords: food fraud, database, vulnerability assessment, mitigation plan

P4

FOOD FRAUD PREVENTION GUIDE FOR AGRIFOOD SECTOR. AN INTEGRITY ASSESSMENT TOOL FOR APPLIED STRATEGIES IN ORDER TO ASSURE FOOD AUTHENTICITY

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Nowadays, consumers are worried about food fraud and its consequences. In addition, the GFSI schemes BRC and IFS include in their current versions, terms related to food authenticity. These two important issues suggested us that the food industry needed a document to help them to control these circumstances. The aim of this guide is dual: on the one hand it provides a tool to the entire food business operators in order to prevent suffering a fraud by their own suppliers. On the other hand, it avoids making further costly mistakes in their own facilities. The system we propose is based in HACCP system, including new items to prevent and control specifically food fraud. The analysis of the vulnerability enables the evaluation of risk considering different aspects of the raw material, the supply chain and the food processing. The system permits the evaluation of the severity of the occurrence. It is important to highlight that to implement the Food fraud prevention Guide is not necessary to invest neither time nor resources. The main advantage of our system is that it fits perfectly with all type of companies, in any case at any time. This document was developed in the context of a public-private partnership, between the Agriculture Department (Government of Catalonia) and Premiumlab. We also have the support of the University of Barcelona, the Universitat Autònoma de Barcelona and the ACCA, the Catalan organization of food science.

Keywords: food fraud, prevention, food sector, guide

P5 MULTIDISCIPLINARY APPROACH FOR FOOD FRAUD DETECTION

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The problem of food fraud is getting a rapidly increasing interest. There is often a relationship with safety issues, but fair trade, a level playing field and transparent information for consumers are important issues on their own as well. Fighting food fraud is a very complicated area caused by several elements. The possibility to detect fraudulent activities depends on the type of fraud, ranging from altered composition and labelling, via geographic origin, mixtures and mimicking supplements to claims of sustainable production or unethical actions. A supporting approach is the possibility to predict the type of fraud primarily based on type of product and country of origin (1). Different types of fraud request the application of different methods. "Out of the box" thinking is important to battle the creativity of fraudsters. Methods additional to the established toolbox might give a relevant improvement of the chance to detect food fraud successfully (2). This lecture will present some examples of multidisciplinary analysis to detect food fraud and trace their origins. Emphasis will be given to the combination of chemical analysis and visual inspection. Chemical limits are set for certain parameters of both saffron and manuka honey. Also in both cases complementary analysis by visual inspection is described (3) or is part of a general framework for honey analysis in the EU (4). In other cases, visual examination will successfully complement primary (chemical) analysis. Based on the presented examples a general approach for multidisciplinary analysis will be pointed out. This approach will consist of several elements: order of application of different methods, detection of excess of limits and identification of fractions in the sample.

[1] Bouzembrak and Marvin, Food Control 61 (2015): 180-187.

[2] Olsen, Book of Abstracts 7th RAFA Conference (2015): 93.

[3] ISO 3632-1:2011.

[4] Directive 2001/110/EU.

Keywords: fraud detection, multidisciplinary analysis, visual examination, honey, saffron

P6

THE VIRTUAL FOOD AUTHENTICITY NETWORK

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The Food Authenticity Network is a UK Department for Environment, Food and Rural Affairs (Defra) initiative to help bring together those involved in food authenticity testing. The network aims to raise awareness of the tools available to check for mislabelling and food fraud and to ensure that the UK has access to a resilient network of laboratories providing fit for purpose testing to check for food authenticity so consumers can have confidence in the food they buy. The UK Government committed to setting up a Network in response to Recommendation 4 of the Elliott Review into the Integrity and Assurance of Food Supply [1] which highlighted the need for standardised testing approaches. It was intended that the creation of the Network will help bring together the UK's expertise in a range of food authenticity testing techniques and ensure better collaboration and data sharing between all those with an interest. LGC limited is the coordinator of the network; the network website (<http://www.foodauthenticity.uk/>) was built, piloted and launched in July 2015. The network now has over 370 members and is a one-stop-shop for anyone involved in food authenticity testing, the food industry, government, academia, enforcers and consumers alike. With all the relevant material together (e.g. policy and law section, 72 UK authenticity research reports, 58 standard operating procedures, 25 UK authenticity surveys, 22 UK nitrogen factor studies are now (as of January 2016) available directly from the website) in one place, it is much easier to access and disseminate information on methods and new techniques to the food authenticity community while also helping facilitate communication and understanding between those working in the area via the network's news & events pages and discussion boards. In December 2015, 14 UK Centres of Expertise for food authenticity testing were published on the network together with direct contact details of technical experts in each of the organisations thus making them accessible to the entire food authenticity community. Please visit the network website and sign up to become a member if you have an interest in food authenticity testing.

Keywords: food, authenticity, network, methods, SOPs

Acknowledgement: Department for the environment, food and rural affairs (Defra)

P7

ARE THEY AT RISK AND DO THEY KNOW? FOOD SAFETY KNOWLEDGE OF POULTRY MEAT CONSUMERS IN SLOVENIA

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In the last ten years campylobacteriosis is registered as the most frequent bacterial enteric infection in humans, with increasing prevalence in Europe (EFSA, 2016). The illness is mainly transmitted by contaminated food/water with pathogenic *Campylobacter* species; the main is *C. jejuni* – a commensal of birds' intestines. Since slaughtering of broilers and further processing of meat do not reduce the risk, but may even contribute to cross-contamination, campylobacters are present on meat surface, survive along the whole chain and pose public health risk because of low infective dose in humans. So, the main risk factors contributing to high rates of campylobacteriosis are improper preparation and cross-contamination during preparation of poultry meat and other food in the kitchen. The aim of this study were to investigate Slovenian consumer awareness of microbiological risk with poultry meat, their knowledge about campylobacters and their hygiene practices during preparation of poultry meat. In total 680 consumers of poultry meat who also frequently prepare it in the kitchen (69.3% female, 30.7% male), from all age groups (41.5% 60 years), of different education (basic or less 7.0%, upper secondary 38.3%, tertiary 54.7%) from different demographic areas (51.2% rural, 20.3% suburban, 28.5% urban) in Slovenia participated in the survey. The questionnaire included 27 questions, 14 of them required explanation, with the aim to assess consumer knowledge and awareness about microbiological risk and to determine gaps in their safe food handling practices. The results are somehow worrying. Only about 50% of respondents pay attention to the origin of poultry meat and over 60% of them do not use insulation bag when buying it. More than one third of respondents do not use different cutting boards for different foods (38.8%) and do not find incorrect preparation of poultry meat at home as health risk (29.0%). They are not aware that it can be contaminated with microbes multiplying at room temperature (21.7%) or in even in the fridge (26.3%). More than a half responded their practice of thawing frozen poultry meat on kitchen surfaces at room temperature (50.5%). Results demonstrate poor consumer awareness and critical violations regarding safe food handling practices. Only 16% of respondents have heard for *Campylobacter*, even less knew any facts about them. Since knowledge about safe food preparation was confirmed as mainly acquired at home (42%), in comparison to schools and magazines/books (17.5%) or other ways (internet, work, 10–13%), life-long education of consumers is crucial to reduce foodborne illnesses. Increasing the awareness of consumers about microbial risks and of importance of good hygiene practice in domestic environment via public information campaign is suggested, especially concerning poultry meat.

Keywords: food safety, consumer awareness, microbiological risks, poultry meat, *Campylobacter*

Acknowledgement: The authors thank Slovenian Research Agency for financial support of the projects.

P8 BIG DATA FROM A SMALL LAB: COMPLETE HOLISTIC/NON TARGETED/FINGERPRINTING OVERVIEW USING ALTERNATIVE INSTRUMENTAL APPROACH FOR FOOD AUTHENTICATION AND FRAUD PREVENTION

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Traditional strategies for the food fraud control have relied on the detection of specific marker compounds and the comparison to genuine reference data.

Furthermore, an adulterant can be detected only if it is known beforehand and specifically searched by the analyst, but in this way a new adulterant will not be never unveiled.

These limitation of actual control system were underlined by the European Commission in the Report on the food crisis, fraud in the food chain and the control thereof 2013/2091(INI) where they stated that:

- 53 Is convinced that a change of attitude is needed within the competent authorities, moving from an administrative and veterinary approach towards a policing approach ...

- 55 Calls on the Commission and Member States to further stimulate European and national research and development programs to develop and implement technologies and methods used to detect food fraud, such as sensor technology, data analysis and the fingerprinting of products, and to facilitate the commercial availability of tests in the short term; acknowledges the existing European research projects on food integrity and authenticity, such as TRACE and AuthenticFood;

The TRACE project identified some important fingerprint analytical techniques as NMR, LC Mass Spectrometry, IRMS, ICP-MS, NIR, MIR, Raman Spectroscopy.

The general assumptions are that the spectral data contains useful and relevant information, the spectrum can be related to food properties and thus to its authenticity. But only one technique is not sufficient because does not contain all the necessary information.

The aim of our work was to combine many of the techniques suggested, or some alternatives, in a very small lab (<50m²), able to process rapidly the samples for both quantitative and fingerprinting data collection.

The keywords that define most of the techniques identified and in use in our lab are:

- small footprint
- high flexibility (no sample treatment no matrices effect)
- high sample throughput
- high number of detectable parameters
- possibility of retrospective analysis
- Greenest (low energy consumption, solventless, low waste)

Actually we work combining the results coming from different instrumentation as:

- NIR for general composition (moisture, fat, ash, protein, carbohydrate)
- TXRF instead of ICP-MS for metals and other main elements included Sr and Rb or Br
- DART/Orbitrap instead of LC-MS for small molecules identification, a very powerful fingerprinting results
- HS SPME-GC for aroma or off-flavor profile
- CZE for small polar ions or polar compounds
- Color CIE-Lab for instrumental measurement
- Texture Profile Analysis for mechanical properties

This fingerprinting approach may provide rapid and complete screening high-throughput analyses and seem to be an excellent tool for food authentication and fraud prevention. It was applied with success to many different food (meat product, cheese, fish, coffee, chocolate, virgin olive oil, honey).

Keywords: DART/Orbitrap, TXRF, NIR, food authentication, fraud prevention, green analytical chemistry

P9

MAPPING THE BEEF SUPPLY CHAIN FROM FARM TO FORK FOR TRANSPARENCY

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Food fraud/crime prevention and food authentication is now at the forefront of academic research and industrial application. However, food fraud is a complex issue engulfed within multifaceted food supply chains and is difficult to control in these complicated food systems where there are multiples actors, activities and intermediaries involved. The food supply chain can be defined as a series of movements and transactions of food and/or food products upstream and/or downstream by a network of stakeholders from the original supplier of the food and/or food products in simplest form to the final customer in the intended form of use. Globalisation means that food supply networks can now span across not only several countries but also continents. While food supply chains can be assumed to involve upstream and downstream movements that are linear in nature, in actuality this is not the case. Food supply networks or chains are complex and fragmented with many intricate stages and steps spanning over thousands of miles with numerous actors, activities and intermediaries involved in getting raw materials or final produce from A to B by the most efficient and cost effective means possible. An example of such complexity in the food system is the UK beef supply chain. It is represented by many steps starting with animal feed and primary production and passes onwards through many fragmented and intricate stages before reaching the final consumer. In addition to this 'main' chain, there are several 'side' chains that impact on the workings of other supply networks. These 'side' chains involve the import and exporting of beef products into and out of the UK beef network, as well as rendering and Animal By-Product activities associated with 'waste' or unfit cuts of the animal. These cuts or 'waste' go on for destruction or further processing destined to be used in other industries such as animal feed, pet food or textile industries. Consumer acceptable cuts of meat are transported through the supply chain to final consumers. However, edible co-products of animals such as fat and bones also make their way to the final consumer via other food industries, most notably tripe (fat) and gelatine/collagen manufacture (bones) and thus form additional 'side' chains. A poster depicting the movement of goods and the actors involved in the UK beef supply chain from feed to consumer is presented. Production figures, as well as import and export figures are also presented on this poster. By mapping food supply chains, such as the UK beef supply chain as presented in this poster, it helps academics and industry stakeholders to clearly identify vulnerable nodes and emerging threats in the beef network. This will enable the development and employment of strategies to safeguard both consumers and reputable food companies against fraud and crime in food supply networks and thus support the successful longevity of the food industry.

Keywords: beef, supply chain, mapping, transparency

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P10 **REAL-TIME PCR FOR SALMON IDENTIFICATION**

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Seafood consumption is at an all-time high globally. In addition to providing almost a quarter of the global intake of animal protein, and a tenth of the world's population lives off income from fisheries and aquaculture. Sustainable management of this industry is critical to ongoing global food security. The pressure to meet global demands for seafood, and the complexities of the global supply chain, make it increasingly important, and difficult, to ensure the authenticity of products on the market. Mislabelled seafood causes economic and health impacts to consumers. Additionally, it represents a means for products of Illegal, Unreported and Unregulated (IUU) fishing to enter global markets. IUU fishing prevents accurate management of fish stocks, and threatens sustainability. A means for accurate and rapid identification of seafood, including processed products, is critical for confirming species identity and therefore compliance with regulations. DNA barcoding has revealed cases of suspected fraud in market surveys on every continent, and represents one of the most effective means for determining species authenticity. The Fish Barcode of Life campaign has resulted in a sequence database of DNA barcodes that includes most commercial fish species. However, DNA sequencing is not practical for all seafood identification. There is a need for more rapid, portable testing methods that can be accessed by industry and regulatory groups. Real-time PCR presents a means of addressing this need. Portable platforms, simplified protocols and streamlined analytical software allow analysis by non-experts in field settings. Kits for identification of various salmon species, developed from DNA barcode sequences, have been designed for the Hunter real-time PCR platform in partnership with InstantLabs. These kits have been tested on commercial salmon products of various processing levels. The results illustrate the possibilities for commercial, on-site testing of seafood for species authenticity. These tools advance the ability to identify seafood, supporting improved stock management and the development of more rigorous supplier verification programs.

Keywords: real-time PCR, seafood, salmon, DNA barcoding, authenticity

P11

APPLICATIONS OF VIBRATIONAL SPECTROSCOPY FOR FEED SAFETY CONTROL: DETECTION OF ANIMAL ORIGIN MATERIAL BY NIR AND RAMAN SPECTROSCOPY

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Currently, the European legislation prohibits the use of animals meals in feedstuffs in order to prevent bovine spongiform encephalopathy (BSE) infection and diffusion (with the exception of some particular cases) [1], however the legislation is rapidly moving towards a partial lifting of the existing “feed ban” [2] and the competent control organisms are urged to develop suitable analytical methods able to avoid food safety incidents related to animal origin products. Nowadays, the official methods for animal components in feed are light microscopy and polymerase chain reaction (PCR) in accordance with Regulation (EC) n° 51/2013. Both methods have some limitations (i.e. subjectivity of results, difficult automation, high-qualified operators, low selectivity, non-quantitative techniques etc.) which suggest exploring new analytic ways to get reliable results in a short time. The combination of spectroscopic techniques with optical microscopy could lead to the development of an individual particle method, and reach the sensitivity required (0.1 %w/w). NIR and Raman spectroscopies are promising techniques because of their specificity; indeed the complex pattern of signals proper of a vibrational spectrum contains a lot of information, as it represented the chemical fingerprint of the sample [3]. Together with the Italian reference laboratory on animal proteins (IZSTO), a spectroscopic method based on Fourier Transform spectroscopy (Raman and NIR) coupled with chemometrics was developed. Robust classification models were obtained (and validated) by applying the discriminant analysis (DA) method. In addition, the availability of rapid and simple control methodologies is also important in view of the possible introduction of insects as a feed compound in the future. This possibility is drawn because of the nutritional properties and the economic and environmental benefits of insects, as suggested by the Food and Agriculture Organization (FAO) [4]. For these reasons, NIR imaging and DA were also applied to set-up a method for the detection of insect meal traces in feed. The automatic spectroscopic imaging (both Raman and NIR) enables the operator to manage his time in a more efficient way, leaving to the instrument the commitment of scanning the samples in search of the animal fragments. This approach could be very useful for in-situ applications such as product acceptance at the arbors in case of import from third countries, as well as for customs inspections. In particular, time and costs of analysis would be drastically reduced if it was possible to avoid the sample delivery to a dedicated laboratory.

[1] <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:300:0001:0033:EN:PDF>

[2] http://ec.europa.eu/food/food/biosafety/tse_bse/docs/roadmap_2_en.pdf

[3] D. Pavino, S. Squadrone, M. Cocchi, G. Martra, D. Marchis, M.C. Abete, Food Chemistry 121 (2010) 826–831

[4] <http://www.fao.org/docrep/018/i3253e/i3253e.pdf>

Keywords: processed animal proteins, feed safety, near infrared spectroscopy, Raman spectroscopy, discriminant analysis

Acknowledgement: This work was supported by the "Ricerca Corrente 2013" project entitled "Utilizzo degli insetti come fonte proteica sostenibile nei mangimi: uno studio di fattibilità", identifying code "IZS PLV 08 /13 RC".

P12

PEPTIDE PROFILES AS NOVEL AND HIGHLY SENSITIVE MARKERS FOR THE HEAT TREATMENT OF MILK

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Reliable and sensitive methods are required for example by food industry to assure the integrity of the food chain or by food safety authorities to detect adulteration. Peptide profiling with MALDI-TOF mass spectrometry is a rapid and sensitive analytical approach for complex matrices such as milk and milk products. In this work, a method was developed which combines quick peptide purification by StageTip extraction and peptide profiling by MALDI-TOF mass spectrometry to monitor peptide profiles of milk and milk products. There are several industrial heating processes which result in different milk types, such as: traditionally pasteurized milk, ESL milk, UHT milk and sterilized milk. All these techniques are well established, validated and accepted by international norms to reach marginal loss of quality and nutritional value. However, a high intensity of heat treatment has an adverse effect on many milk nutrients. The aim of the present study was therefore evaluation of heat induced changes of the native peptide profile of milk in order to identify highly sensitive marker peptides to monitor industrial heat treatment. For a training set commercially available pasteurized (n=20), ESL (n=29) and UHT (n=29) milk samples were collected from all over Germany and their peptide profiles were analyzed by our newly developed method. In order to determine the influence of the milk type on the peptide profile a high number of samples was necessary to exclude differences resulting from regions, lactation state, season or breed. Additionally the dependence of the relative intensity of putative marker peptides from the thermal impact was investigated by heating experiments with raw milk samples. Analysis of the commercial products as well as the heating experiments enabled us to determine 13 promising marker peptide candidates and to define cut-off limits related to the two main groups of milk types: mildly heated (pasteurized and ESL) and UHT milk. Afterwards an independent blind test set was created to validate the quality of the defined cut-off limits and 10 of 13 newly developed markers were found suitable as indicators for different heating methods of industrial milk samples. Within our method it is possible to predict thermal processing conditions of unknown commercial milk samples. Additionally, peptide profiling can be applied to evaluate adulteration of milk from different species and also for authenticity control of other milk products.

Keywords: marker peptides, peptide profiling, MALDI-TOF MS, milk, heating

Acknowledgement: This study was financed by a scholarship from the Heinrich-Stockmeyer Foundation.

P13**GC-MS DETERMINATION OF CYCLOPROPANE FATTY ACIDS: A NEW TOOL AGAINST PARMIGIANO REGGIANO COUNTERFEIT****Angela Marseglia¹, Marco Nocetti², Veronica Lolli³, Gerardo Palla⁴, Augusta Caligiani^{5*}**^{1,3,4,5} Department of Food Science, University of Parma, Parma, Italy² Consorzio del Formaggio Parmigiano Reggiano, Reggio Emilia, Italy

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Cyclopropane fatty acids (CPFA) as lactobacillic acid and dihydrosterculic acid are components of bacterial membranes discovered for the first time three years ago in milk and dairy products (concentration of 100-1000 ppm on milk fat). Data collected on more than 2000 dairy samples showed empirically that cyclopropane fatty acids (CPFA) were present only in dairy products from cows fed with silages (1,2), and their determination has been demonstrated to be a powerful tool for the authentication of PDO cheeses, as Parmigiano Reggiano, where the use of silages is forbidden. In this context, an application for an official standardization of the method has been proposed by our research group and by Consorzio del Formaggio Parmigiano Reggiano and is currently under validation study. The quantitative GC-MS method developed was applied to 304 samples of PDO cheeses of certified origin, comprising Parmigiano Reggiano (Italy), Grana Padano (Italy), Fontina (Italy), Comté (France), Gruyère (Switzerland). The cheese database we are constructing demonstrates that CPFA are always absent in all PDO cheeses for which the use of silages is forbidden and always present when silages in cow's feeding are admitted. In particular, all the authentic Parmigiano Reggiano samples showed values of CPFA lower than 0.006 mg/100 mg of cheese fat (LOD of the method), while cheese samples from milk of cows fed with silage always showed contents higher than 0.030 mg/100 mg cheese fat. The method is able to detect the counterfeiting of Parmigiano Reggiano with other cheeses until 10-20 %. These results comfort the hypothesis that CPFA can be used as a marker of silage feedings for cheese, and the present method, proposed for Parmigiano Reggiano cheese, can be easily extended to all Italian and European PDO cheeses that forbid the use of silage feeding in their Product Specification Rules.

[1] Marseglia A, Caligiani A, Comino L, Righi F, Quarantelli A, Palla G. (2013) Cyclopropyl and ω -cyclohexyl fatty acids as quality markers of cow milk and cheese. *Food Chem*, 140, 711-716.

[2] Caligiani A, Marseglia A, Palla G. (2014) An Overview on the Presence of Cyclopropane Fatty Acids in Milk and Dairy Products. *J Agric Food Chem* 62, 7828–7832.

Keywords: authentication, animal feeding, milk and dairy products, cyclopropane fatty acids, Parmigiano Reggiano

P14

MINIATURIZED NIRS FOR NON-DESTRUCTIVE AUTHENTICATION OF PACKAGED CHICKEN FILLETS

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Food fraud is a significant and growing problem, driven by globalization, economic opportunity, and the low probability and severity of punishment. On-site identification of food products suspect to adulteration is complicated for food safety inspectors, because products visually do not deviate from their authentic counterparts. Furthermore, laboratory analysis are costly, restricting the amount of samples which can be analysed. Near-infrared spectroscopy has been used as a technique for fast pre-screening of samples. These techniques rapidly provide information on macro-component level, and can be linked to a chemical parameter for which legal limits are established. A disadvantage is that inhomogeneous samples (meat, bakery goods, fruits, vegetables, etc.) need to be homogenized prior to analysis. In order to circumvent these problems, we investigated the application of miniaturized NIR devices for on-site and non-destructive authentication of (I) the freezing history (fresh-thawed), (II) the legal moisture – protein ratio of chicken fillets and (III) prediction of the expiration date without opening the package. For experiments I and II, sampling was performed over a time-span of 6 weeks, for two retailers, including fillets from different growth systems. Multiple multi-variate data analysis algorithms were tested and support vector machine (SVM) classification and regression algorithms were found to give the most promising results. This probably due to the inhomogeneity of the samples resulting in non-linear data. For classification of chicken fillets in the classes fresh and thawed, false negatives and false positives were in the 1% to 15% range depending on preferred model settings after repetitive validation. Furthermore, for moisture-protein quantification, small deviations from the actual values obtained using the corresponding ISO methods were found (1 - 5%). Determination of the expiration date (III) was piloted with a limited sample set, but results are promising for further investigation. As a prospectus, the ongoing size- and price reduction of these types of vibrational spectroscopic devices will result in more consumer and professional users. The resulting data streams and multivariate models may lead to new approaches towards assurance of the authenticity of foods.

Keywords: NIR, Raman, food authenticity, chemometrics, machine learning

P15

VALIDATION CRITERIA FOR SIMULTANEOUS MULTI COMPONENT QUANTITATIVE NMR ANALYSIS AND NMR FINGERPRINTING METHODS

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The goal of this work was to set up a new quality control parameter suitable for performance assessment in simultaneous multi component quantitative NMR analysis and NMR fingerprinting methods. In order to achieve the goal, two inter-laboratory comparisons (ILCs) were organized.

The first one consisted in the analysis of wheat and flours aqueous extracts (4 samples) and was aimed to ascertain the statistical equivalence of the scaled NMR spectra. 780 NMR spectra were produced by 32 participants using 39 different NMR spectrometers. Seven signals were submitted to univariate internationally agreed statistics typically applied in performance assessment of ILC participants.

The second ILC regarded a model mixture made up of five compounds. In particular, a model mixture made up of five compounds [Aldicarb, Methamidophos, Oxadixyl, Pirimicarb and 3-(trimethylsilyl)-2,2,3,3-tetradeutero-propionic acid sodium salt (TSP)] dissolved in deuterated water was submitted to NMR analyses. 1260 NMR spectra were produced by 30 participants using 34 different NMR spectrometers. The analytical target of the second ILC was the quantification of analytes by the calibration line method. Such a method was chosen as it allows for identification of a theoretical line to be taken as reference in performance assessment.

Results show that quantitative NMR is a robust quantification tool. Performance assessment was carried out on single component quantification, by the popular and traditional z-score, and on multi-component analyses by means of a new performance index (named Qp-score) which is related to the difference between the experimental and the consensus values of the slope of the calibration lines. By an analogous reasoning followed for z-score, performance assessment by Qp-score is considered satisfactory when $|Qp| \leq 2.0$, questionable when $2.0 < |Qp| < 3.0$ and unsatisfactory when $|Qp| \geq 3.0$.

This study introduces a new quality control parameter, Qp-score, suitable for harmonization of fingerprinting protocols and simultaneous quantitative multi component analysis. Such parameter, that was designed considering consolidated internationally agreed statistics, represents an unbiased evaluation tools for NMR method validations. Qp-score accounts for laboratory performance in terms of both instrumental adequacy and operator skill and enables laboratories to pooling of NMR data in suitable databanks. Moreover, Qp can be valuable for the development of multi-laboratory metabolomic platforms.

Keywords: simultaneous multi-component quantitative analysis, fingerprinting, NMR spectroscopy, interlaboratory comparison, performance assessment

P16 NEW IMMUNOASSAYS FOR MAJOR MILK FRAUD

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Due to big price differences between milk and milk products of different species, fraudulent mixing stays attractive. Bovine milk (from cows and buffalos) is the most abundant (96%) and the cheapest compared to milk of other species and sources. The most produced bovine milk product is cheese and bovine rennet whey (BRW) is the high-volume and low-cost by-product which is, reduced to powder (BRWp), much less costly than milk powder and the cheapest bovine milk product. For the detection of both bovine milk and BRW(p), new immunoassays have been developed in different formats but all applying one or more of the three monoclonal antibodies (mAbs) raised against bovine κ -casein (RIKILT), an important milk protein on the surface of casein micelles. The corresponding linear epitopes and the dominant amino acids (AA) were found on the para- κ -casein part (1 mAb) and the glycomacropeptide (GMP) part (2 mAbs) with ultrahigh-density peptide microarrays (Schafer-N) by which the specificity towards other milk proteins and species and sources could be predicted. Inhibition immunoassays were developed in the traditional microtitre plate ELISA format (EuroProxima) and in innovative optical multiplex biosensor formats, based on field-deployable smartphone-controlled interferometers (Demokritos) and portable imaging Surface Plasmon Resonance (iSPR; Plasmore), using solid phases coated with the protein or with the response increasing epitope-containing synthetic peptides. Due to the small epitopes (5-7 amino acids) towards the mAbs react and the applied inhibition assay format, the assays were found to be suitable for raw and heat-treated (protein denatured) bovine (cow and buffalo) milk and BRWp in the milk and/or cheese of other species and sources with measurement ranges from percentages down to the ppm level. The addition of BRW(p) to bovine milk (powder) could be detected via the presence of the GMP after a trichloroacetic acid (TCA) protein precipitation. This work was supported by the EU-funded projects "FOODSNIFFER" (www.foodsniffer.eu) and "IMPRESS" (www.foodimpressor.eu).

Keywords: immoassays, milk species, bovine milk, bovine rennet whey, fraud

P17
**FREE RANGE FRAUD: HOW MODERN ANALYTICAL TECHNIQUES
CAN BE USED IN CONJUNCTION WITH AGRICULTURAL EXPERTISE
TO AUTHENTICATE FREE RANGE AND ORGANIC EGG
PRODUCTION**

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In March 2010 Keith Owen was jailed for 3 years and fined £3m for fraudulently passing off more than 100m battery farmed eggs as free range or organic eggs. 6 years on, with free range eggs accounting ~44 % of egg production in the UK and a price premium of ~50% compared to enriched cage production the economic incentive for “free range fraud” is still significant. Furthermore, consumers who choose to purchase free range and organic eggs may do so for ethical reasons based on higher welfare concerns. European laws define the minimum standards for free range egg productions. By measuring the stable isotope composition and trace element concentrations in eggs, it is possible to build up an environmental fingerprint of the sample that is related to feed, forage, water supply and living environment. Free range chickens that have access to range outside pick up an environmental fingerprint from the dirt, insects, plants and rainwater that are otherwise unavailable to barn or colony chickens. Chemometrics can be applied to the multi isotope and trace element composition in order to differentiate between production systems based on these environmental fingerprints. Attendees to this presentation will be introduced to the work undertaken by Food Forensics Ltd to develop a robust production system authenticity test for eggs based on stable isotope and trace element analysis. During development a number of anomalous observations were encountered that could only be explained through a thorough investigation of agricultural practice at the sites in question. These observations include the effect of feed composition used in Northern Ireland on the classification of organic eggs and the effect of poor ranging behaviour in free range hens on the classification of free range hens. The later observation raises the more fundamental question, at what point does free range production become barn production?

Keywords: food fraud, free range, stable isotope ratio analysis, trace elements, authenticity

P18

SCREENING AND IDENTIFICATION OF FOOD SUPPLEMENT ADULTERANTS USING LIQUID CHROMATOGRAPHY WITH HIGH-RESOLUTION MASS SPECTROMETRY

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Addition of active pharmaceutical ingredients and their analogs to certain food supplement products is an economically motivated adulteration done to develop or intensify the claimed biological effect. Sexual enhancement and weight loss supplements are the two most adulterated product categories. The latter product group is being adulterated with synthetic weight-loss drugs with anorectic or laxative effects (such as sibutramine and its analogs) and also with antidepressants to suppress side-effects of these drugs. Sexual enhancement supplements are often adulterated with synthetic phosphodiesterase type 5 (PDE5) inhibitors, such as avanafil, lodenafil carbonate, mirodenafil, sildenafil, tadalafil, udenafil, or vardenafil, which are used as prescription drugs to treat erectile dysfunction but have a long list of potential side effects and harmful interactions with other prescription drugs. Many adulterated products have been found to contain analogs of the approved PDE5 inhibitors, in which case the health risks are mostly unknown. Moreover, detection and identification of both known and novel analogs pose a significant analytical challenge. This presentation will discuss screening, identification and quantitation of weight-loss adulterants and PDE5 inhibitors in food supplement products and ingredients using ultra-performance liquid chromatography coupled to a Q-Exactive Plus accurate-mass/high-resolution mass spectrometric (MS) instrument. A combination of full scan MS-data dependent MS/MS and all ion fragmentation (AIF) was employed to acquire data for both known (targeted) and unknown (non-targeted) compounds. Software-facilitated identification of targeted analytes was conducted based on retention time, accurate mass and isotopic pattern of pseudomolecular ion, and accurate masses of fragment ions using in-house developed compound database. Detection and identification of non-targeted compounds and analogs were performed by retrospective evaluation of MS and AIF experimental data. The method for PDE5 inhibitors was validated according to the AOAC International Standard Method Performance Requirements (SMPR) 2014.010, 2014.011 and 2014.012 and was approved as the AOAC Official First Action method 2015.12 for screening, identification and quantitation of PDE5 inhibitors in food ingredients and supplements.

Keywords: adulterants, food supplements, non-targeted analysis, high-resolution mass spectrometry

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FRAUD DETECTION IN MARINE PRODUCTS WITH MOLECULAR ANALYSIS TECHNIQUES. A CASE STUDY IN ELASMOBRANCHII**Anastasia Imsiridou^{1*}, Styliani Maradidou², Dimitrios Loukovitis³, George Minos⁴**^{1,2,3,4} Alexander technological educational institute of Thessaloniki, Thessaloniki, Greece* Corresponding author - E-mail: imsiri@otenet.gr, Phone: 00302310013381

Several studies have detected fraud in fish markets, mainly by substituting species with others of a different price or identity. Species identification cannot be properly controlled unless genetic markers are employed, especially when the commercial product is not the whole specimen. A simple PCR amplification of the nuclear 5S rDNA gene and/or the PCR analysis of mtDNA genes followed by direct sequence analysis of the amplified fragment, are genetic methodologies available for species identification. Both of these approaches have been employed in the present study. A total of twenty four fish fillets were purchased from different open markets of Thessaloniki city (Northern Greece), labelled with the common Greek name "galeos". Marine products labelled as "galeos" have higher commercial value (17 euros/kg), in contrast to other Elasmobranchii species known as "sapounas" with much lower value (5 euros/kg). White muscle tissue was taken from each specimen and stored at -20°C. Total DNA was extracted from frozen fish tissue muscles, according to the CTAB methodology. The PCR amplification of the 5S rDNA gene was performed using the universal Pendas et al. (1994) primers. Two non species – specific patterns were revealed for the 5S rDNA gene: the first one consisted of a single band of 200 bp (eighteen samples) and the second one consisted of three bands with sizes of around 200, 400 and 550 bp (sixteen samples). The amplification of the mitochondrial 16S rDNA gene was done with the universal Palumbi (1996) primers. The size of the PCR products was approximately 600 base pairs for all specimens. A sequencing analysis on a 3500 Genetic Analyzer (Applied Biosystems) followed. In total, 566 base pairs at the 5' end of the mtDNA 16S rDNA gene were sequenced. Eight different haplotypes were revealed among the twenty four specimens and were deposited to GenBank (accession numbers: KU577277- KU577284). The eight haplotypes were entered to the BLAST engine and revealed an almost 99% maximum identity with eight different species: six samples were classified to *Hexanchus griseus*, four samples were classified to *Mustelus griseus*, four samples were classified to *Prionace glauca*, two samples were classified to *Alopias vulpinus*, two samples were classified to *Mustelus manazo*, two samples were classified to *Scyliorhinus canicula*, two samples were classified to *Squalus acanthias* and two samples were classified to *Squatina squatina*. These species belong to seven different families of Elasmobranchii. According to the Fishbase, four species correspond to the Greek common name "galeos": *Mustelus mustelus*, *Mustelus punctulatus*, *Mustelus asterias* and *Galeorhinus galeus* and none of these species was identified in the "galeos" samples of the Greek markets. Analysis of the mtDNA 16S rDNA gene was proved suitable for species authentication and fraud detection. The results obtained, clearly support the usefulness of genetic tools for application in seafood traceability.

Keywords: Elasmobranchii, food fraud, galeos, 16S rDNA gene, sequencing analysis

P20**TACKLING FISH FRAUDS: STRATEGIES TO DISTINGUISH FRESH FROM FROZEN FISHERY PRODUCTS**

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Selling fish products as fresh when they have actually been frozen and thawed is a common fraudulent practice. Moreover, fish intended for raw consumption must be previously frozen according to Regulations EC 853/2004 and EU 1169/2011, in order to kill parasites. Thus, besides being a commercial fraud, non compliance to this rule also represents a sanitary fraud. Since 2008 we have been evaluating the most performing techniques (NIRS, FFFS) in distinguishing fresh from frozen fishery products in order to make available reliable tools both for official control plans and for self-control activities of suppliers. A microscopic method, with high validity values assessed on 35 fish species (i.e. sensitivity 90.70% - C.I. 82.49-95.9% and specificity 92.59% - C.I. 75.71–99.09%), and showing the best predictive power when compared to spectroscopic techniques, was set up. The high performances of the method, allow its application in routine monitoring and surveillance programmes, performed by national regulatory authorities and food business operators, to evaluate the prevalence of the food fraud on the market and to prevent its spread. A specific monitoring plan, implemented in North-Western Italy in 2013-2014 in order to estimate how widespread the fraud phenomenon was among restaurants and retailers, showed that the proportion of storage mislabelling was up to 27% in 2014. However some major challenges have to be considered: for marinated fish we could assess the same method reliability, while for smoked products, for which technological treatments (i.e. freezing at -7°C for at least 30 min to cut the products into slices) are needed, misclassification could be possible, especially considering that a legislative gap is now present regarding storage temperatures from -2 to -20°C. Also transportation and storage time and temperatures should be taken into account. Thus only a tight collaboration with food business operators and control authorities will allow to correctly consider all the processing conditions that have to be distinguished from true frauds. Cephalopods and crustaceans cannot be correctly classified adopting nor the microscopic approach, neither other methods with the expected validity up to date. Thus we recently applied a FFFS technique to distinguish fresh from frozen bonitos and common octopuses, resulting in 85% and 93% correct classification of the samples, respectively, and a proteomic approach to distinguish fresh from frozen musky octopuses, whose promising results obtained will be further investigated. Nevertheless interspecies variability and time after fishing are the most crucial parameters that have to be considered in the evaluation of methods reliability. Opportunities and limits of the adopted techniques in official control will be discussed.

Keywords: fish, fraud, fresh, frozen-thawed

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P21 **¹³C-SNIF-NMR - A COMPLEMENTARY TOOL IN FOOD AUTHENTICITY CONTROL**

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Stable isotopic analyses are now routinely used for the authentication of fruit-based products. Site-specific isotope ratios are directly measurable by SNIF-NMR (Site-specific Natural Isotope Fractionation - NMR), a technique which for many years was limited to deuterium/hydrogen ratios and which is official for wines, juices, vanillin, vinegar and maple syrup. The obstacles linked to the NMR technology itself have recently been resolved and ¹³C-NMR is now a robust means of measuring site-specific ¹³C/¹²C isotope ratios, opening up new possibilities in the detection of economic adulteration. In fruit juices for example, the technique is able to distinguish for the first time between C4 plant sugars (cane, maize) and CAM plant sugars (pineapple, agave). It has also been used to improve the control of agave syrup and to check the absence of exogeneous cane/maize sugar. It also gave useful information to control Tequila beverages.

Keywords: NMR, isotope, SNIF NMR, beverages

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SENSITIVE DETECTION OF ECONOMICALLY MOTIVATED ADULTERATION OF HONEY BY BULK AND COMPOUND SPECIFIC ^{13}C ISOTOPE RATIO MASS SPECTROMETRY USING LIQUID CHROMATOGRAPHY AND ELEMENTAL ANALYSIS INLET DEVICES**Jens Griep-Raming^{1*}, Dieter Juchelka², Andreas Hilkert³**^{1,2,3} Thermo Fisher Scientific, Bremen, Germany

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Honey is considered a value-added food of natural origin, yet it is simply structured regarding its composition, which makes it prone to economically motivated adulteration by addition of sugars of other sources. Testing for adulteration can be done using various methods, including melissopalynological pattern analysis, sensory analysis, amino acid profile analysis, and others. The limit of detection of these methods is generally in the double digit percent range, depending on the type of sugars added. The introduction of bulk ^{13}C isotope analysis by White and Doner in 1978 was a major step towards establishing better methods sensitivity. Still, it is possible to adulterate honey undetected by bulk ^{13}C isotope analysis and sugar profile analysis by carefully selecting a mixture of sugars that mimic both, the bulk ^{13}C composition ($\delta^{13}\text{C}$) and the sugar profile of the natural product. This work describes a multi-parametric method, looking at both, bulk and compound specific $\delta^{13}\text{C}$, identifying adulteration based on inconsistencies in the parameter set itself.

The basic concept of the method is based on the fact that in natural polyfloral honey, the isotopic composition of the individual sugars, di-, tri- and oligosaccharides as well as the protein fraction contained in the honey sample are of same biological origin and thus have very similar isotopic carbon composition. The deviation of a single (or more) of these parameters from the others is an indicator of adulteration.

The $\delta^{13}\text{C}$ of bulk honey is determined by analyzing pure honey samples in a Thermo Scientific Flash 2000 elemental analyzer coupled to a Delta V mass spectrometer and a ConFlo IV referencing device (all Thermo Fisher Scientific, Bremen, Germany). Samples are prepared by encapsulation in tin foil and introduced into the elemental analyzer without additional treatment.

The protein fraction is prepared by Na_2WO_4 precipitation from aqueous sample solution, dried and analyzed using the configuration described above.

$\delta^{13}\text{C}$ isotopic values of individual sugars and sugar fractions are determined by chromatographic separation on a Phenomenex Rezex RCM (Ca^{2+}) 300 × 8 mm column using water as solvent. The organic compounds in the eluate are chemically oxidized to carbon dioxide using a Thermo Scientific LC Isolink device. Dissolved CO_2 is extracted and dried using the gas exchanger and Helium counterflow gas drier of the same device. The resulting CO_2 in He is analyzed by the Delta V mass spectrometer (see above). Individual $\delta^{13}\text{C}$ values are being determined for the following sugars and sugar groups: i) glucose, ii) fructose, iii) disaccharides, iv) trisaccharides, and v) oligosaccharides.

The resulting maximum difference between the following values is being calculated: a) bulk $\delta^{13}\text{C}$, b) protein $\delta^{13}\text{C}$, and c) the individual compound group $\delta^{13}\text{C}$ i) through v) above. A large difference in the values (on the order of 1‰ or greater) might indicate adulteration and requires further investigation.

Keywords: honey adulteration, isotope ratio mass spectrometry, LC–IRMS, EA–IRMS

P23 HONEY-PROFILING™ - TAKING AUTHENTICITY TESTING TO THE NEXT LEVEL

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Honey is a product valued for being purely natural and healthy. But it is also a high priced product which makes it very interesting for fraudsters, who try to increase profit by dilution of honey with cheap sugar syrup or maximizing honey production by questionable means. Authenticity of honey inevitably has become an important issue for the honey industry in the past years. To ensure quality and authenticity of honey, it has to be tested with several different methods. Classical ways of testing mostly focus on the presence of certain marker compounds, thus only covering single aspects of a possible adulteration. The absence of a marker compound does not necessarily prove authenticity of a honey, as targeted methods are blind for anything outside the target. The Honey-Profiling™ developed by QSI GmbH (Bremen, Germany), Bruker BioSpin GmbH (Rheinstetten, Germany) and ALNuMed GmbH (Bayreuth, Germany) is based on comparison of an entire NMR-spectrum with a database consisting of NMR-spectra of authentic honeys. Additionally, a broad range of specific substances is quantified as well. More than 4,000 samples have been analysed with classic (non-NMR) analyses so far to ensure their suitability for the database. The analyses included amongst others the analysis of sugars, isotopic composition, pollen analysis and analysis of enzyme activities. Samples from over 50 countries and more than 20 botanical varieties were acquired. The NMR analysis is used in combination with multivariate statistics to check unknown samples for their authenticity and yields information on adulteration, processing steps as well as on geographical and botanical origins.

Keywords: NMR, honey, authenticity, screening, multivariate statistics

Acknowledgement: Many thanks for their valuable contributions and discussions to the colleagues from ALNuMed GmbH and Bruker Biospin GmbH.

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THE USE OF THE $^{87}\text{Sr}/^{86}\text{Sr}$ ISOTOPE RATIO MASS SPECTROMETRY (TIMS) TO AUTHENTICATE TOMATO ORIGIN: A CASE STUDY

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Nowadays the tomato processing industry needs new and improved tools for producing better information for the consumer and a more efficient protection of typical Italian products. This paper presents a case-study of the application of the Sr isotopic technique to the geographical traceability of tomato. Aim of this work was to set up an analytical methodology able to discriminate, with certainty, between the origin of tomato derivatives coming from China and from Italy. More than 100 samples were analyzed: 88 from Italy (the provinces of Piacenza, Parma, Ferrara and the regions of Puglia and Campania) in the form of berries, "passata", cherry tomatoes and double concentrate; 30 double concentrate tomato paste samples from Xingjiang (NW) and Hubei (E), two Chinese Provinces. This technique proved to be effective in the geographical discrimination of the two populations, with statistical significance ($p < 0.01$); six blind samples were examined and correctly assigned to the Italian cluster. Within the Italian samples, the technique was able to discriminate almost all the provenances, in virtue of the diverse geology of the sites. This study, with the setting up of a data-base, in virtue of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio as a chemical indicator, can also represent a useful tool for the legislator in order to avoid possible frauds in this food sector, often diffuse nowadays.

Keywords: $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio, food traceability, mass spectrometry, MC-ICP-MS, TIMS

Acknowledgement: Mutti S.p.A., Montechiarugolo, PR, Italy for the financial support

P25 FATTY ACID COMPOSITION AND $\delta^{13}\text{C}$ ISOTOPIC RATIO CHARACTERIZATION OF PUMPKIN SEED OIL

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This study aimed to verify the authenticity and geographical origin of pumpkin seed oil using chemical and isotopic characterization combined with chemometric analysis. Thirty-eight pumpkin seed oils collected from various parts of the world and 17 samples of authentic pumpkin seed oil blended with rapeseed, sunflower and soybean oil. The fatty acid composition was analysed by gas chromatography (GC), while the carbon isotope composition of the major fatty acids was determined using gas chromatography-combustion-stable isotope ratio mass spectrometry (GC/C/IRMS). Pumpkin seed oils are highly unsaturated, oleic acid varies from 26.8% to 43.6% and the content of linoleic acid is between 37.2% and 54.9%. The average $\delta^{13}\text{C}$ values of the four main fatty acids are $-29.2\pm 1.2\text{‰}$, $-30.3\pm 1.6\text{‰}$, $-27.9\pm 1.7\text{‰}$ and $-28.1\pm 1.7\text{‰}$ for C16:0, C18:0, C18:1, C18:2, respectively. To determine adulteration, rapeseed, sunflower and soybean oil, that are cheaper and belong to different botanical families, were added to pumpkin seed oil in varying percentages (1–10%). A 100% correct classification of both geographical and botanical origin was achieved based on the composition and $\delta^{13}\text{C}$ values of fatty acids. Principal component analysis (PCA) and regularized discriminant analysis (RDA) analysis gave comparable results. The results suggest that PCA is a useful approach for determining the geographical origin and authenticity of pumpkin seed oil when having a large set of samples.

Keywords: pumpkin seed oil, fatty acids, geographic origin, authenticity, stable isotope ratio

Acknowledgement: Authors wish to thank to the the U.S. Department of Agriculture and various oil producers for collected samples.

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PDO PARMIGIANO REGGIANO CHEESE: NON TARGET MASS SPECTROMETRY, CHEMOMETRICS AND THE FUTURE PATH TO DETECT FRAUD

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According to the list of the European Commission report from 2013, milk products are the fourth-most frequently adulterated products in Europe. Within this group, premium cheeses are top of the list. Especially Italy has a large number of cheeses which carry a protected designated origin (PDO) label. The most prominent one is Parmigiano Reggiano, a PDO cheese from a specific geographic area of northern Italy. It is well known that there may be several products sold to consumers which claim to be Parmigiano Reggiano but are often parmesan cheeses of lesser quality. Two independent systems were used to characterize numerous authentic samples of Parmigiano Reggiano according to geographical origin based on analysis (untargeted approach) of small molecules with LC–HRMS and large molecules with MALDI–TOF/TOF. Using chemometrics, raw, untargeted results were processed with multivariate analysis to generate a significant and reliable prediction model. After proper outliers removal, a very high level of significance and reliability was reached with the PCA for classes model 86% of recognition ability. Targeted and Non-Target Approach combined with a suitable chemometric data analysis will take into account the production process, the geographic origin as well as the type of animal feed to assess the authenticity. This work will help producers and consumers by delivering a highly specific method to identify authentic Parmigiano Reggiano and fraudulent products thereof.

Keywords: non target mass spectrometry, chemometrics, fraud, PDO, Parmigiano Reggiano

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STABLE ISOTOPES AS TRACERS OF GEOGRAPHIC ORIGIN OF PLANT SEEDS AND OILS

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Geographic origin of natural and food products has become an important issue as a consequence of high quality product demand by markets and consumers. Among analytical procedures for determination of geographic origin, isotopes are one of the best tools for fingerprinting. Stable carbon, oxygen and hydrogen isotope composition is altered during vegetation- and soil-atmosphere exchange processes, such as evapotranspiration, carbon assimilation and respiration. Particularly the multiple-isotope approach, i.e. the simultaneous measurements of stable isotope composition of different elements (d^2H , $d^{18}O$, $d^{15}N$, d^3C and also heavy isotopes as ^{88}Sr), provides a unique way to integrate, record and trace fundamental biological processes related to environmental seasonal variations, agricultural practices and other human impacts, allowing the discrimination of origin and food authenticity even within small regions. The isotopic composition of oxygen and deuterium of precipitation varies systematically with latitude and altitude, largely as a result of temperature-driven enrichment of the heavier isotope from humid air masses as they move to the cooler, higher latitudes or over orographic barriers. Recent work has developed methods for integrating observational data with information on these geographic patterns to produce high-fidelity maps of water dD and $d^{18}O$ over a range of spatial scales (isoscares). As the $d^{18}O$ and dD of plants reflects the $d^{18}O$ and dD of the water, plant material may be used to identify the geographical origin of samples and in some cases, processing techniques involved in manufacturing. While ^{13}C is depleted during photosynthesis, leaf water is enriched in ^{18}O during transpiration, which is passed on to organic molecules. It was detected a variety of fractionations during nitrogen transformation in soils and plants. Plant $d^{15}N$ can also be used as a tracer for different traditional agriculture practices. In addition to stable isotopes, radiogenic isotopes such as $^{87}Sr/^{86}Sr$ have been used in geographic origin and traceability studies. In this study, three economically important plant species are selected as case-studies, namely Coffee plant seeds, the argan tree (*Argania spinosa*) and the holm oak (*Quercus rotundifolia*). The geographic origin of these products will be evaluated through an array of different methodological data based on stable isotopes techniques. The results will allow to differentiate producing regions and to reinforce the quality and commercial value of the different products and sub-products, as well as give us an understanding at how plant fruits integrate the effects of extreme local climates. Factors such as altitude and distance to the coast will also be addressed. Clearly, we illustrate an example of how increased knowledge of ecosystem ecology, ecosystem goods chemistry and biology leads to an environmental fingerprint of high quality products.

Keywords: stable isotopes, geographic origin, environmental fingerprint, seeds

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IMPLEMENTING MULTI-ELEMENT AND ISOTOPIC FINGERPRINTING AS TOOL FOR FOOD AUTHENTICATION IN AUSTRIA: SCIENTIFIC BACKGROUND, POTENTIAL AND RELEVANT LEGAL ASPECTS

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“Multi-element and isotopic fingerprinting” methods have been recognized for their considerable potential for tracing the geographical origin of food. Especially the potential of isotopes of the light elements C, N, H or O have been explored widely for this purpose. It has been found, that most of the light isotopes follow seasonal variations of their isotopic composition, which might be changed during storage or processing, as well, what is limiting their potential. Therefore, geology/soil-born element- and isotopic information in soil and food is being increasingly explored for its potential to serve as reliable geographical tracer for food origin. Especially the isotopic composition of the elements Sr and Pb show a distinct regional variability and are stable over time. Moreover, they allow for a direct link of soil to plant. To regionalize locally gathered data of food, soil and water, a relation between the elemental and isotopic composition of the soil and water and the chemical composition of the food is needed.

In special cases, for example in fish, due to the concentric “tree-ring” like growth of the ear stones (‘otoliths’), the environment can be linked in a time-resolved manner to the ear stone chemistry and thus to the fish’s life history. In turn, this enables the verification of new regulations regarding the labeling of fish from aquaculture according to their origin (EU-regulation 1379/2013).

Therefore, within the CSI: TRACE your FOOD! project, one key objectives is to determine the relationship between environmental factors (geology, soil, water chemistry, sea level, etc.) and the chemical composition of selected foods. The project is funded by the Sparkling Science program of the Austrian Ministry of Science, Research and Economy supporting research projects jointly conducted by research institutions and schools in Austria. In a Citizen Science based research approach students from 10 schools in all nine provinces of Austria and one school in Hungary developed the regional basis for the study (identifying local products, collection of environmental data and samples, etc.) as scientists using mainly online tools.

Finally, regional chemical maps of soil, water and agricultural products are established. A statistical online tool will allow for the provenancing of samples of unknown origin.

To ensure the practical and legal relevance of the results, the project is conducted in close cooperation with the Austrian Agency for Health and Food Safety (AGES) and the Agrarmarkt Austria Marketing GesmbH (AMA-Marketing).

The presentation gives insights into the scientific background of the study, shows the potential of using the multi-element and ⁸⁷Sr/⁸⁶Sr isotopic fingerprint for tracing vegetables and fish from regional Austrian production and discuss relevant legal and organizational aspects relevant for the establishment of the multi-element and isotopic fingerprint for tracing and authenticating of Austrian food.

Keywords: isoscape, food provenancing, strontium isotopes, citizen science, Austria

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P29**VOLATILE PROFILE ANALYSIS AS A TOOL FOR GROUND BLACK PEPPER AUTHENTICITY SURVEY****Jaromir Hradecky^{1*}, Eliska Kludska², Diana Cencialova³, Jana Hajslova⁴**

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Black pepper, as a precious commodity on worldwide market, may become a subject of fraud. Cases of adulteration of ground black pepper with cheaper plant materials or mislabeling have been reported. Smart approaches for the testing of spices authenticity are needed to disclose such practices and protect consumers. One of possible authentication strategies is profiling of volatile compounds. The evaluated set of samples in our study consisted of 35 ground black peppers – 13 authentic and 10 wholesale samples were delivered by spice trading company and other 12 were collected in retail markets in the Czech Republic. Volatile profiles of other materials derived from black pepper (oleoresin, spent – residual material of oleoresin production, pepper peels) that can be under certain conditions used for adulteration and samples labeled as “spice mixture” containing ground black pepper, spent and oleoresin were also evaluated. Black pepper volatile profiles were obtained using head-space solid-phase microextraction coupled to gas chromatography/mass spectrometry (HS–SPME–GC/MS). Time of flight mass analyzer was used for primary data acquisition (TruTOF, LECO, USA). Automated deconvolution & peak find algorithm were carried out using ChromaTof software by LECO. Statistical Compare feature of the software aligned signals in all off measured samples and after the normalization of their areas, the statistical analysis was performed in Simca software (Umetrics). All authentic ground pepper samples and those from retail market grouped together using principal component analysis. Some of wholesale samples, tend to group with samples of “spice mixture” spent or peels. Interestingly, those wholesale samples were labeled as “suspect” by our commercial partner, based on their low price or nonstandard flavor. For one of “suspect” samples, organic solvents used for oleoresin isolation were the most decisive compounds. Other separated samples showed similar profiles of volatiles to those of pepper related materials mentioned above. It was observed that relative abundance of beta-myrcene was decreasing with the aging of ground sample.

Keywords: black pepper, spice, authenticity, SPME–GC/MS

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P30 PROVENANCING OF FRUIT RAW PRODUCTS USING ELEMENTAL AND STRONTIUM ISOTOPIC FINGERPRINTS

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The demand for methods capable for the determination of food origin and authenticity is constantly gaining public interest. Within this study, a method based on elemental and strontium isotopic analysis of strawberries was developed following prior established protocols. Strawberry raw products from 7 different countries were analysed for their elemental as well as Sr isotopic composition using ICP–MS. The ⁸⁷Sr/⁸⁶Sr isotope ratios gave a clear differentiation between the investigated samples originating from different sites of growth. In combination with multielement pattern, an almost 100% distinction was possible. No significant within-site heterogeneity was observed in either cases. In addition, the influence of soil liming on the final Sr isotopic composition in the fruit was investigated in a controlled experiment. Additional influence parameters, such as the irrigation water and rainwater, were taken into account, as well. Although the ⁸⁷Sr/⁸⁶Sr isotope ratios of the lime, the irrigation water and the rain water were significantly lower than the isotope ratio of the soil (0.71609±0.00071, U (k=2) – before liming and 0.71599 ± 0.00079 – after liming U (k=2)), the ⁸⁷Sr/⁸⁶Sr isotope ratios of the plants (0.71331±0.00089, U (k=2)) and strawberries (0.71363±0.00092, U (k=2)) showed a clear pattern: Sr isotope ratios in plant and fruits were shifted from the original signature before planting (0.70867±0.00141, U (k=2)) towards that of the soil. Further, differences of the elemental pattern between limed and non-limed soil were insignificant. These results confirm that the elemental and Sr isotopic information in the bioavailable fraction in soils is not altered by the investigated agricultural practices under the present conditions. In a second step, we show first results of experiments on processed strawberries (e.g. storage of strawberries in yogurt) and the impact of the storing conditions on the elemental and Sr isotopic fingerprint. Laboratory experiments of strawberries processed to yoghurt showed that the original Sr isotopic fingerprint could be retrieved even in a small piece of strawberry extracted from the yoghurt after processing.

Keywords: provenance, traceability, element, isotopes, ICP–MS

P31**AUTHENTICATION OF VIRGIN OLIVE OIL QUALITY BY A SPME-GCMS VALIDATED METHOD****Inmaculada Romero del Río^{1*}, Celia Oliver-Pozo², Noelia Tena³, Ramón Aparicio-Ruiz⁴, María T. Morales⁵, Ramón Aparicio⁶, Diego L. García-González⁷**^{1, 2, 3, 6, 7} Instituto de la Grasa (CSIC), Seville, Spain^{4, 5} University of Seville, Department of Analytical Chemistry, Seville, Spain* Corresponding author – E-mail: dluisg@cica.es, Phone: +34 954611550

Volatile compounds are responsible for the aroma of virgin olive oils (VOO) that is determinant for their classification into categories: extra-virgin, virgin and lampante-virgin olive oil. VOO qualified with the highest quality (extra-virgin category), which are obtained from health olives harvested at their optimum ripeness and processed under the best conditions, have a distinctive profile of volatiles in comparison with VOO qualified with some sensory defects. The high number and different nature of volatile compounds drive to the need of a reliable analytical method that allows their authentication. The standard method, based on sensory assessment by trained assessors working in a sensory panel, has been questioned over years because its inherent subjectivity and the need of a strict training of panelists. However, it continues being the only standard methodology for VOO sensory quality assessment. That explains the need of a further research in flavor study of virgin olive oil aroma to develop chemical methods that can be used as support to panel test. Although there are some analytical solutions available, they have not been validated and the regulatory bodies are reluctant to adopt them. In this regards, the European Union has encouraged the validation of these analytical tools through the research program Horizon2020, which involves gaining knowledge from the analytical properties of the chemical methods for sensory assessment. Solid phase microextraction (SPME) is the most used system in the isolation and preconcentration of volatiles because of its simplicity, and the separation and quantification of volatile compounds of VOO is usually carried out by gas-chromatography (GC). This work is focused on the analytical validation of the methodology used to determine the actual concentration of volatiles in virgin olive oils when applying SPME–GCMS. The analytical validation was focused on the evaluation of the linearity with the building of the calibration curves, the limits of detection and quantification (LOD and LOQ respectively), the working ranges, the accuracy estimated as trueness and precision, the sensitivity and the selectivity of the method. The validation process includes the calibration curves for 32 volatile compounds responsible for the most common sensory perceptions in virgin olive oils. Sixty-seven percent of the compounds presented a relative standard deviation in repeatability lower than 10%, and this percentage rises to 95% in lampante virgin olive oils. The accuracy was established in 97% of the studied volatile compounds. Once the analytical aspects of each volatile marker were studied, an empirical example of the ability of the method to discriminate virgin olive oils of different categories (extra virgin, virgin, ordinary and lampante) by the quantification of their volatiles was provided.

Keywords: virgin olive oil, quality categories, volatile compounds, SPME–GC–MS

P32**CHARACTERIZATION OF WINE VINEGARS WITH PROTECTED DESIGNATION OF ORIGIN BY ATR-FTIR SPECTROSCOPY****Rocío Ríos-Reina¹, Celia Oliver-Pozo², José M. Amigo³, Raquel M. Callejón⁴, Diego L. García-González^{5*}**

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Vinegar is a product obtained by a double fermentation process (alcoholic and acetous fermentation or acetification) by using a wide variety of methods and different raw materials (wine, honey, cider, etc.). In the past, vinegar was considered as a secondary product in the family of fermented products and lacked of any recognized quality standard. Nowadays, vinegar is considered as a high quality product by many consumers. In fact, new protected designations of origin (PDO) are being approved for wine vinegars, and this lead to the need for authentication tools that protect against falsifications or mislabeling. Thus, the characterization of wine vinegars with a PDO is crucial to defend and certify their quality and authenticity. For this purpose, new spectroscopy techniques such as Fourier Transform mid infrared spectroscopy (FTIR), equipped with Attenuated Total Reflectance (ATR), has been used to investigate the potential of this technique as a rapid, cost-effective and non-destructive tool. Spectra from 84 wine vinegars belonging to three different PDO ("Jerez", "Condado de Huelva" and "Montilla-Moriles") including different ageing and categories have been analysed and compared in the infrared region of 4000–600 cm⁻¹. Changes associated to aging and categories were observed in the bands located in the "fingerprint" region of the FTIR spectrum (1800-900 cm⁻¹). These bands were assigned to certain specific compounds of vinegars that increase during aging in wood barrels (e.g. alcohols, esters, ethers) or due to special compounds of Pedro Ximenez categories (e.g. sugars, furfural). The ability of these bands for assessing the aging and categories of PDOs was checked by principal component analysis. This analysis revealed the importance of the region associated to acetic acid (1800–1680 and 1475–1230 cm⁻¹) and the bands at 1085, 1045 and 1015 cm⁻¹, characteristics of ethanol, alcoholic and esters compounds.

Keywords: wine-vinegars, protected designation of origin, vinegar ageing, ATR-FTIR, fingerprint region

P33 STABLE ISOTOPE RATIO ANALYSIS FOR AUTHENTICATION OF RED YEAST RICE

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Red yeast rice (RYR) is a non-prescription dietary supplement used in traditional Chinese medicine, obtained from rice fermented with the mold *Monascus purpureus* (*Aspergillaceae* family). Depending on the fermentation conditions, the products may contain monacolins, pigments and citrinin as secondary metabolites. The pharmacological compound Monacolin K is a naturally occurring hypocholesterolemic statin used to prevent cardiovascular diseases. The homologous prescription biosynthetic statin, lovastatin, cultured with *Aspergillus terreus* under patented and carefully controlled conditions, is not distinguishable from monacolin K. There is therefore a suspicion that RYR products are spiked with lovastatin, without being declared. As reported by different authors [1,2]. Stable Isotope Ratio Analysis represents a fast and simple way of checking whether or not a sample is of natural origin. We therefore collected around 10 samples of red yeast rice powder and 10 samples of synthetic lovastatin. Monacolin K was isolated from rice using preparative HPLC and together with lovastatin, was subjected to analysis of the isotopic ratio of C using an Isotope Ratio Mass Spectrometer interfaced with an Elemental Analyser. We found that $^{13}\text{C}/^{12}\text{C}$ is able to clearly distinguish lovastatin (-17.3‰) from monacolin K (-29.8‰). In order to have an overall picture of the market, we also investigated the authenticity of 20 samples of commercial products containing RYR.

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Keywords: red yeast rice, monacolin K, lovastatin, IRMS, adulteration

P34**DETERMINATION OF CARBON ISOTOPE RATIO OF ETHANOL IN CHINESE SPIRIT BY LIQUID CHROMATOGRAPHY COUPLED TO ISOTOPE RATIO MASS SPECTROMETRY****Zhong Qiding^{1*}, Wang Daobing²**^{1,2} China National Research Institute of Food and Fermentation Industries, Beijing, China

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Chinese Spirit (so called Baijiu) is a Spirit with long history and deep cultural foundation, which listed as the world's six distilled spirits with brandy, whiskey, vodka, rum and gin. Chinese Spirit has a large market with the annual sales revenue upto 700€ in 2014, however, just as illegal added sugar and water in honey, juice and wine, the Economic Motive Adulteration also threaten the consumption and production of Chinese Spirit. Nowadays, the carbon isotope ratio of ethanol plays an important role in authenticity evaluation of alcoholic beverage, following this advantage, method for the determination of ethanol ¹³C/¹²C isotope ratio of Chinese Spirit by liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS) was established and parameters were optimized with the Milli-Q water mobile phase. Although there are many types of high alcohols and esters in Chinese Spirit, baseline separation of ethanol and other organic compounds can be achieved when working at 30°C and 0.25mL/min, in addition, just as Krummen reported the maximum load of the system was just 2000 ng C, the Chinese Spirit sample should be diluted thousands of times before injection, thus the high alcohols and easters can be neglected. The influence of column temperatures were investigated, results showed that the maximum standard deviation of measured ¹³C/¹²C value from 30°C to 70°C was lower than 0.06%, it should be emphasized that the retention time decreased by 62s for 70°C than 30°C, thus the working column temperature 30°C was optimized. Optional flow of mobile phase (0.25mL/min to 0.40mL/min) was evaluated, and results showed no significant difference on the standard deviation but different measured values: the δ¹³C value depleted with the increased mobile phase speed. However, as the "identical principle" was followed, this difference can be ignored. The precision of the system was tested, the standard deviation (1σ) for triplicate analysis of both standard solutions and Chinese Spirit samples was less than 0.09%, results from five independent analyses on the same ethanol solution and Chinese Spirit sample on four different days showed the reproducibility was very good, indicating the robustness of the method; the accuracy of the method was valid by the official method (OIV oeno 2009) and a very strong correlation(R²=0.999) can be obtained for 10 Chinese Spirit samples.

Keywords: Chinese spirit, ethanol, stable carbon isotope ratio, LC-co-IRMS**Acknowledgement:** This research was supported by The International S&T Cooperation Program of China (Grant No.S2015ZR1061) and by the FP7 project Foodintegrity "Ensuring the integrity of the european food chain" (Grant No.613688) from European Union.

P35 THE CRITICAL COMPARISON OF GC-HRMS AND DART-HRMS POTENTIAL FOR THE WHISKY AUTHENTICATION

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In the recent years, the interest in alcoholic beverages has been rapidly growing. However, some of the specifically high value spirits such as Scotch whisky may become a subject of fraud. While adulteration of whisky is relatively easy to perform, the detection is a great challenge for chemical laboratories. In general, the adulteration of whisky may occur in several ways, e.g. mixing it with ethanol originated from a raw spirit or even synthetic ethanol, adding certain ingredients with flavouring properties. Alternatively, a cheaper whisky may be bottled and sold as a more expensive whisky. Several methods have been used for whisky authentication such as UV/VIS spectroscopy, near red spectroscopy, capillary electrophoresis or gas chromatography coupled to mass spectrometry (MS). In our previous study, we analyzed a large set of whisky samples using solid phase microextraction (SPME) with GC coupled to high resolution mass spectrometry (HRMS). The results showed some low molecular phenolic compounds might be important markers for some whisky group. In following experiments realized within present study, we decided to extract phenolic compounds using ethylacetate and eliminate abundant whisky components not responsible for group classification such as water and alcohols. The preconcentrated ethylacetate extracts were directly injected in GC-HRMS. For a rapid screen of phenolic fraction also an ambient ionization technique DART (Direct Analysis in Real Time) coupled to HRMS was employed. A unique set of samples analysed in this study consisted of Scotch blended whiskies (n=77) and Scotch single malt whiskies (n=71) was obtained from The Scotch Whisky Research Institute. For single malt whiskies information on maturation cask (cask used for the bourbon or sherry production), production area (Speyside, Islay, Highland, Lowland), technological production (peated, non-peated) and ageing period (8-21 years) were available. To assess variability among different whisky groups according to the technological production process, production area, maturation cask and aging period, chemometric tools such as principle component analysis (PCA), partial least square regression (PLS-DA) and orthogonal PLS were used for the processing of generated data. The potential of both GC-HRMS and DART-HRMS for whisky authentication will be discussed in a detail.

Keywords: whisky, authenticity, adulteration, DART-HRMS, GC-HRMS

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P36

ASSESSING SAFFLOWER ADULTERATION IN SAFFRON (*CROCUS SATIVUS* L.) BY REAL-TIME PCR**Caterina Villa¹, Joana Costa², M. Beatriz P.P. Oliveira³, Isabel Mafra^{4*}**^{1,2,3,4} REQUIMTE-LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal

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Saffron (*Crocus sativus* L.) is the most expensive spice in the world, being greatly appreciated in food preparation for its unique color, taste and aroma. Owing to its high market value, saffron has been frequently associated with an unrivaled degree of adulteration, for which orange-flowering plants, such as safflower (*Carthamus tinctorius*) are commonly used [1]. Thus, the detection of this type of adulterants becomes a very important issue for assuring the consumer's protection against fraudulent practices. Recently, molecular biology techniques have proved to be well suited for unequivocal identification of species in foods [2], spices [3] and medicinal plants [4]. DNA markers, such as SCAR [1], or DNA barcoding to identify particular species [5] are major approaches. The main objective of this work was to develop specific molecular markers for saffron authentication, exploiting ITS regions to detect safflower adulteration. For this purpose, different leaf and stigma samples of *C. sativus* L. were used, as well as a set of binary mixtures as references prepared by adding known amounts of safflower (dried flowers) to saffron stigmas in the range of 0.1 to 20%. DNA was extracted using the Qiagen Plant kit. New primers were specifically designed and applied to qualitative PCR and real-time PCR targeting: a SCAR marker of *C. sativus* L. to amplify a 274 bp fragment; and two DNA barcode loci of *C. tinctorius* (ITS1 and ITS2) to amplify 382 bp and 620 bp fragments, respectively. The SCAR marker proved to be specific for *C. sativus* L. without any cross-reactivity with other plant species. Regarding the safflower detection, the qualitative PCR results showed sensitivities down to 0.1% and 1% with ITS1 and ITS2 primers, respectively. The real-time PCR approach with EvaGreen dye and melting curve analysis was applied to ITS1 region, showing absolute and relative sensitivities of 0.4 pg of *C. tinctorius* DNA and 0.1% of safflower in saffron. To develop a robust quantitative methodology, the parallel amplification of both ITS1 sequence and an endogenous control gene enabled the development of a normalised quantitative calibration model based on ΔC_t method in the range of 20–0.1%. The method exhibited adequate real-time PCR performance parameters (PCR efficiency=94.9% and $R^2=0.9991$). It is possible to conclude that new useful and effective tools were proposed for the specific detection of *C. sativus* L. stigma and quantification of safflower adulteration in saffron and for its authentication.

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Keywords: saffron, authenticity, DNA barcode, ITS, *Carthamus tinctorius***Acknowledgement:** This work was supported by a FCT grants UID/QUI/50006/2013 and COST Action FA1101. Joana Costa is grateful to FCT grant (SFRH/BPD/102404/2014) financed by POPH-QREN (subsidised by FSE and MCTES). Samples were kindly supplied by Bank of Plant Germplasm of Cuenca.

P37 DIFFERENTIATION OF COD-LIKE SPECIES BY HRM ANALYSIS

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Approximately 18% of the world's total marine catch regards fishes from the Gadiform order, corresponding to almost 6.5 million tons in 2011. Particularly, Gadidae family represents an important marine resource, comprising species with commercial relevance, such as *Gadus morhua*, *Gadus macrocephalus*, *Pollachius virens* and *Theragra chalcogramma*. These species are highly consumed worldwide, being usually prone to adulteration issues because of their phenotypic similarity [1, 2], stressing the need for the development of specific analytical tools for authenticity purposes. The present study intends to evaluate the use of a DNA barcode of cytochrome b (cytb) gene as a potential molecular marker for the discrimination of four genetically related codfish species by means of high resolution melting (HRM) analysis. Prior to the experimental work, *in silico* barcoding analysis was performed for the design of universal primers targeting a cytb region of the selected species, namely Atlantic cod, Pacific cod, Alaska Pollock and Saithe. Other fish, crustacean and mollusc species, as well as meat and plant species were used for specificity testing purposes. DNA was extracted with the NucleoSpin Food kit and yield/purity were evaluated by UV spectrophotometry in a micro-volume plate accessory. Specificity and sensitivity of the designed primers were assessed by qualitative PCR and sequencing. Afterwards, a real-time PCR assay using EvaGreen dye coupled to HRM analysis was developed. The real-time PCR results with conventional melting curve analysis showed one group of melt peaks at temperatures ranging from 76.5–78°C that did not allow species differentiation. On the other hand, the application of HRM analysis enabled discriminating all the four fish species, which were included into distinct clusters with high level of confidence (>99%). DNA sequencing explained the results from HRM analysis and confirmed the authenticity of all Gadidae fish species. These findings suggest that cytb gene is an effective gene marker for the authentication of Gadiform species. To our knowledge, it was shown for the first time that using mini-barcodes coupled to HRM analysis allowed the rapid discrimination of cod-like species and can be applied to fish-containing foodstuffs as a robust and highly sensitive methodology.

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Keywords: DNA barcoding, cytb, high resolution melting analysis, *Gadus morhua*, Gadidae

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P38 HIGH RESOLUTION MELTING ANALYSIS AS A NEW TOOL TO AUTHENTICATE PLANT FOOD SUPPLEMENTS: THE CASE OF ARTICHOKE (*CYNARA SCOLYMUS*)

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Artichoke (*Cynara scolymus* L.) is a medicinal plant mainly used for its antioxidant, diuretic, choleric and hepatoprotective properties, being frequently included in herbal infusions and plant food supplements (PFS) marketed for weight-loss (Lattanzio et al, 2009). Both types of products can be adulteration targets, either by the deliberate substitution of other lower-cost plant species, or by the accidental swap of plants owing to misidentification. Therefore, to ensure consumer's safety, analytical methods for plant species identification in complex matrices are crucial. For this purpose, DNA-based methods have been reported as the most adequate tools for plant authentication. Genetic composition of each plant is unique and independent from the part of the plant used (Kazi et al., 2013). Moreover DNA molecules are very stable, not affected by the plant's age, physical conditions or environmental factors, in opposition to chemical markers. In this work, a molecular approach based on real-time PCR coupled to high resolution melting (HRM) analysis to discriminate *C. scolymus* from other *Cynara* species was developed and applied to the analysis of herbal mixtures and PFS labelled as containing artichoke as ingredient. For this purpose, different *Cynara* voucher species (*C. scolymus*, *C. cardunculus*, *C. humilis* and *C. syriaca*) were obtained from germplasm banks, while samples of herbal infusions (6) and PFS (8) were acquired at local herbal and dietetic stores. DNA from plant material and PFS was extracted using the commercial NucleoSpin Plant II kit. For *Cynara* spp. differentiation, new primers were designed on a microsatellite region of *C. cardunculus* (GenBank EU744973.1) for the development of qualitative polymerase chain reaction (PCR) and real-time PCR assays. Prior to the specific PCR assays, DNA extracts were positively tested targeting a universal eukaryotic sequence (18S rRNA gene). The qualitative PCR results were specific for *Cynara* genus. Further development of real-time PCR coupled to HRM analysis showed that the tested *Cynara* spp. were grouped in three distinct clusters with a level of confidence above 99.4%, thus enabling the discrimination of *C. scolymus* from the others. The analysis of commercial samples showed that, with the exception of one PFS sample, all samples were positive for the presence of the universal eukaryotic gene. All herbal infusions and three PFS were positive for the presence of *Cynara* spp. based on the qualitative PCR assay. The application of the proposed method of HRM analysis confirmed the unequivocal presence of *C. scolymus* with high level of confidence (>98.8%) in the tested samples. To our knowledge, this is the first successful attempt for the rapid discrimination of *C. scolymus* in PFS.

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Keywords: *Cynara* spp., artichoke, authenticity, DNA-based methods, plant food supplements

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P39 DNA MINI-BARCODES COUPLED TO HIGH RESOLUTION MELTING (HRM) ANALYSIS FOR THE BOTANICAL AUTHENTICATION OF ROSEMARY HONEY

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Honey is a natural product highly consumed for its taste, nutritional value and health benefits. Monofloral honeys are the most appreciated by consumers and frequently attain high market values, thus being prone to fraudulent practices. Therefore, the development of methodologies to assess and authenticate the botanical origin of honey is of utmost importance. For this purpose, traditional methods based on pollen identification by microscopic analysis are still being used, but they are time-consuming and greatly dependent on the experience/skill of trained analysts. As an alternative, the use of DNA markers represents promising approach for the identification of botanical species in honey. Currently, DNA barcoding has been regarded with increasing interest for the taxonomic identification of plants, with two plastidial genes (*matK* and *rbcl*) being proposed for their differentiation (Bruni et al., 2012). Thus, the objective of this work was to identify the botanical species in rosemary honey using mini-barcode regions coupled to high resolution melting (HRM) analysis. For this purpose, different plant species (*Lavandula spp.*) and ten mono- and multifloral honeys were used. Three DNA barcoding loci, namely the plastidial coding genes *rbcl* and *matK* and the noncoding intergenic *trnH-psbA* region, were used to design primers targeting *Lavandula spp.* (GenBank Z37408.1, KJ196360.1 and HQ902822.1). DNA from plants and honeys was extracted with NucleoSpin Plant II kit (method A), according to Soares et al. (2015). The specificity and sensitivity of the designed primers were assayed by qualitative polymerase chain reaction (PCR) and real-time PCR. Prior to the specific amplifications, DNA extracts were positively tested targeting a universal eukaryotic sequence (18S rRNA gene). Results from specific PCR assays were further confirmed by real-time PCR amplification using EvaGreen fluorescence dye. The application of HRM analysis allowed discriminating *Lavandula spp.* into distinct clusters with high level of confidence. When applying the developed methodology to rosemary honey, samples were classified on the same cluster of *Lavandula stoechas* (endemic species in Portugal), therefore confirming its botanical origin. To our knowledge, this is the first study using HRM analysis for the rapid discrimination of plant species in honey.

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Keywords: honey, botanical identification, authenticity, DNA mini-barcodes, HRM analysis

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P40**AUTHENTICITY OF GARLIC ORIGIN USING METABOLOMIC APPROACH BASED ON HIGH RESOLUTION MASS SPECTROMETRY**

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Depending on a variety and growing conditions, garlics may fairly differ in a content of flavour significant compounds and other biologically active components. To confirm the compliance with trader's declaration on respective commodity at the market, authentication method based on metabolomic fingerprinting has been developed. For the purpose of non-targeted screening of all ionisable low molecular weight substances extracted from garlics grown in the Czech Republic, and those with declared country of origin Spain and/or China, following instrumental platforms employing high resolution mass spectrometry (HRMS) were investigated: (i) ambient mass spectrometry utilizing direct analysis in real time ionization (DART) ion source coupled to mass spectrometer with an orbitrap mass analyser; (ii) electrospray ion source – mass spectrometer with time of the flight (TOF) mass analyser, direct infusion (DI) of sample into ion source; (iii) high performance liquid chromatography (HPLC) – electrospray ion source (ESI) – mass spectrometer with a TOF mass analyser. The metabolomic fingerprints were used to develop statistical models (Orthogonal Partial Least Squares-Discriminant Analysis, OPLS-DA,) for the differentiation of the country of origin. While DART-HRMS fingerprints (mass spectra of sample extracts) did not enable sufficient classification of tested garlics and only slightly better results were obtained by DI-HRMS, models obtained by processing of data generated by HPLC-HRMS provided satisfactory results, moreover, characterisation and/or identification of present metabolomics components was enabled.

Keywords: garlic, authenticity, high resolution mass spectrometry, direct analysis in real time (DART), liquid chromatography-mass spectrometry (LC-MS)

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P41 THE ADULTERATION OF SPIRIT DRINKS IN TERMS OF METHANOL PRESENCE

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As adulteration of spirit drinks we can consider if in the production of spirits was used alcohol, which is not of agricultural origin; in the labeling is given any false data; spirits category in the classification does not correspond; into spirit drinks are added synthetic aromatic compounds and flavoring substances by in a non-approved way; absolute value of deviation in concentration of ethanol expressed in volume percentage compared to ethanol declared on the packaging does not meet the requirements etc. It is well known that methanol is toxic to man. At concentration of 400 mg.l⁻¹ in human blood severe poisoning occurs, that may cause permanent health consequences. The methanol concentration in the blood of 1000 – 2500 mg.l⁻¹ is lethal to humans. In this work in cooperation with the Slovak Veterinary and Food Administration we assessed the methanol content in 97 samples of specific groups of spirits, with the use of liquid chromatography. As the monitored spirits were different kinds of vodka, gin, plum, pear or other fruit spirits, liqueurs, brandy that originated from the market in Nitra region, but also home made fruit spirits were included. The results achieved have been assessed in accordance with the requirements given for the maximum limits for methanol content in different kinds of spirits that laid down in the European Parliament and Council Regulation (EC) No. 110/2008. One sample of domestic fruit spirits contained 10.1 g.l⁻¹ vol. % methanol and at the second sample was found methanol content 11.2 g.l⁻¹ vol. %, so they exceeded the requirements of the legislation (10 g.l⁻¹ obj. % methanol). One sample of plum spirit also did not meet that requirement because it contained 10.6 g.l⁻¹ vol. % methanol. Other non-compliant sample was brandy, which was found to contain methanol 2.2 g.l⁻¹ vol. % (requirement is not more than 2.0 g.l⁻¹ vol. % methanol). Three samples of gin also exceeded the required content of methanol (max. 0.05 g.l⁻¹ vol. % methanol in gin) and observed levels of methanol varied from 0.59 to 2.4 g.l⁻¹ vol. %. Although vodka samples met the requirements of the legislation on maximum methanol content, but not all samples met the requirements on minimum alcohol content, which also can be considered a method of spirit fraud and misleading in the product labeling. It can be concluded that the highest methanol content was detected in domestic fruit spirits, on average 7.02 g.l⁻¹ vol. %. The methanol content at relatively high level was found in other fruit spirits as well. The lowest content of methanol was monitored in vodka (less than 0.10 g.l⁻¹ vol. %).

Keywords: adulteration, spirit drinks, methanol, alcohol, vodka

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AUTHENTICATION POSSIBILITIES OF WINES OF BLAUFRÄNKISCH VARIETY ORIGINATING FROM DIFFERENT AREAS**Martina Fikselová¹, Peter Czako^{2*}, Alica Bobková³, Vladimír Vietoris⁴, Lucia Zeleňáková⁵, Zuzana Krvavá⁶, Jozef Golian⁷**^{1, 2, 3, 4, 5, 6, 7} SUA, Nitra, Slovakia

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The aim of this work was to detect selected parameters of the Slovak wines of Blaufränkisch variety in purpose its possible authentication or differentiation. Wines were produced in the same year (2012) and originated from different places of the same region. Assessment was based on chemical parameters such as total acidity, total sugar, alcohol content, acetic acid, citric acid, saccharose content and sensory quality of wines. Wines originated from the Južnoslovenska wine region from 3 different areas/producers, produced as varietal wines. Determination of chemical parameters was done by ALPHA Wine Analyzer designed for rapid analysis of wines, which works on the principle of FTIR spectroscopy. Sensory evaluation of wines was assessed by the electronic nose, then by profile method and by 100 points of the international rating system O.I.V (100 to 87 points - excellent, 86-73 points - very good; 72 to 57 points - good, 56-41 points - satisfactory, less than 40 points - insufficient) Following chemical properties of wines, there were found some differences regarding observed parameters. At total acidity of wines the mean results ranged from 5.0 g.l⁻¹ (area 2) to 5.8 g.l⁻¹ (area 3). Alcohol content of wines varied from 11.3 % (area 2) to 12.4 % (area 3), total sugar content from 0 (area 1,2) to 2.7 g.l⁻¹ (area 3), acetic acid 0.65 (area 1) to 0.79 g.l⁻¹(area 3), citric acid 0.16 g. g.l⁻¹ (area 2) to 0.57 g.l⁻¹ (area 1), and saccharose content 0.6 g.l⁻¹ (area 2) to 1.4 g.l⁻¹ (area 3). From the sensory point of view by 100 points system, samples originating from area 1 were rated by the highest counts of points (81), so these wines can be assumed with very good quality. Samples originated from the remaining two areas were given by points ranging from 72 to 56, so they can be evaluated as with good quality. By profile method as the best accepted were again samples from the area 1, which obtained the highest score due to its smell that is characteristic for these wines, namely aroma of ripe cherries, less blackberry and cinnamon. Sensory evaluation of wines was also completed by electronic nose testing, and documented by PCA analysis. Sensory analysis demonstrated connection between the results achieved by measuring the chemical and sensory evaluation. Because of sensory evaluation is often associated with sulfur dioxide content, total sulfur dioxide results of wines were also determined and compared with the Commission Regulation (EC) No. 606/2009 as well.

Keywords: wines, authentication, alcohol, FTIR, sensory analysis

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VERIFYING THE DECLARED ORIGIN OF TIMBER USING STABLE ISOTOPES, MULTI-ELEMENT ANALYSIS AND CHEMICAL PROFILING

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One of the European Union's aims is to halt the import of illegally acquired and endangered timber and specific legislation is place to support this. The FLEGT (Forest Law Enforcement Governance and Trade) regulations were introduced in 2003 with the intention of reversing the rate of destruction of the world's forests and the timber trade law (regulation (EU) 995/2010) stipulates that importers of tropical timber must be able to identify the origin of timber used in their products, and as of 3rd March 2013, it is a criminal offence to sell endangered tropical timber in the European Union without a FLEGT licence. The procedure to determine the declared origin of timber currently involves verification of shipping documents and visual checks of common timber species for origin identification. There are only a few analytical methods available to support this process and this poster describes three such methods: elemental analysis isotope ratio mass spectrometry (EA-IRMS), inductively coupled plasma mass spectrometry (ICP-MS), and chemical profiling using liquid chromatography time of flight mass spectrometry (LC-TOF-MS). Samples of Sapele, Rosewood and Ebony cores were taken from across West Africa and Madagascar. Data were acquired from the ²H, ¹³C and ¹⁸O stable isotope analysis of cellulose, the trace element profile, and chemical profile of the powdered timber samples. In order to assess the potential for geographical origin discrimination based on the combined profile of stable isotope ratios and multi-trace element concentrations, data acquired from the analysis by EA-IRMS and ICP-MS were processed by canonical discriminant analysis (CDA) resulting in a cross validation classification rate of 87%. Twenty elemental variables were selected by the CDA for the multivariate analysis which provided maximum discrimination between the timber samples originating from Ghana, Cameroon, The Congo, The Democratic Republic of Congo, and Madagascar. A statistical model using principle component analysis was also developed using the data acquired from the LC-TOF-MS chemical profiling enabling the determination of the country of origin of the tropical timber samples and a cross validation classification rate of 92%. Hence, a combination of the methodologies of stable isotope ratio, trace element analysis and chemical profiling offers an effective approach in verifying the declared origin of timber for those samples tested.

Keywords: illegal logging, trace element, stable isotope, EUTR, chemical profiling

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P44**RAPID AND NONDESTRUCTIVE TECHNIQUE FOR DETECTING FRAUDULENT PRACTICE OF MISLABELING FROZEN/THAWED TUNA AS FRESH****Marlon M. Reis^{1*}, Ekaitz Martinez², Miguel A. Pardo³, Angela Melado⁴, Eduardo Saitua⁵, Raquel Rodriguez⁶, Izaskun Pérez⁷, Idoia Olabarrieta⁸**¹ AgResearch, Hamilton, New Zealand^{2, 3, 4, 5, 6, 7, 8} AZTI, Derio, Spain

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An important driver in the price of seafood is whether the product is fresh or frozen. Seafood at grocery stores is often labeled fresh, though it is generally previously frozen. Fresh fish is typically more expensive than frozen, specifically red tuna could reach up to three times the price. Yet experts from the seafood industry suggest it is not always in better condition. Freezing the fish just after the catch and in ultra-low temperatures preserves the taste and quality of the fish by slowing cell degradation, whereas fresh fish on ice continue to degrade in quality until they reach the consumer. Tuna is not always properly labelled: frozen/thawed could be sold as fresh, meaning it is sold more expensive than it should be. Therefore, it is important to prevent the fraud of the mislabeling in order to not to deceive the consumer. Sometimes this fraudulent practice found in the market is difficult to detect, because when freezing and thawing operations are carried out at proper conditions it is difficult to differentiate between fresh and frozen/thawed fillets. This study investigates the ability of Visible-Near InfraRed Spectroscopy (Vis-NIRS), in the ranges from 400 to 2200 nm, to detect, in a rapid and a non-destructive way, whether a sample of tuna is fresh or if it has been frozen/thawed. Fresh fillets were locally obtained, 45 samples were prepared, scanned by Vis-NIRS and subsequently frozen. After one week of storage time, the samples were thawed at 4°C for 24 hours and re-scanned, i.e. each sample was scanned at 16 °C before and freezing/thawing. An extra measurement was done at different temperature in order to study the effect of temperature in the correlation model and to find out whether it is a confounding factor. A fraudulent practice could be to cut off the superficial (and darker) layer of the thawed fillet to make it look like bright red and thus look fresher. In order to check the ability of vis-NIR-spectroscopy for detecting this practice, and for studying the effect of meats blooming after cutting, the exterior layer was cut and measured after one hour. Chemometrics was used for multivariate data analysis. Principal Component Analysis was applied to evaluate the effect of blooming on visible spectral range (400–700 nm) concluding that the oxygenation of the meat after cutting off the surface does not affect the results. Furthermore, the temperature factors were analyzed and no effect was found in the result. Partial Least Square Discriminant Analysis (PLS–DA) classification method was applied using repeated double cross-validation showing that there is a probability of more than 85% that a fresh sample is predicted correctly as fresh and more than 90% that frozen/thawed is really a frozen/thawed. The results suggest that Vis-NIRS is a nondestructive measure analytical method able to detect the difference between fresh and frozen/thawed tuna samples.

Keywords: fraud, fresh tuna, frozen/thawed, chemometrics, vis-NIRS**Acknowledgement:** The financial support from Royal Society New Zealand within the New Zealand-EU International Research Staff Exchange Scheme (IRSES)/REPLAY is fully appreciated. Ekaitz Martinez acknowledges the scholarship of the Department of Environment, Territorial Planning, Agriculture and Fisheries of the Basque Country Government for the development of this work. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n^o (613688) FoodIntegrity Project.

P45 ISOTOPES AND TRACE ELEMENTS FOR DAIRY PRODUCTS ORIGIN CONTROL

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The measurements of stable isotope composition (carbon, hydrogen, oxygen, nitrogen and sulfur) of food provide a very sensitive method for control of their origin and authenticity. The aim of the study is to explore the relationship between isotope composition and trace elements concentration of milk and its geographical origin and seasonal variation of those parameters. The stable isotope composition of milk is strictly related to different isotopic composition of feed. The different composition of cow fodder (grass, maize and hay) and different composition of drink water are the reason of seasonal and regional variation of isotopic composition of fresh milk and finally of dairy products like UHT milk, cheeses etc. The isotope composition allows detecting the origin from the geographic point of view or from the point of view of the production processes (organic or commercial). The measurement of various isotopic ratios (D/H, $^{18}\text{O}/^{16}\text{O}$, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$) in different fractions of a product (water, protein, fat) enables characterization of the origin of raw milk. The aim of this study was to demonstrate the differences in regional and seasonal variations of isotopic and trace elements composition of milk. The mass spectrometry technique is the main method for measurement of the stable isotopes content in food and beverages. The DELTAplus mass spectrometer connected with an H/Device instrument was used to determine hydrogen isotope ratio. Oxygen isotope ratio was determined in water samples on a Gasbench instrument connected with a mass spectrometer. For the determination of C, N, and S in solid materials elemental analyzer coupled with a mass spectrometer was used. The measurements of trace elements concentration were performed on ICP-MS for a limited group of ecological and industrial products. Finally, application of the multielement (isotopic and trace element concentrations) method for origin control of dairy products requires additional research on a larger population of dairy product samples, from organic and commercial farms. After the construction of the big database for isotopes and for trace elements concentrations, the method can be used as a standard for control of authenticity of Polish (organic and commercial) dairy products.

Keywords: dairy products, stable isotopes, trace elements

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P46 ISOTOPIC COMPOSITION OF CO₂ IN SPARKLING DRINKS

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Stable isotope analyses have been useful tool for food authenticity control. Important limitation of the application isotopic method for food authenticity control is a lack of database of stable isotope composition for different origin food. Most popular European alcoholic sparkling drinks are: sparkling wine, cider and beer. Nonalcoholic sparkling beverages are: natural and artificial carbonated mineral waters and a lot of carbonated soft drinks. For sparkling wine is allowed only natural methods of bubbles CO₂ production by addition of sugar to fermentation. The addition of sugar to produce of CO₂ bubbles in wine is allowed during the 1st fermentation or 2nd fermentation. The addition of beet sugar (C3 plants - Calvin cycle) or cane sugar and corn syrup (C4 plants – Hatch-Slack pathway) result in different isotopic composition of CO₂. Artificial carbonated drinks typically using CO₂ from industrial source results in lower δ¹³C values. Subject of the study was to investigate the stable carbon isotope composition of the CO₂ bubbles of sparkling drinks for control of the drinks authenticity. Basic aim was an identification of the source of carbon dioxide in these drinks. Our method was a measurement of the δ¹³C values of CO₂ for authentic sparkling drinks. The Gasbench vials were initially filled by flushing with helium 5.0. After that, 50 µl of gas CO₂ was taken from the headspace of the bottle with the sparking drinks by the use the gastight syringe and was transferred to the Gasbench vials through septum cap. The isotopic composition was determined using Gasbench (ThermoQuest) connected in continuous flow mode to DELTAplus (FinniganMat) mass spectrometer. Every sample was measured sixth time for carbon isotopic composition. The standard deviation of the values obtained from measurements for δ¹³C was less than 0.15‰. Big differences in carbon isotopic compositions δ¹³C in CO₂ in every group of products were observed. This is connected with different origin of the CO₂. Biggest difference was find for mineral waters: for water contained a natural gas from spring and for water carbonated by industrial gas. The final product of the study is a simply method for origin control of CO₂ in sparkling drinks. The sensitivity of the method for big population samples with good origin confirmed was tested. The study are continued for different group of commercial sparkling drinks and the data base for Polish mineral waters, ciders and beers is constructed. The correlation between a carbon isotopic composition δ¹³C in CO₂ and in C₂H₅OH for different authentic, alcoholic drinks was tested and compared with literature data.

Keywords: CO₂, sparkling drinks, δ¹³C

Acknowledgement: The work has been supported by the statutory activity of the Institute of Nuclear Chemistry and Technology.

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A NOVEL APPROACH FOR AUTHENTICATION OF DURUM / COMMON WHEAT BASED ON LIQUID CHROMATOGRAPHY HIGH-RESOLUTION TANDEM MASS SPECTROMETRY MERGED WITH CHEMOMETRICS

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FAO estimation for global and annual wheat production was at a record approximately 600 million tonnes, while for the European Union, the world's largest producer, was around 150 million tonnes. The importance of wheat has been mainly attributed to its ability to be ground into flour and semolina, which form the basic ingredients for bread and other bakery products, and pasta. Focusing on Pasta, Italian law establishes that pasta must be exclusively made by durum wheat semolina and water, and a maximum contamination of 3% from common wheat flour in durum wheat flour is allowed. The problem of durum wheat adulteration with common bread wheat is of particular interest in the Italian, French and Spanish markets, where semolina is the only allowed constituent for pasta. Nowadays, wheat is mainly authenticated by genomics and proteomics approaches. This fraud has a huge impact on quality and economy. On the one hand, this adulterated flour produces lower pasta quality since leads to a product with a scarce resistance to cooking. On the other hand, the price of durum wheat is about 25% higher than that of common wheat. Therefore, useful tools for the detection of the adulteration of durum wheat flour with common wheat are highly required. In this research, a novel strategy was developed. A total of 172 wheat samples harvested in Parma and Bologna (Italy) were analysed using a metabolic fingerprinting method. In the first phase, a solid liquid extraction procedure was optimized, the aim was to isolate as maximal representation of small molecules contained in wheat. In the next step, attention was paid to an optimal setting of detection method for which liquid chromatography coupled with high-resolution mass spectrometry, UHPLC–TripleTOF 5600 (Sciex), was performed. Consecutively, data processing was carried out using various software packages, such as Marker View (Sciex) and SIMCA (Umetrics). Unsupervised pattern recognition technique, Principal Component Analysis (PCA) was initially employed, and durum wheat, common wheat and admixtures samples were nicely distinguished. Afterwards, orthogonal partial least square discriminant analysis (OPLS–DA) was favorably used to discriminate between durum wheat and common wheat. In parallel, admixtures containing 3% and 10% of common wheat were also evaluated in the validated model.

Keywords: UHPLC–HRMS, metabolic fingerprinting, wheat, adulterated samples

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FISH SPECIES IDENTIFICATION IN COMPLEX PREPARATIONS THROUGH NEXT GENERATION SEQUENCING MTDNA BARCODING

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The identification of the species of origin in food preparations is a major concern to identify frauds that might have economic, environmental, ethical and health implications. Our current method for species identification includes amplification and sequencing of the cytochrome C oxidase subunit I gene (COI) and comparison to the GenBank and to the Barcode of Life Data System. This technique is based on Sanger sequencing with the limit, when applied to complex mixtures, of providing only the most represented DNA sequence or uninterpretable mixed DNA sequencing chromatograms. In our routine work, we recently received fish sticks for analysis; for some of the aliquots it was not possible to retrieve a clean DNA sequence, probably due to a mixed preparation. Next Generation Sequencing (NGS) can overcome this limit and provide a complete picture of the DNA composition in complex food preparations. Here we present a preliminary workflow for amplicon sequencing on seven samples from certain species (*Epinephelus diacanthus*, *Pangasius hypopthalmus*, *Thunnus albacarens*, *Thunnus obesus*, *Thunnus thynnus*, *Tursiops truncatus*, *Stenella coeruleoalba*), three equimolar DNA mixtures (mix-A: *E. diacanthus* and *P. hypopthalmus*; mix-B: three *Thunnus spp.* and *S. coeruleoalba*; mix-C: three *Thunnus spp.* and *T. truncatus*), and four aliquots of the fish sticks. DNA was processed by COI amplification and purification. Amplicons were then processed according to Illumina Nextera XT protocol. Library pool was sequenced on Illumina MiSeq (2x300 cycles). Reads were assembled by Trinity RNA-Seq v2.1.1 into contigs that were compared to the nr database with Blastn. Reads were then mapped against the contigs for relative abundance computation. As expected, samples with individual species were fully classified without misidentification. Sequencing of mix-A provided identification of *P. hypopthalmus* and *E. diacanthus*. In mix-B and mix-C, due to the high similarity between *Thunnus* species, only one species of tuna fish was identified, but in both cases the correct cetacean was identified. Sanger sequencing results, which suggested a mixture of species composing the fish sticks, were resolved by NGS: *Gadus chalcogrammus* was correctly assigned, but also *Bos taurus* was detected, probably due to powdered milk, declared in the ingredients list. A few reads mapped also to *Triticum aestivum*, used for breeding. This preliminary work represents a novel approach to species attribution in complex fish preparations to detect frauds. Although tuna species were not discriminated under the genus level, this method was able to detect a different species (*T. truncatus* or *S. coeruleoalba*) when present. Furthermore, it allowed species identification in a complex sample such as fish stick, not achievable by Sanger sequencing. The mtDNA barcoding approach is very promising, and it may be also applied to meat products, providing a very powerful tool for fraud detection.

Keywords: next generation sequencing, DNA barcoding, food preparation, fish species identification, untargeted analysis

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EVALUATION OF THE ROASTING IMPACT ON THE IDENTIFICATION OF HAZELNUT (*CORYLUS AVELLANA* L.) ORIGIN: A CHEMOMETRIC APPROACH

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Hazelnuts (*Corylus avellana* L.) belonging to different cultivars or grown in different geographic areas can be differentiated by their chemical profile; however, the roasting process may affect the composition of raw hazelnuts, thus compromising the possibility to identify their origin in processed foods. In this work, we characterized raw and roasted hazelnuts (Tonda Gentile Trilobata, TGT, from Italy and from Chile, Tonda di Giffoni from Italy, and Tombul from Turkey), as well as hazelnuts isolated from commercial products, with the aim to discriminate their cultivar and origin. The chemometric evaluation of selected chemical parameters (proximate composition, fatty acids, total polyphenols, antioxidant activity, and protein fingerprint by SDS-PAGE) permitted us to identify hazelnuts belonging to different cultivars and, concerning TGT samples, their different geographic origin. Also some commercial samples containing Piedmontese TGT hazelnuts were correctly assigned to TGT cluster. In conclusion, even if the roasting process modifies the composition of roasted hazelnuts, this preliminary model study suggests that the identification of their origin is still possible using simple, low cost and largely diffused analytical approaches.

Keywords: corylus avellana l., hazelnut, authentication and traceability, chemotype

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APPLICATION OF PATTERN RECOGNITION TECHNIQUES TO CHEMOTYPING AND THE IDENTIFICATION OF PEPPER (*CAPSIUM ANNUUM* L.) AT ECOTYPE LEVEL

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This work was aimed to recognize and authenticate the "Peperone di Carmagnola" chemotype (*Capsicum annuum* L., Italian product waiting for the PGI recognition) identifying their typical four typologies (Lungo, Quadrato, Trottola, Tumaticot) also differentiating them from other peppers varieties (different geographic origin). These peppers are often processed (preserved in oil, cooked by roasting or transformed in sauce) losing their morphological profile and so requiring analytical techniques for the authentication. Some minor components of pepper (polyphenols; metals; organic acids; sugars), the protein patterns and the antiradical activity were determined. Some samples were thermally treated in laboratory in order to investigate the impact of the thermal processing/cooking on the traceability; moreover, some commercial samples (thermally processed or canned in olive oil) were considered in the study. The identification of Carmagnola peppers was conducted using PCA and more advanced artificial intelligence-based techniques (Artificial Neural Networks), leading to new information about the significance of each group of compounds.

Keywords: *Capsicum annuum* L., pepper, authentication and traceability, chemotype, artificial neural networks

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A METABOLOMIC STRATEGY TO DISCRIMINATE ANCIENT TRITICUM VARIETIES

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Cultivated *Triticum* species are classified into hulled and free-threshing ('naked') forms. Among the latter, bread and durum wheat are the most important *Triticum* species cultivated worldwide. "Hulled wheats", which means that the kernel retains its husk during harvest, are the earliest domesticated wheats by mankind and the ancestors of current wheats. Ancient wheat cultivation had decreased drastically during the 1960s due to dietary and economic changes and the introduction of bread and durum wheat, which are both higher yielding. However, during the past years, the increasing demand for natural and organic products led to the rediscovery of ancient wheat species such as spelt (*Triticum spelta* L.), emmer (*Triticum dicoccum*), and einkorn (*Triticum monococcum* L.). This renovated interest is also due to the desire for a healthy and equilibrated diet, like Mediterranean diet. In fact, hulled wheat is recognized as a very healthy cereal and is recommended in treatment of disease such as high blood cholesterol, colitis and allergies. Compared to the standard wheat, these grains are characterized by a higher contents of soluble dietary fibre, proteins and lipids (mostly unsaturated fatty acid). Moreover, more rapidly digested starch (RDS), a higher starch digestion index (SDI), low glycaemic index value and high satiety value of ancient wheats make them particularly suitable for people affected by diabetes. Concerning trace elements, emmer, einkorn and spelt mainly differed from wheat cultivars for higher contents of Li, Mg, P, Se and Zn. Up to now, only targeted profiling, developed for quantification of a given class of metabolites, has been applied to investigate similarities and difference between ancient *Triticum* varieties. However the increasing power of MS techniques permits today the simultaneous analysis of hundreds of metabolites, allowing a better characterization of the small molecules (up to 1200 Da) composition of complex plant matrices. In the present study 77 hulled wheat samples were analyzed using a non-target metabolomics approach based on reversed phase liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOF MS) and multivariate data analysis. The PCA scores plot obtained for positive and negative ionization mode indicates a pronounced samples clustering according to the varieties: Garfagnana (*T. turgidum* var. *dicoccum* L.), ID331 (*T. monococcum* L.) and Rouquin (*T. spelta* L.). To further confirm and validate the metabolome difference between the three varieties, PLS-DA and OPLS-DA model were constructed. PLS-DA was performed to maximize groups differences and OPLS to highlight key variables and potential biomarkers. High R² and Q² obtained for both supervised models indicates an excellent predictability and suggests that the metabolomics approach applied could potentially allow the determination of adulterations, since emmer and einkorn are more expensive than spelt.

Keywords: ancient wheats, food authenticity, metabolomics, HRMS, chemometrics

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AN INTEGRATED SENSORY AND INSTRUMENTAL APPROACH TO AUTHENTICATE A TYPICAL ITALIAN SALAMI FROM MORA ROMAGNOLA PIG BREED

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In this study, a sensory and instrumental analytical approach for verifying the authenticity of a typical and high-quality Italian salami, manufactured from Mora Romagnola pig breed, was investigated. Mora Romagnola is an autochthonous breed, extensively farmed within a geographically confined area in Italy, located in the east part of Emilia Romagna region. The aim was to highlight any difference, detectable by a sensory or an instrumental evaluation, between conventional and Mora Romagnola salami, thus proposing an integrated approach for quality control. To reach this result the sensory profiles of ten Italian market salami, both conventional and from Mora Romagnola breed, were defined through a Quantitative Descriptive Analysis (QDA[®]), by using selected visual, olfactory, taste and textural attributes. In order to facilitate judges in evaluating the attributes, reference standards and their relative intensity were defined and provided to the panellists. Moreover, an instrumental approach, based on several techniques, was also followed. In particular, a high resolution image analysis was performed by an electronic eye, in order to collect information on the sample appearance. In addition, since textural characteristics are a fundamental aspect for the consumer acceptance, the textural properties of both the conventional and Mora Romagnola salami were analysed. In detail, Texture Profile Analysis (TPA), as well as a tensile test were respectively performed in order to simulate the conditions to which the salami product is subjected during mastication (and thus correlate the results with the sensory evaluations) and to estimate the binding force existing between the fat and meat particles. The findings of this work have returned an integrated profile sheet thus providing a simple tool potentially useful for verifying the authenticity of the Mora Romagnola salami and to distinguish them from the conventional similar products available on the market. In addition, a fast and integrated characterisation of the Mora Romagnola salami might contribute to improve the diffusion of correct and proved quality information on the product, may improve the producers' awareness and can be an instrument to properly promote the product to the consumer, thus preventing fraud.

Keywords: sensory and instrumental approach, high-quality salami, Mora Romagnola salami, food fraud

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DISCRIMINATION BETWEEN BEEF AND PORK MEAT BY OMEGA-CYCLOHEXYL-FATTY ACIDS AND OTHER SECONDARY FATTY ACIDS

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Adulteration of costly meat products with cheaper counterparts is a serious global problem, so there is the need for new, rapid and reliable analytical methodologies and easily quantifiable markers to be used for meat authentication. Current methods for detection of different meat species in beef are based on DNA and ELISA, but also other technologies have also been considered as ultraperformance liquid chromatography, Raman spectroscopy, low-field NMR, mass spectrometry (1). In the present work, we explore the possibility of using ω -cyclohexyl fatty acids and other minor fatty acids as iso-branched chain fatty acids as markers of ruminant meat, and in particular of beef meat. ω -Cyclohexyl fatty acids, 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acid, are microbial fatty acids recently reported in milk (2). Because no data are reported in literature on the presence of cyclic fatty acids in meat, we developed analytical procedures in order to detect them in both meat of ruminant and not ruminant animals. GC-MS method allowed to detect ω -cyclohexyl fatty acids, and with a suitable internal standard, to reach a reliable quantification of these acids in meat samples of different origin. In particular, ω -cyclohexyl fatty acids were detected only in bovine and ovine meat but not in pork and horse meat. They are probably synthesized by ruminal bacteria, therefore they could represent a marker for ruminant meat. Preliminary data showed that the quantification of ω -cyclohexyl fatty acids, combined with that of specific iso-branched chain fatty acids, permits to discriminate beef meat from pork meat and to determine the ratio of beef/pork meat in minced meat, both raw and cooked (ragout).

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Keywords: meat, ω -cyclohexyl fatty acids, authentication, GC-MS, iso-branched chain fatty acids

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VOLATILE PROFILE OF WILD HOPS GROWN IN THE NORTH OF PORTUGAL: COMPARISON WITH A CULTIVAR HOP PRODUCED IN THE SAME REGION**Julio Cesar Machado Junior¹, Sara C. Cunha², Jorge Sá Morais³, Isabel Ferreira^{4*}**^{1,2,4} LAQV/REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal³ Polytechnic Institute of Bragança, Agricultural College of Bragança, Bragança, Portugal

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Cones of Hop (*Humulus lupulus* L.) plants are used in brewery for centuries, because of their aroma and bitterness. There are many varieties of hop cultured around the world, each one with singular characteristics and presenting a different pattern of volatile compounds. The adaptability of hops to a certain region depends on the soil characteristics and climate. In Portugal, is cultured only the Nugget variety, although wild hops grow in different regions of the country. Studying the volatile profile of these wild hops is relevant to increase knowledge about hops botanical origin. Concerning the methodologies used for analyses of volatile compounds, solid phase microextraction (SPME) can be successfully applied for the extraction of volatiles from hops. Current work is focused on the separation and determination of volatile compounds extracted from cones of wild hop varieties and comparison with a domestic variety (Nugget) both produced in the North of Portugal (Bragança). The characterisation of the volatile profile pattern was performed by GC–MS. Two samples of wild hop (W1 and W2) naturally growing in North of Portugal, were analysed and for comparative reasons a sample of the cultivated variety of hop produced in the region was also analysed. Wild hops presented different profiles: W1 presented 76.4% ($\pm 6.5\%$) of myrcene, 5.5% ($\pm 1.4\%$) caryophyllene, 8.0% selinene (α and β) and 10.1% ($\pm 3.0\%$) of other compounds, include selina-3,7(11)-diene ($2.8\% \pm 0.3\%$), aromadendrene ($1.5\% \pm 0.6\%$) and panasinsene ($2.0\% \pm 0.1\%$). W2 presented 56.1% ($\pm 7.1\%$) myrcene, 15.1% ($\pm 1.5\%$) farnesene, 7.5% ($\pm 1.5\%$) caryophyllene, 8.5% selinene (α and β), 3.4% ($\pm 0.6\%$) selina-3,7(11)-diene, 2.2% ($\pm 0.4\%$) panasinsene and 7.2% ($\pm 1.5\%$) of other volatile compounds, include aromadendrene ($1.3\% \pm 0.2\%$). Different profile was found in Nugget variety which contained about 55.7% ($\pm 3.9\%$) of myrcene, 30.0% ($\pm 3.5\%$) of humulen, 8.1% (± 0.9) of caryophyllene and 6.2% ($\pm 0.6\%$) of other compounds. Further studies of characterization of wild hops grown in North Portugal are being performed by analyses of bitter compounds, alfa and beta acids, and others polyphenols, like isoxanthohumul and xanthohumul.

Keywords: *Humulus lupulus* L., wild hops, nugget, myrcene, caryophyllene**Acknowledgement:** This work received financial support from FCT (Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013.

P55 DISCRIMINATION OF GEOGRAPHICAL ORIGIN OF LENTILS (*LENS CULINARIS MEDIK.*) USING ¹H NMR FINGERPRINTING AND MULTIVARIATE STATISTICAL ANALYSIS

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Lentil (*Lens culinaris Medik.*) is the fourth most important pulse crop in the world after bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), and chickpea (*Cicer arietinum* L.). Canada is the world's largest exporter of lentils, while in Italy lentils are a minor legume and can be found in restricted areas. However, Italian lentils present unique and characteristic qualities giving them a higher value, so that many of them have obtained international and national marks linked to their geographical origins, such as "protected geographical indication" (PGI), "traditional food products" (PAT) and Slow Food Presidium. For these reasons, there is a growing demand for analytical methods able to certify the declared geographical origin of lentils, in order to protect consumers and producers from fraud and unfair competition. In the present work, non-targeted ¹H-NMR fingerprinting, in combination with different multivariate statistical analysis techniques, was used to classify lentils according to their geographical origin. In particular, 85 lentil samples from two different countries, i.e. Italy and Canada, were collected from retail markets and analysed by using an optimized ¹H-NMR protocol. Principal component analysis showed partial grouping of samples on the basis of origin with overlapping zones. Therefore, two class-modeling techniques such as Soft Independent Modelling of Class Analogy (SIMCA) and UNEqual dispersed classes (UNEQ) and three discriminant techniques, such as k - Nearest Neighbor (k-NN), Linear Discriminant Analysis (LDA), Partial Least Squares - Discriminant Analysis (PLS-DA), were used and the performances of the resulting models were compared. The best average recognition and cross-validation prediction abilities, 100% and 93.7% respectively, were obtained by the LDA mode I, performed on a set of 20 principal components previously selected by a stepwise decorrelation procedure. The other models, except the SIMCA one, also showed good performances (above 90%). All tested statistical models were validated by evaluating the prediction abilities on an external set of lentil samples. LDA model showed the best results with an external prediction ability of 100%, but also the other models showed remarkable performances (above or near 90%). These findings demonstrated the suitability of the methods developed to discriminate geographical origin of lentils and confirmed the applicability of the NMR data, in combination with chemometrics, to solve geographic origin issues of foodstuffs.

Keywords: lentils, geographical origin, NMR fingerprinting, multivariate statistical analysis

P56 NEAR-INFRARED REFLECTANCE (NIR) SPECTROSCOPY AS A SCREENING TOOL FOR RAPID CHARACTERIZATION OF TRANSGENIC AND NON-TRANSGENIC MAIZE CROPS

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Depending on the intended result transgenic plants may, for instance, be intended for human and animal consumption. The EU has established a legal framework to ensure that the development of modern biotechnology, and more specifically of GMOs, takes place under safety conditions, ensuring a clear labeling of GMOs placed on the market, and enabling consumers or professionals to make an informed choice. Many techniques have been proposed for determination, characterization and authentication of GMOs; however, DNA methods for identification of transgenic products have more confidence and reliability than other methods. These techniques are destructive, tedious, expensive and not suitable for real-time control measures. Near-infrared spectroscopy (NIRS) meets all the requirements for up-to date quality control systems in agro-food products: it combines a high-speed response with a low per-sample cost; requires little or no sample preparation; which has been introduced poorly as a nondestructive method to identify GMOs. Our goal is to test NIRS methodology as a discriminating alternative to PCR for GMO detection in crops. A total of 88 non-transgenic maize samples from different commercial seed brands and local Asturian maize populations and one transgenic maize sample were employed in this study. Two PCR protocols were designed for GMO detection in maize. Samples from Bt11, NK603, Bt176, MON810, Soya Bean RR and a sample from a local Asturian maize population, were used as PCR controls of GM-target and taxon-target presence. For NIRS analysis a Foss NIRSystems 6500 scanning monochromator (Silver Spring, MD) equipped with a transport module was used to measure the reflectance spectra from 400 to 2,500 nm, at every 2 nm employing the rectangular cell that allows 94 cm² of the sample surface area to be irradiated. The maize samples were PCR analyzed for the presence of GM targets. Only PCR positive results from taxon-presence were taken into account. One sample was found to be a GM maize. Samples GM free, were NIRS analyzed with a view to detecting spectral outliers seeing the structure and spectral variability of the full population; the CENTER algorithm included in the WinISI II software package was used for this purpose. This algorithm performs a Principal Component Analysis and then determines the center of the spectral population and calculates the standardized Mahalanobis distance (GH) of each sample from that center. For agroindustrial products, samples for which GH>3 are generally considered spectral outliers. The GH average value of GM free population was 0.8122. The NIRS spectrum of transgenic maize sample was included to perform PCA analysis again and it showed a considerable variability and a great distance from the population center, GH value of 5.939. We believe that NIR spectroscopy can be used as an additional screening method, in the early selection to classified maize samples as transgenic.

Keywords: near infrared spectroscopy, PCR, transgenic and non-transgenic maize, discrimination

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SCIENTIFIC FACTORS RELATED TO CONSUMERS HEALTH AS NEW TOOLS FOR CONFIRMATION OF AUTHENTICITY OF CYPRIOT/ROMANIAN WINES**Rebecca Kokkinofta^{1*}, Despo Christodoulou², Naso Economidou³, Eleni Tzioni⁴, Maria Constantinou⁵, Yiota Hadjiloizou⁶, Katerina Damianou⁷, Panayiotis Constantinou⁸**^{1, 2, 3, 4, 5, 6, 7, 8} State General Laboratory, Nicosia, Cyprus

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The production of winemaking in Cyprus and in Romania dates back to ancient times and had an important role in the everyday life, in both countries. The aim of the study was to characterize the special identity of Cypriot and Romanian wines, especially the factors related to consumers health, as markers in order to study their authenticity. The study included a total of 66 authentic and commercial wines from the two countries and 10 wines with different origin. The approach was holistic by using advanced technology such as SNIF-NMR, IR-MS, LC-MS/MS, ICP-MS and an integrated isotopic, elemental, phenolic and anti-scavenging profile was established, related to their load in pesticide residues. We present here the results of the measurements made at the General State Laboratory of Cyprus. The stable isotope data (D/H, $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$) after statistical evaluation by the use of different chemometric techniques, showed as more useful discriminators the dependency of the deuterium/hydrogen ration of the methylene site in the ethanol molecule (D/H) and also the $\delta^{18}\text{O}$ values of the wine water, able to distinguish the samples according to their origin. The elemental content and the load in heavy metals is able to distinguish mainly the botanical origin, but not the geographical origin of the wines. An accredited multi residue method with ethyl acetate has been used for the determination of approximately 170 pesticides in wine, using liquid chromatography tandem mass spectroscopy (LC-MS/MS). Within the framework of this project the method has been modified in order to introduce the analytical system GC-MS/MS. Validation studies for the GC-MS/MS analysis have been carried out for up to 79 pesticides at two levels 0.05 mg/kg and 0.1 mg/kg for both red and white wines. Within the project thirty one (31) samples of Cypriot wines and twenty eight (28) Romanian wines have been analysed. The 71% of the Cypriot wines and 86% the Romanian wines were found to be positive. All results were below the MRL's according to the European Regulation 396/2005. The 50% of the samples contained more than one pesticide. The pesticides found in the Cypriot wines were Carbendazim, Triadimenol, Spinosad, Metalaxyl, Fenhexamid and Pyrimethanil whereas in Romanian wines were Carbendazim, Iprodi one, Dimethomorph, Pyrimethanil and Metalaxyl. The above holistic methodology can be used to investigate the authenticity of commercial wines when they are compared to the authentic samples from the same region.

Keywords: wines, authenticity, stable isotopes, pesticides, chemometrics**Acknowledgement:** The work is co-funded by the European Regional Development Fund and the Republic of Cyprus through the Research Promotion Foundation (Project: Bilateral/Cy-Ro/071). We thank Chara Savvidou, Charalambos Louca, Olympiada Kourouzidou, Eleni Kyprianidou and Charalambos Ioannou for excellent laboratory work and Dr. Popi Kanari for useful discussions.

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**STRONTIUM ISOTOPIC RATIO IN AGRICULTURAL PRODUCTS:
RESEARCH GAPS AND FUTURE INVESTIGATIONS FOR ITS USE IN
GEOGRAPHICAL TRACEABILITY****Agnese Aguzzoni^{1*}, Francesco Comiti², Tanja Mimmo³, Peter Robatscher⁴,
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A geographical indication (GI) is a widely known mark that relates a product to its specific district of origin and production process, attesting an intrinsic quality and authenticity. Furthermore, it is a useful tool to promote and protect rural economy. Therefore, GI is an added value that has acquired the status of guarantee not only for producers but also for consumers. However, due to its large use, GI is exposed to misuse and counterfeiting, thus several efforts have been made to detect and fight this fraud in the last decades. Among the analytical tools available, the use of the strontium isotope ratio ($^{87}\text{Sr}/^{86}\text{Sr}$) is gaining increasing importance in the field of geographical traceability, since it is related to the geological features of the district in which agricultural products are grown. Moreover, several studies reinforced this connection proving that strontium keeps unaltered its original isotopic fingerprint up to the end product, even after processing, with no isotopic fractionation. One of the most important processes through which this element is transferred in the soil-plant system is root uptake. Strontium shows some common chemical features with calcium (Ca) since they are both alkaline earth elements. For this reason, they are absorbed together by organisms, even if from a biological point of view calcium is required as an essential mineral with physiological role while strontium is not. Several investigations conducted so far have examined the potential of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio, for instance comparing the fruit with its parent material or agrifood from different areas. Nevertheless, knowledge gaps are still present and until now few works have investigated the influence of the strontium concentration and isotopic composition of irrigation water and fertilizers used in agriculture on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of end products. The aim of the current contribution is to focus the attention on the strontium uptake in fruit trees, particularly to identify the major source from which this element is absorbed. As a case study, apple trees from South Tyrol were selected, considering that apple is one of the most valuable products for the regional economy with a production area of 19000 ha in 2014. Moreover, apples from South Tyrol have been certified with the “protected geographical indication” (PGI) sign since 2005. This work serves as a background for a PhD project focused on the isotopic characterization of agricultural food products. The comprehension of the process of strontium uptake is fundamental to acquire a better knowledge of the absorption of strontium in the soil-plant system, and will allow further investigations, in addition to strengthening the use of $^{87}\text{Sr}/^{86}\text{Sr}$ ratio as a geographical tracer.

Keywords: strontium, geographical tracer, apple tree, protected geographical indication (pgi), root uptake**Acknowledgement:** The Autonomous Province of Bolzano, Department of Innovation, Research and University (Decision n. 1472, 07.10.2013) is gratefully acknowledged for financial support and Eco-research srl for collaboration.

P59 FOOD HEMP PRODUCTS: A WAY OF SMUGGLING CANNABIS OR NOT?

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Hemp and Marijuana both come from the same plant – *Cannabis Sativa* L. The term 'Hemp' commonly refers to the industrial/commercial use of the cannabis stalk and seed for textiles, foods, papers, body care products, detergents, plastics and building materials. The term 'marijuana' refers to the medicinal, recreational or spiritual use involving the smoking of cannabis flowers. The flowers of the plant and to a lesser extent the leaves, stems, and seeds, contain Δ^9 THC (Tetrahydrocannabinol) which is the main psychoactive component of the Cannabis plant. According to EU regulation 1122/2009 the maximum permissible content for industrial hemp is 0.2% THC while for marijuana the THC content is much higher than 0.2%. The sale and consumption of hemp foods is permitted and seems to be increasing, but there is no harmonized legislation at an EU level. THC limits are not the same for all EU countries. THC content in food is generally controlled via using low hemp cultivars, with some countries also setting their THC limits in foods. Recently the SGL in Cyprus came across a case of an import of alcoholic drink (vodka) named after cannabis, containing a whole leaf, as well as other parts of the cannabis plant. Some of these parts also contained flowering tops. To investigate the case and clarify whether this was a Hemp product of smuggling of cannabis the Laboratory proceeded in finding the % of THC content of the alcoholic liquid and separately the THC content of the plant material. Results showed a great variance in % of THC content of the plant material from bottle to bottle, while their batch number was the same. This indicated inhomogeneity of the batch. Variance was also shown with the THC content of the plant material where in a number of plant material the content was above 0.2% and in some below this level. THC content of Alcoholic liquid was less than 0.005 mg/L. In the absence of an EU legislation on hemp food products, interpretation of results concerning the % of THC content of the sample, raises serious legal issues. The alcoholic drink has a very low % of THC content which is acceptable as food hemp product. The fact that this product had also a plant material which was, in a number of bottles of the same batch, considered as cannabis, an illegal drug, made the case even more legally complex.

Keywords: hemp, cannabis, vodka, THC

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METABOLOMIC FINGERPRINTING AS A TOOL FOR WHEAT AUTHENTICATION

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The authentication of agricultural commodities and products thereof is a relevant topic of food science. Development of new modern approaches that could improve the expensive and time consuming methodologies is highly desired. One of the modern approach used for food authentication and fraud revealing is metabolomics, which is focused on target or non-target analysis of small molecules (<1500 Da). Two complementary approaches are usually used here: metabolic fingerprinting and metabolite profiling. While the metabolomic profiling focuses on the analysis of selected group of metabolites, the fingerprinting methods assess the whole chemical pattern of the sample by using the advanced pattern recognition mathematical techniques. The genetic background of agricultural commodities and various environmental or other external influences affect the fingerprint of food matrices dramatically. Within this study Direct Analysis in Real Time (DART) ion source coupled to high resolution Orbitrap Mass Spectrometer (DART–OrbitrapMS) was applied on a wide set of wheat (*Triticum durum*) samples grown in two different cropping systems, conventional and organic, in year 2012 and 2013 (altogether 90 samples from Italy and 8 French test samples were analysed). Acquired data were treated using a multivariate analysis: Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). Sample groups clustering was evident in both obtained score plots. For example, the PCA of metabolomics fingerprints revealed clear differences between samples from Basilicata, Tuscany, Molise and Emilia-Romagna regions (Italy) as well as a difference between years of harvest. French samples were completely separated from other samples. Also partial differences between types of cultivation were observed from PLS-DA. However, the year of production had a more significant impact on the measured metabolomic fingerprints in comparison with the farming system. Metabolomics fingerprinting realized by DART-OrbitrapMS is very useful tool for authentication of raw materials.

Keywords: authenticity, DART-MS, metabolomic fingerprinting, durum wheat

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MISLABELING IN ONLINE MARKET IN CHINA: SUBSTITUTION OF SABLEFISH (*ANOPLPOMA FIMBRIA*) WITH PATAGONIAN AND ANTARCTIC TOOTHFISH (*DISSOSTICHUS ELEGINOIDES* AND *D. MAWSONI*) REVEALED BY DNA BARCODING

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China's rapid economic development has determined profound changes in seafood consumption patterns, and nowadays besides the traditional luxury seafood, also high-quality marine fish (such as salmon, cod and tuna) are consumed. Among these is *Anoplopoma fimbria* (Sablefish), a commercially important ground fish distributed in the North Pacific reaching very high prices on the Chinese market. A recent molecular survey on products sold online in China found that all the analyzed products sold as Yin Xue, one of the terms used to indicate *A. fimbria*, were in fact *Dissostichus* spp., a genus of Antarctic ground fish extremely vulnerable to overfishing (Xiong et al. 2016, Food Control, 60, 519-532). Considering this and due to the lack of a standardized naming system for seafood species in China, an initial search was conducted to identify all the possible Chinese names used to indicate *A. fimbria*. Then, the DNA barcoding of a ~655bp region of the mitochondrial cytochrome c-oxidase I (COI) gene was employed to verify the identity of 42 products sold on e-commerce platforms under these names. In addition, the information reported on the webpage and on the label was analyzed in the light of the Chinese regulation in force. The aim of the present study was to assess the challenges of the online market with regards to frauds for fish species substitution. In particular, on the basis of an accurate analysis of all the collected data, we speculated the causes of mislabeling and we discussed the need for the enforcement of a traceability system in China, able to increase the trade transparency and close the markets to products deriving from Illegal Unreported and Unregulated (IUU) fishing, often affected by overexploitation. All the PCR products gave readable sequences. By using the IDs analysis on BOLD and the BLAST analysis on GenBank all the samples were unambiguously identified at the species level. Of the 42 products sold as Sablefish, only 6 (14.3%) were molecularly identified as this species, while 32 (76.2%) were identified as *Dissostichus eleginoides* (Patagonian Toothfish) and 4 (9.5%) as *D. mawsoni* (Antarctic Toothfish), highlighting an alarming overall misrepresentation rate of 85.7% and implications for the management of these species' fisheries. In fact, the identification of mislabeled Patagonian and Antarctic Toothfish raises for the first time the hypothesis of China being the final market for these species through substitution of Sablefish, reaching higher prices in this market. The combined analysis of all the information collected from the webpages and the labels (denominations, producers, origin) allowed us to hypothesize both unintentional and intentional mislabeling. In particular, our findings suggest the possible existence of a trade pattern enabling IUU fishing operators to launder illegal catches of Patagonian and Antarctic toothfish through mislabeling, while maintaining sale prices and therefore, maximizing profits.

Keywords: sablefish, toothfish, DNA barcoding, Chinese E-commerce, seafood frauds

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CHEMICAL PROFILING OF WHISKIES USING ORBITRAP GC-MS

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Whisky is a premium distilled spirit beverage that is produced using long established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, has high commercial value, and can be economically important in the regions of the world where it is produced and consumed. As such, it is essential that whisky producers are able to obtain an accurate and comprehensive chemical profile which is characteristic to their individual product. This information can then be used to identify adulterated or counterfeit products and enable action to be taken, with confidence, to protect the product and brand. Gas chromatography coupled to mass spectrometry represents one of a number of different analytical approaches that can be applied to meet this objective. It is important to consider both the sample preparation protocol employed and the parameters used in the instrumental analysis. In this study, we used GC-Orbitrap mass spectrometry for profiling components contained in ethyl acetate extracts prepared from several whiskies differing in origin and age. The key objective of this 'proof of concept' study was to analyse samples from different geographical regions and ages and to demonstrate the potential of the Thermo Scientific Q Exactive GC system to provide comprehensive information on the occurrence of both low and high intensity components, which is needed for sample classification. Non-target full range high resolution mass data was acquired and then evaluated using accurate mass deconvolution and spectral matching along with sophisticated statistical tools to detect any chemical differences between the samples. The measurements were performed in EI full scan (m/z 50–600) at a mass resolving power of 60,000 (FWHM, m/z 200). In addition to the experiments above, each sample was analysed singly using positive chemical ionisation to obtain molecular ion information to further support the proposed identity of unknown component peaks. The data was deconvoluted to extract components present at low and high concentrations and to provide clean spectra. The cleaned spectra were then searched against nominal mass spectral libraries and further filtered based on accurate mass matching of the proposed hits. The sub 1 ppm mass accuracy, across peaks of different intensity, enabled proposed compound identities to be quickly confirmed or eliminated. The combination of EI and PCI spectra provided information for the identification of unknown peaks in the chromatogram. The complete chemical characterisation of both low and high intensity components provided a comprehensive profile of the whisky samples. Further details will be presented in the poster.

Keywords: chemical profiling, whisky, Orbitrap mass spectrometry

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MUSKY AND CURLED OCTOPUS: ARE THEY FRESH OR FROZEN-THAWED? CHANGES IN PROTEOMIC PROFILE COULD HELP US TO FIND THE TRUTH

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In recent years food security and the fight against food fraud have become a top priority for public health and the economy. Providing information about the preserving method of a fish product is of enormous importance for the perception of its quality by the final consumer. The substitution and sale of frozen-thawed fish labelled as fresh is a widespread and difficult to unmask commercial fraud, and a potential danger for the consumer's health. Histology is a valid method to differentiate fresh from frozen-thawed fish, but not for all species, in particular for cephalopods. Proteomics could help to identify markers for rapid screening of samples to find fraud, as we did on tissue samples of musky and curled octopus (*Eledone moschata* and *Eledone cirrhosa*) with mono- and two-dimensional electrophoresis (1DE and 2DE). Samples of 10 *E. moschata* and 13 *E. cirrhosa*, certainly fresh, provided by official veterinarians, were divided into two aliquots, the first aliquot was immediately processed to extract the proteins, while the second aliquot was frozen at -20 °C for 72 hours and subsequently thawed and analyzed. Different approaches have been used to improve our method, including two different extractive protocols and two different staining (Blue Silver and Oriole fluorescent stain®). Protein maps were acquired and analysed with BioNumerics® 2D Gel Types software, to detect qualitative and quantitative differences between the samples. The 1DE analysis showed a clear change in the protein banding patterns of *E. moschata* and *E. cirrhosa* samples, allowing us to easily differentiate the samples belonging to these species. No protein differences have been evidenced between fresh and frozen-thawed samples and so the 1DE analysis seems to be not useful for this purpose. The 2DE comparative analysis conducted on *E. cirrhosa* samples, stained with Blue silver, showed in 11/13 samples a significant reduction of two spots with a molecular weight of about 45–50 kDa and an isoelectrical point of about 6,5-7. *E. moschata* samples showed a different protein map profile and these two spots are not present in this species, either fresh or frozen-thawed, while other differences are under evaluation on more replicates to eventually confirm data. The use of an extractive method with an additional protein concentration step and the fluorescent staining increase, in both species, the number of spots revealed and further analysis are ongoing to search other potential biomarkers linked with the two different stages of preservation. The confirmation of the two spots of *E. cirrhosa* as biomarkers associated also to other proteins will open the way for future studies to develop a rapid and accurate test, providing a useful tool for producers and official authorities.

Keywords: two dimensional electrophoresis, fresh, frozen-thawed, musky octopus, curled octopus

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ESTIMATION OF THE AUTHENTICITY OF DIFFERENT TYPES OF SERBIAN BRANDY APPLYING CHEMOMETRIC TOOLS

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Serbian fruit brandy is the national, traditional alcoholic drink, which brings authenticity and distinctive feature of the region from which it originates. The quality and quantity of produced fruit brandies, Serbia occupies a leading place in the world, a brandy becomes the subject of forgery due to increasing success in the global market. Fraud in the production of fruit brandies is based on the use of sugar non- fruit origin (made of sugar beet or cane sugar) during fermentation in order to increase the yield of ethanol. Also, one of the ways to forge brandy is the use of fruit that does not lead to the geographical origin of the area in which the production takes place. In order to successfully detect counterfeits in the production of fruit brandies in Serbia in the past several years, used the method of determining the stable isotopes $\delta^{13}\text{C}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$. The instrument for stable isotope analysis consists of the Elemental Analyzer (FlashEA 1112 HT) and Isotope Ratio Mass Spectrometer (ThermoFinnigan DELTA V Advantage). In SP Laboratorija, we have made control samples as laboratory brandies from different kind of fruits, such as plum, pear, apple, white and black grape, from different geographical origin (South and North part of Serbia) and brandies from sugar cane and corn. At the same time, we have prepared laboratory samples of fruit brandies with different amount of added beet sugar (5%, 10%, 20% and 50% sugar was added on the weight of fruit). The values for $\delta^{13}\text{C}$ in ethanol (from -23 to -30) have confirmed the use of only C3 plants in the production of alcoholic beverages and give important information about botanical origin of ethanol in alcoholic beverages. The combination value of the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ ratios enabled us to designate the geographical origins of alcohol derived from the same kind of fruit. The application of chemometric methods for processing the results it is possible to verify the authenticity of different types of fruit brandies. Linear regression analysis was used to investigate the correlation between the amount of sugar and value of the stable isotope of hydrogen, and for the formulation of a mathematical model that describes the dependence. The value of percentage of added sugar was transformed in the negative logarithm of the molality (-log B) of added sugar to obtain a linear relationship between the variables. For the $\delta^2\text{H}$ values which are quite low, the use of the described equation will show a high content of added sugar derived from sugar beets. By applying this equation, it is possible to calculate the amount of added sugar in the unknown sample. The obtained results allow database establishment and classification of commercial fruit brandies based on botanical and geographical origin.

Keywords: botanical origin, geographical origin, isotope ratio mass spectrometer, chemometric method

P65 QUALITY CONTROL OF FRUIT JUICES

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Fruit juice is the product obtained by mechanical processing of one or more types of fruit. Another process of production is from concentrated fruit juice. This process is achieved by adding adequate amount of water to concentrated fruit juice. Checking the quality and authenticity of fruit juices in SP laboratory accredited is in accordance with ISO/IEC 17025 is based on the check parameters defined in national regulations. To determine whether fruit juice correspond to the terms of hygienic safety checked ethanol, volatile acid, D / L lactic acid and hydroxymethylfurfural (HMF). Special requirements for quality are taken from the Code of Practice of the European association of manufacturers of fruit juices (A.I.J.N- European Fruit Juice Association). These requirements apply to determine the authenticity, by checking the relative density, dry matter (^oBx), L-malic acid, potassium, total phosphorus, formol number, D-isocitric acid and sugar-free extract. Continuous quality control of hundreds samples of fruit juices, mixed fruit juices and concentrates, was carried out during the year 2015. The results showed that 4% of the samples did not meet the requirements in terms of authenticity, while in 20% of non-compliant samples showed a deviation of three parameters. Lower content of malic acid, which is an indicator of overripe fruits, occurred in 39% of the total number of parameters that were not in line with reference values. It was also observed that grape juice concentrates and grape or apple juices in combination with other fruits juices in most cases did not meet the specific requirements for quality. The reasons for the occurrence of non conforming product may be dilution of juice, replacement of some fruit by another undeclared kind of fruit, non compliance with product declaration or inadequate quality of starting raw material. Checking the quality and authenticity is very important both for the consumer and for the producers themselves. The results of the analysis can show flaws in the production and be a warning to producers to improve the quality of their products.

Keywords: fruit juices, quality, authenticity

P66 BIOCHEMICAL AND CHROMATOGRAPHIC FINGERPRINTING OF HERBAL FOOD SUPPLEMENTS

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The current authentication of herbal food supplements is being done by high performance biochemical and chromatographic fingerprinting that allows the identification of metabolic markers for the authentication of plant active compounds (WHO, 2007). Based on the data obtained from the instrumental and biochemical fingerprinting of *Hypericum perforatum* L., an experimental program has been designed in order to test some intermediate products and final products – plant food supplements. Herbal supplements based on *H. perforatum* L., as single ingredient or in complex mixtures with other medicinal plant species have been selected and tests correlated with extensive phytochemical characterization of the plant species *H. perforatum* L. have been performed. The steps of the experimental study were as following: (1) preparation of solutions from raw materials, intermediate and the final products (tablets); (2) qualitative analysis by High Performance Thin Layer Chromatography (HPTLC) – for the identification of selected markers; (3) quantitative analysis of phytochemicals compounds – selected as markers. The chromatographic analysis of the results was done by comparative evaluation and correlation of the content through the marker compounds in raw materials, intermediate and final product whose composition is found. The dosage methods used were either those described in the European Pharmacopoeia, Romanian Pharmacopoeia or the ones developed and validated (in house) in the company's laboratory. If for the final products, conditioned into a solid form (as tablets), identification of plant species with the obtaining of chromatographic and biochemical fingerprints can be done, for the complex plant mixtures (with many plant species as powder/in fluid extract), a deeper and detailed qualitative analysis is necessary as well as the use of other analytical methods. Based on the results achieved, "an extensive phytochemical and chromatographic characterization diagram" was obtained which takes into account the correct identification of medicinal plant species and derived complex herbal food supplements.

Keywords: biochemical fingerprinting, herbal food supplements, High Performance Thin Layer Chromatography (HPTLC), *Hypericum perforatum* L.

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DNA BARCODING OF MEDICINAL PLANT SPECIES FOR THE MOLECULAR AUTHENTICATION OF COMPLEX HERBAL FOOD SUPPLEMENTS

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The market of herbal food supplements is highly competitive since sale and consumption is experiencing constant growth. The incentive for contamination, fraudulent market substitution and the use of unlabeled fillers has increased. As such, these products are frequently mislabeled, adulterated, and sometimes even do not contain the claimed active ingredients, as species of a lower market value are substituted for those of a higher value (Newmaster et al., 2013). This practice is a direct threat to consumer safety and comes at a time when consumers are becoming increasingly concerned about the authenticity of the products they purchase. Eroding consumer confidence is driving the demand for research and market testing through reliable, but cost-effective, methods of authentication. Both the US FDA and European EMA support the use of innovative methods but have not issued specific instructions on the use of DNA barcoding for this purpose. Recent studies in the field of DNA barcoding of herbal supplements have highlighted widespread species substitution, adulteration and the use of unlabeled fillers (de Boer et al., 2015). Our collaborative research project (PhytoAuthent) is designed to address, investigate and evaluate the safety concerns posed to consumers by herbal food supplements containing one or more plant species. We are testing, developing and applying innovative molecular analysis methodologies for plant identification in herbal products, in real life case scenarios. We focus on five plant cases (or complexes) in which substitution has varying significance: (1) replacement of *Hypericum perforatum* by other *Hypericum* species; (2) replacement of *Veronica officinalis* by other *Veronica* species, which in both these two cases are likely to yield a less effective product; (3) replacement of the threatened and protected *Gentiana lutea* by the poisonous *Veratrum album*, a substitution with potentially lethal effects; (4) exchange substitution of some Echinacea species depending on availability and price, which could lead to mislabeled products; (5) replacement of *Dactylorhiza orchids*, that are both CITES-listed and protected in most countries, by synthetic congeners, a case of fraudulent substitution. Using DNA barcoding, but with emphasis on second-generation high throughput sequencing (HTS), we aim to (1) develop DNA barcoding systems for identification of species and substitutes in the selected plant cases; (2) develop DNA metabarcoding methods using Ion-Torrent platform to identify and monitor plant ingredients in processed herbal food supplements and medicines; and (3) evaluate the time, cost and accuracy of different authentication methods, in order to identify and promote the overall most effective method. Starting from these five plant cases, we intend to develop high-throughput DNA barcoding for species-level identification and authentication of complex herbal food supplements.

Keywords: DNA barcoding, molecular authentication, medicinal plant species, herbal food supplements, safety

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P68 DEVELOPMENT OF A REAL-TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR RAPID DETECTION OF PORK

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Meat adulteration and mislabeling of processed meat products were concerned by consumers. Especially, the authenticity of pork species in commercial meat product is crucial for economic, religious and health perspectives. Rapid and accurate detection methods to identify pork in meat products should be developed. In this study, a loop-mediated isothermal amplification (LAMP) technique was used to develop on-site detection method using a portable real-time fluorometer without the need for DNA extraction. Pork-specific and eukaryotic primer sets were designed based on the mitochondrial D-loop regions and the nuclear 18S rRNA genes respectively. A universal 18S rRNA primer set was used as an endogenous control in the PCR reaction. The pork-specific primers amplified without any cross-reactivity with non-target animal species. The LOD was 1 pg for pork and 0.1 % of pork in binary mixtures with beef, lamb and chicken. This assay was applied to 42 processed meat products to verify for labelling compliance. All meat products were successfully identified pork by this assay within 30 min without the DNA isolation. Thus, the developed method can be served as a useful on-site tool for discrimination of food adulteration.

Keywords: real-time loop-mediated isothermal amplification (LAMP), pork, authentication, mitochondrial DNA, 18S rRNA

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RAPID SCREENING FOR OIL AUTHENTICITY USING IR SPECTROSCOPY FOLLOWED BY TRIGLYCERIDE ANALYSIS

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Edible oils and fats play fundamental roles in our nutritional habits and health. High quality edible oils such as extra virgin olive oils and argan oils are expensive and thus prone to adulterations. Sophisticated methods for the authenticity analysis are used in specialized laboratories, including spectroscopy, liquid- and gas-phase chromatography, often hyphenated with mass spectrometry. Triglycerides, fatty acid, waxes and sterols are analyzed for such purposes. Nevertheless, fast and simple screening methods are needed for the rapid analysis of large number of samples at low cost. We present such a screening method based on medium infrared spectroscopy (ATR FT-MIR), high pressure liquid chromatography with evaporative light scattering detection (HPLC-ELSD), and multivariate analysis. A set of 87 edible oils from eight botanical species (olive, argan, nut, hazelnut, sunflower, canola, peanut and pumpkin seeds) was collected and analyzed by ATR FT-MIR and HPLC-ELSD. A drop of oil was directly deposited on an ATR-FT-MIR spectrometer. Spectral data was acquired from 4000 to 400 cm^{-1} . Before liquid chromatography, the sample was diluted in a 65% acetonitrile (AcN) solution prior to injection. Triglyceride separation was performed on an a reverse-phase column in gradient mode, using a 65% AcN (solvent A) and 50% 1-propanol 50% 2-propanol solution (solvent B). Additional oils (n=45) and mixtures thereof were analyzed by ATR FT-MIR for cross-validation. Multivariate analysis was performed using the software SIMCA from Umetrics. The spectroscopic and chromatographic data acquired on the 87 oils were both analyzed using principle component analysis (PCA) and orthogonal partial least squares (OPLS). With ATR FT-MIR, all eight botanical origins were clearly separated. A peak table with 42 major compounds was constructed from the HPLC data. PCA and OPLS permitted a clear separation of all oils. An additional set of 45 olive oils analyzed by ATR FT-MIR was correctly classified. Preliminary data also suggest that adulterations of olive oils with 20% of sunflower or canola oils can be detected from the ATR FT-MIR data. Interestingly, flavored oils (olive oils with basil, rosemary, chili peppers, etc.) were also correctly classified with our model. Our data shows that a rapid spectroscopy screening can be used as a first pass method on large number of oil samples to assess their authenticity. This first pass allows selecting possible cases of adulterations or mislabels. The selected samples can then be analyzed by HPLC-ELSD for confirmation, which involves also minimal sample preparation and handling. The combined workflow represents a fast and simple tool for oil authenticity screening of large numbers of samples.

Keywords: oil authenticity, triglycerides, ATR FT-MIR, HPLC-ELSD, multivariate analysis

P70 SIMULTANEOUS IDENTIFICATION OF LAMB, BEEF, AND DUCK IN MEAT MIXTURES USING MULTIPLEX-PCR ASSAY

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Beef and lamb are two of the most highly consumed meats, and food fraud incidents related to beef and lamb gained public attention regarding both raw and processed meat product types. In those incidents, duck meat was mixed into lamb or beef in most cases due to its low price and ease of accessibility. In this study, a multiplex PCR technique was applied for the simultaneous identification of duck, beef and lamb meats in meat mixtures. To simulate food adulteration, lamb and duck meat were mixed into beef and beef and duck meat were mixed into lamb. Species-specific primer sets were designed from the cytochrome b gene of mitochondrial DNA. A universal 18S rRNA primer set was also applied as a positive control in the PCR reaction. The sizes of the PCR products for eukaryotes, lamb, beef and duck were 99, 133, 166 and 204 bp, respectively. All primer sets showed specificity in the PCR using template DNAs from 16 animal species. The sensitivity for both single and multiplex PCR was 5 pg. The detection limit for target species in raw and heat-treated meat mixtures was 1%. This multiplex PCR method successfully discriminated raw and cooked lamb, beef and duck meat samples.

Keywords: multiplex polymerase chain reaction, simultaneous identification, lamb, beef, and duck, food authentication

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THE OLIVE OIL SUPPLY CHAIN UNDER THE MAGNIFYING GLASS: THE MULTI-DISCIPLINARY FRAUD VULNERABILITY ASSESSMENT APPROACH

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Recent food fraud scandals around the world have further increased the need to strengthen companies' ability to combat fraud within their own organizations and across their supply chain. However, current food safety management systems (FSMS) are not specifically designed for fraud detection or mitigation. A first step to include measures in FSMS and to carry out adequate fraud vulnerability assessments is the identification of relevant risk factors. This requires a multiple perspectives approach. Although product-related, technical factors and economic drivers are usually considered, eventually the behavioural side is often overlooked. We have identified key food fraud risk factors based on the principles of the criminological routine activities theory (Felson & Boba, 2010) which takes into account a suitable target (opportunities), a motivated offender (motivations) and (lack of) guardianship (controls). Olive oil is susceptible to fraud, as we all know cases are resurfacing frequently. Therefore, we selected the extra virgin olive oil supply chain to examine the vulnerability of companies along the chain. To assess the fraud vulnerability and establish generic vulnerability profiles, the science-based fraud vulnerability self-assessment tool that was developed previously (<http://www.pwc.com/foodfraud>) was applied. This tool assesses the fraud vulnerability of companies and is considering opportunities, motivations and current control measures at different environmental layers (own company, supplier, customers, supply chain, wider environment, etc.). Companies at different stages of the supply chain (manufacturing/packaging/wholesale/retails) were invited and interviewed. The results show that different risk factors determine the fraud vulnerability of companies at different stages of the supply chain. The earlier the stage companies are engaged in, the more opportunities they have to commit fraud. Motivation related risk factors and control measures were showing less consistent patterns along the supply chain. These two elements were proven to vary from company to company, and from country to country. The fraud vulnerability assessment data resulted in recommendations to lower fraud vulnerability to the interviewed companies, and the overall sector.

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Keywords: fraud risk, olive oil, vulnerability assessment

P72**NON-TARGETED METABOLOMIC PROFILING ANALYSIS BY HR-Q-TOF MS ANALYSIS FOR FOOD AUTHENTICITY DETECTION****Jens Luetjohann^{1*}, Anna Bauer², Eckard Jantzen³, Juergen Kuballa⁴**^{1,2,3,4} GALAB Laboratories GmbH, Hamburg, Germany

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Food quality and traceability are issues of wide relevance to customer protection and manufacturers. The quality of food products is often linked to their origin as, for example, French wine or Spanish ham, which have a substantial influence on the market price of the product. Another factor causing differences in the intrinsic value of food is its way of production e.g. in an organic or conventional way. Thus, to guarantee the traceability of these items becomes essential, not only for customers but also for manufacturers and retailers. To detect the fraud in the manufacturing chain specific and robust analytical methodologies are needed. Stable isotope analysis (HR-IR-MS) is the classical method for authenticity detection evaluating differences in isotope ratios of the main elements. In dependency of the geographic region and the practice of cultivation there are differences in the isotope ratios of like elements H, C, N and O giving proof of the authenticity of the origin and way of cultivation. Unfortunately, this methodology is limited regarding specificity and robustness and may be affected by artificial irrigation and fertilizers. The aim of the project, funded by the German Federal Ministry of Food and Agriculture (BMEL), is the establishing of a methodology for authenticity testing by metabolomic profiling by UPLC–HR–Q–ToF MS. Plant-derived commodities of different geographical origin grown under organic and conventional conditions are sampled. Generic sample extracts are generated and measured by UPLC–HR–Q–ToF MS in MSE mode with different polarity (ESI- and +). Acquired data sets of each subgroup are subjected to a non-targeted metabolomic profiling approach using chemometric multivariate data analysis strategies like PCA and PLS-DA for evaluation of the respective discriminative models to characterize the data. The validation approach is carried out using wider samples sets and samples originating from at least two different crop years. The individual models represent a valuable tool to verify the authenticity of crops from different regional origin, varieties and from organic and conventional production. A database covering different varieties and farming conditions is a basic principal to verify the authenticity. Additionally selective marker compounds are identified by libraries and public database search and finally confirmed by reference standard compounds.

Keywords: foodomics, metabolomic profiling analysis, multivariate data analysis, food authenticity, HR-Q-ToF MS

P73**PREPARATION AND FUNCTIONAL CHARACTERIZATION OF FISH BONE GELATIN AND COMPARISON WITH COMMERCIAL GELATIN****Venous Sanaei Ardekani^{1*}, Abdul Salam Babji²**

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In the present study, gelatin extraction from the bone of freshwater catfish (*Clarias gariepinus*) was conducted and determined the functional properties of gelatin and compared with bovine gelatin (commercial gelatin). Result showed that the catfish bone gelatin had high content of protein of 82.10%. Catfish bone gelatin exhibited greater viscosity (4.64 mPa.s) than bovine gelatin (3.17 mPa.s). The viscoelastic properties of bovine gelatin were higher than catfish bone gelatin. Gelling and melting point of bovine gelatin (20.6, 27.5°C) were higher than catfish bone gelatin (17.8, 25.1°C). SDS-PAGE of bone gelatin contained α -chain and β -components. The isoionic points of gelatin extracted of catfish bone (8.71) were higher than bovine gelatin (5.35). FTIR spectra of catfish bone gelatin exhibited major adsorption bands in amide band region. The major absorption bands of catfish bone gelatin were found at 3309–3310 cm^{-1} (amide A), 2926–2929 cm^{-1} (amide B), 1644–1645 cm^{-1} (amide I), 1550–1563 cm^{-1} (amide II) and 1240–1241 cm^{-1} (amide III). The results of this experiment showed the potential of catfish bone as raw material for gelatin production.

Keywords: gelatin, fish bone, functional properties, freshwater fish

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FINDING UNDECLARED ALLERGENS: AN IMMUNOHISTOCHEMICAL APPROACH TO DETECT SOY PROTEINS IN MEAT

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Addition of foreign ingredients to meat must be declared on labels. The addition of vegetal proteins is commonly practised for technological and economic reasons; specifically, soy proteins are extensively used in raw and processed meat, because of their multiple favourable properties. An undeclared addition of soy proteins represents a commercial fraud for adulteration and also a potentially sanitary risk, even serious, for allergic consumers. The aim of the present study was to develop an optimised immunohistochemical method for detection of undeclared soy proteins in meat preparations and products. As reference materials, we used chicken breast samples, respectively injected with 10% soy and tumbled with 1% soy, prepared and provided by the RIKILT Wageningen UR, Netherlands. We prepared positive samples mixing minced bovine meat with soy protein burgers and negative controls, using fresh minced meat. All samples were formalin-fixed, paraffin-embedded, cut into sections of 4±2 µm and tested. Citrate buffer (pH 6) antigen retrieval performed for 20 minutes at 37°C and at 97°C was tested, as well as a protocol without antigen retrieval. As blocking agents we tested BSA (bovine serum albumin) 5% and anti-goat serum 5%. A polyclonal antibody anti-soy proteins by Sigma-Aldrich (St. Louis, USA, product number S 2519) was employed; the dilutions tested were 1/50, 1/100, 1/250, 1/500, 1/750, 1/1000, 1/2000. The EnVision System Kit (Dako) was used. As visualization agent DAB (3,3'-diaminobenzidine) was tested for different times, i.e. 2, 3 and 4 minutes. After counterstaining with hematoxylin-eosin, all samples were observed at optical microscopy at 10x, 20x, 40x and subjected to a qualitative evaluation of the presence of soy proteins (positive/negative). Soy proteins were successfully stained. The protocol that proved to be the most effective, providing an intense specific staining, was the following: BSA 5%, 1/1000 dilution of the primary antibody, followed by DAB application for 4 minutes, without antigen retrieval. The method will be further evaluated on a statistically significant number of samples of meat preparations and products of various animal species (e.g. pork and bovine sausages), in comparison with a commercial ELISA assay. In parallel, application of an image analysis software to obtain a quantitative determination of soy proteins will be performed. Considering the need of reliable methods allowing the detection of allergenic ingredients, a functional specific immunohistochemical method could be an evaluable tool in comparison with the more popular ELISA assays.

Keywords: soy proteins, immunohistochemistry, allergens, meat

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P75 FRONT-FACE FLUORESCENCE SPECTROSCOPY: A PROMISING TOOL FOR DISTINGUISHING FRESH FROM FROZEN-THAWED FISHERY PRODUCTS

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The assessment of the potential exposure of consumers to both sanitary and commercial frauds is a key point in the tackling of food crime. According to Regulation (EU) 1276/2011, fish and cephalopods intended for raw consumption are expected to be frozen at -20°C for 24 h or -35°C for 15 h, in order to reclaim parasites and prevent human health risk. Moreover, store distribution chains can be interested into verifying the state of freshness of the food provided by their suppliers, using self-monitoring plans. For these reasons, a valid, cost-effective and high throughput method able to discriminate between fresh and frozen-thawed fish and cephalopods is required. We already showed accuracy and reliability of a histopathological method to differentiate between fresh and frozen/thawed fish, whose application for cephalopods did not succeed. Here the potential of front-face fluorescence spectroscopy (FFFS) was investigated to differentiate fresh from frozen-thawed fish and cephalopods. We randomly collected at a seafood market 30 bonitos (*Sarda sarda*) and 30 common octopuses (*Octopus vulgaris*), caught in FAO fishing area 37. From each fish two samples of muscular tissue were obtained, as well as two samples of tentacle from each cephalopod. 60 samples for each species were frozen for 104.5 hours at -20°C and thawed at +4°C for 16 hours, the other 60 samples were refrigerated. All 120 samples were tested with the spectrofluorometer FluoroMax[®]-4 (HORIBA Jobin Yvon S.A.S., USA). Spectra of samples mounted between two quartz slides were recorded at 20°C. The emission spectra of tryptophan residues (305–450 nm) and nicotinamide adenine dinucleotide NADH (360–600 nm) were recorded with the excitation wavelengths set at 290 and 340 nm, respectively. For each sample, 3 spectra were recorded. With “leave one-out cross-validation”, the same sample is used for both model estimation and testing. Spectra were statistically examined with the method “partial least square discriminant analysis” (PLSDA), that has been applied using the software MATLAB R2013b (The Mathworks Inc., Natic, MA, USA) and the PLS- Toolbox v. 7.5 (Eigenvector Research). Considering tryptophan spectra, correct classification of the investigated samples was obtained for 76.5% of bonitos and 86.8% of octopuses. Considering NADH spectra, correct classification was observed for 85% and 93% of samples, respectively for bonito and octopus species. Our results suggest that FFFS can be considered as a promising tool for the reliable and rapid differentiation between fresh and frozen-thawed fish and cephalopods. FFFS offers multiple attractive advantages, e.g., no preliminary sample preparation is needed prior to measurement and results are obtained in a couple of seconds. Further investigations are required: the next step will be the testing of the performance of the method on other species of fish and cephalopods.

Keywords: front-face fluorescence spectroscopy, fresh, frozen-thawed, fishery products

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ARE YOU SURE THAT YOUR BLACKCURRANTS ARE NOT ARONIA BERRIES?

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Over the last few years, several major food incidents of global importance, such as the melamine crisis, have clearly shown the limits of the current approach used for food analysis. At present routine food analyses are based on targeted methods and are not able to address novel fraud or adulteration issues or to tackle unlisted contaminations. Recently, new developments in analytical methods have raised the possibility of using non-targeted fingerprinting methods to ensure food and feed safety. The development of such non-targeted methods for several food commodities is the goal of the Agrifood GPS (Global Protection System) project, a 5-year project started in January 2012 with the financial support of the BPI (Banque Publique d'Investissement). AgriFood GPS aims to create an early warning system, which will highlight non-compliance or unwanted contamination and help food companies to make rapid decisions regarding the acceptance or rejection of a batch. The analytical methods developed are based on a metabolomics approach, initially developed in the health sector: using statistical tools, each new sample is compared with an existing database to check whether or not it falls within the range of the statistical model. If the sample is not compliant with the model, the method makes it possible to highlight markers responsible for its differentiation from the existing database. In this poster, an example of the application of non-targeted methods is presented with the detection of adulteration of blackcurrant concentrate with less expensive aronia berry concentrate. Using a "dilute and shoot" approach, aronia berry and blackcurrant concentrates were analysed using UPLC–TOF–MS (Ultra High Performance Liquid Chromatography – Time of Flight – Mass Spectrometry). PCA (Principal Components Analysis) and ICA (Independent Components Analysis) analyses were able to differentiate them and to identify polyphenolic markers. The detection level of aronia berry concentrate in blackcurrant concentrate was also determined using the same analytical and statistical methods.

Keywords: UPLC–TOF–MS, non-targeted analysis, chemometrics, fruit juices

P77**COMPARISON OF TWO DISCRIMINATION MODELS FOR THE DETERMINATION OF GEOGRAPHICAL ORIGIN OF CAVIAR****Sophie Guyader¹, Freddy Thomas^{2*}, Eric Jamin³, Michele Lees⁴, Clément Heude⁵, Martial Piotto⁶, Philippe Benoit⁷**^{1, 2, 3, 4} Eurofins, NANTES, France^{5, 6} Bruker Biospin, Wissembourg, France⁷ STURGEON, Saint Sulpice et Sameyrac, France* Corresponding author - E-mail: freddythomas@eurofins.com, Phone: +33251832100

As part of the Agrifood GPS project, untargeted screening methods were developed to authenticate caviar as a tool to protect the PGI (Protected Geographical Indication) "caviar d'Aquitaine". Using an untargeted approach, authentic caviar d'Aquitaine and foreign origin caviar were analyzed using ¹H NMR spectroscopy (Nuclear Magnetic Resonance). Two discrimination models of caviar d'Aquitaine versus other origins were determined using an OPLS-DA (Orthogonal Partial Least Square by Discriminant Analysis) approach with the SIMCA P software and a PLS-DA using the Matlab software.

Keywords: NMR, profiling, chemometrics, fish product

P78**SIMULTANEOUS DETERMINATION OF BIOGENIC AMINES AS INDICATORS OF FRESHNESS RATE IN FISH BY DIRECT SAMPLE ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY****Francesca Martucci^{1*}, Simona Sciuto², Giovanna Esposito³, Pier Luigi Acutis⁴**^{1, 2, 3, 4} Istituto Zooprofilattico Sperimentale PLV, Turin, Italy

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Fish in poor conservation conditions sold as fresh is an important fraud to control, given the possible implication for consumers' health. For the evaluation of fish spoilage, different techniques have been developed so far, as physical, microbiological or chemical methods. The last ones are based on the detection of substances such as biogenic amines, a group of alkaline compounds stable against heat and acid condition. The principal amines are histamine (HIS), trimethylamine (TMA), dimethylamine (DMA), ipoxantine (IPO), putrescine (PUT) cadaverine (CAD), tryptamine (TRY). Determination of biogenic amines in fish is strongly influenced by the selection of an appropriate extraction solvent. Many procedures are described in literature mainly involving extraction by organic solvents and the analysis by mean of high performance liquid chromatography with diode array or fluorescence spectroscopy. We developed an easy and fast method to extract and simultaneously detect biogenic amines in fish by Direct Sampling Analysis (DSA) coupled with a High Resolution Mass Spectrometer (AxION2 TOF-Perkin Elmer). We selected three species (sea bream, octopus and red mullet), among the most commonly consumed fish, mainly involved in fraud concerning freshness. For the assessment of the method samples were bought just after fishing at the local fish market of Savona (Ligurian sea), as a guarantee of the initial freshness rate. Amines extraction from fish was obtained by mixing 5 g of homogenized muscle and 10 ml of methanol:water (1:1). Samples were vortexed for 5 minutes and then centrifuged. The extraction procedure was performed daily from time 0 (fresh fish) to 15 days. Samples were stored at 4°C. 5 µl of sample were deposited directly onto the stainless mesh of the AxION DSA for N2 ionisation and analysis. Measurement was run in positive ionization mode with flight voltage of -10,000V. Mass spectra were acquired in a range of m/z 20-1200 at an acquisition rate of 1 spectra/s. To obtain higher mass accuracy, calibration solution was infused into the DSA source at 10 µl/min. The biogenic amines investigated were correctly identified in pure solvent and from fish samples extraction. As most important result, we registered an increase of signal intensity of mass ions related to biogenic amines rate in the range of time analysed confirming a good correlation between fish freshness and its alteration. The ability to detect the presence of key compounds to monitoring fish spoilage is of special interest to ensure that consumer and manufacturer rights are protected from fraud and the method proposed in this study seems to be a very promising tool for rapid screening.

Keywords: biogenic amines, fish freshness, mass spectrometry, food fraud**Acknowledgement:** This work was founded by the Italian Ministry of Health (grant no. IZS PLV 01/14 RC). The authors are grateful to dr. G. Fazio for his help in collecting samples at the local fish market.

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SCREENING OF VETERINARY DRUGS IN FEEDSTUFFS BY DESORPTION ELECTROSPRAY IONIZATION-HIGH RESOLUTION MASS SPECTROMETRY

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Veterinary drugs are widely used across developed countries to treat animals and protect their health. Despite the requirements set for feed business operators (Regulation EC No 183/2005), it is generally acknowledged that during the production of mixed feeds, a certain percentage of a feed batch remains in the production circuit and the residual amounts can contaminate the subsequent feed batches. This cross-contamination may result in drug exposure to non-target animal species and, therefore potential risk of animal health as well as the presence of drugs residues in food products of animal origin may occur. For the analysis of cross-contaminated feedstuffs, extensive sample treatment and confirmatory methods, mainly based on LC-MS/MS, are required. In this context, agricultural and food laboratories demand fast screening methods for the identification of veterinary drugs and other illegal preparations in feed samples. The recent introduction of ambient ionization techniques in mass spectrometry, desorption electrospray ionization (DESI) and direct analysis in real time (DART), open the possibility for the direct analysis of samples, thus acquiring the mass spectra from bulk samples in their native state and without tedious sample treatments or chromatographic separation. In DESI, a spray of charged liquid droplets is directed into the sample and creates a solvent film on the sample surface, thus allowing in situ solid-liquid extraction and splashing secondary droplets that contain the analytes into the mass spectrometer inlet. The relative easy operation, the simplicity of sample manipulation and the capability for fast screening, make DESI-HRMS a suitable technique for rapid, selective and in situ analysis of samples in many application fields. In this work, a desorption electrospray ionization-high resolution mass spectrometry (DESI-HRMS) screening method is developed for fast identification of veterinary drugs in cross-contaminated feedstuffs. DESI-HRMS conditions (geometrical parameters, electrospray solvent and mass spectrometry parameters) are optimized to achieve the best sensitivity and selectivity. Several strategies, based on accurate mass measurements and the use of a custom-made database, are tested to direct analyzed samples and to identify veterinary drugs in cross-contaminated feedstuffs. Finally, DESI-HRMS results are compared with those obtained by well-established LC-MS/MS methods and advantages and limitations of DESI-HRMS for routine analysis of veterinary drugs cross-contamination in feedstuffs are also presented in this communication.

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Keywords: ambient mass spectrometry, DESI, veterinary drugs, cross-contamination, feed analysis

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SPECIES IDENTIFICATION OF FISH PRODUCTS USING DNA BARCODING AND NEXT-GENERATION SEQUENCING

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Recent studies in Europe and in North America have discovered extensive mislabeling of fish products. Fillet of lower-priced fish is often sold as higher priced species. These fraudulent activities carry potentially health concerns for consumers as well as the wellbeing of the oceans and vulnerable fish populations. To ensure that the seafood sold is safe, legal and correctly labeled, national food authorities need to increase seafood inspections and testing, improve documentation and verification and require better seafood traceability. This project aimed to implement DNA-based methods for species identification of fish products commercially sold in Norway. First, to document single species products, a DNA barcoding approach was used. Fish fillet from a number of species were purchased from local supermarkets, and identification ensured by DNA isolation, PCR amplification of a mitochondrial gene (the cytochrome c oxidase subunit 1 gene, COI) and sequencing based on an approach established by the US FDA. To document relevant species, a database consisting of COI sequences of about 100 locally sold fish species was established, gathered from the NCBI GenBank or own-sequenced DNA. With this approach, we were able to identify all species tested. Secondly, to document species content in mixed fish products, next-generation sequencing methods were evaluated. Fish cakes consisting of minced fillet of several species were purchased, or made in house with known composition, and the percentages of each species were attempted determined by DNA isolation and next-generation sequencing. An approach attempting to sequence the PCR amplicon of the COI gene was however not successful. Using the Illumina MiSeq, about 2 millions 300 bp reads in each direction were sequenced from each sample, and different bioinformatics pipelines and mapping strategies were tested (OTU clustering using Uparse and Bowtie mapping with Express read counting) were tested. Most fish species were identified, however the percentage of each species in the mixed products poorly reflected the original content. Potential pitfalls when sequencing PCR amplicons include poor homogenization, biased introduced by the PCR reaction with degenerated primers and varying mitochondria numbers in the cells. We are currently trying out a shot-gun sequencing strategy that include increased sequencing depth and additional bioinformatics tools. In conclusion, this project successfully implemented a method to identify single fish species based on DNA barcoding, and continue evaluating next-generation sequencing methods to identify species in mixed fish products.

Keywords: fish product mislabeling, DNA identification, DNA barcoding, next-generation sequencing

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RAPID QUALITY AND AUTHENTICITY TESTING OF OLIVE OILS
FROM HARVEST TO FINAL PRODUCT BY IR AND NIR
SPECTROSCOPY

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Over three million tons annually of olive oil are produced worldwide, with approximately 75% of this being produced in Spain, Italy, and Greece. Quick and easy analysis of oil quality is vital to maintain process efficiency. Rapid, reliable analysis can contribute to process and quality improvements in numerous ways and ultimately improve productivity. Extra Virgin Olive Oil (EVOO) is a premium product that can command a higher price than “standard” olive oils. Adulteration of EVOO with lower quality olive oils is frequently reported in the media. This makes it highly susceptible to fraudulent activity; over 267 oil adulteration incidents reported to the U.S. Pharmaceutical Food Fraud Database, with the vast majority occurring over the past three years.

Keywords: food fraud, authenticity, FT NIR, food quality

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DETERMINATION OF MEAT AUTHENTICITY USING A COMPREHENSIVE PROTEOMIC STRATEGY, DATA-INDEPENDENT ACQUISITION AND HIGH-RESOLUTION MASS SPECTROMETRY**Claudia Martins¹, Francis Beaudry^{2*}, Alberto Ruiz³, Erik Husby⁴, Dipankar Ghosh⁵**^{1, 4, 5} Thermo Fisher Scientific, San Jose, California, United States of America^{2, 3} Université de Montréal, Montreal, Canada

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Due to the internationalization of food production and distribution, there has been a significant increase of food fraud in recent years. Food fraud can have serious health implications, and occurs when food manufacturers implement unethical practices such as making false label claims as well as using additives and fillers within their products to increase profitability. Mass spectrometry is considered a workhorse in protein research, and can be used as a method for detecting marker proteins that support animal tissue identification. Meat adulteration was examined using a well-defined proteogenomic annotation, carefully selected surrogate tryptic peptides and high-resolution MS. Meat samples (beef, pork, horse and lamb) were homogenized in water (1:5; w:v) at high speed and the mixtures were sonicated for 30 min. Proteins were precipitated and the pellets were dissolved in 100 mM ammonium bicarbonate (pH 8.5). Proteins were heated denatured, reduced (DTT) and alkylated (IAA) followed by a trypsin digestion (100:1 ratio). The reactions were stopped and supernatants analyzed using Q-Exactive Orbitrap MS. The chromatography was achieved using a 30 minutes linear gradient along with a BioBasic™ C8 100 × 1 mm column at a flow rate of 75 µL/min. The Q Exactive MS was operated in Data Independent Acquisition (DIA) mode including one full scan and 12 DIA MS/MS scans to cover the mass range 600–1200 m/z. Identification of biomarker proteins representative of a particular species has been performed. We have methodically analyzed in silico Myoglobin, Myosin-1, Myosin-2 and β-Hemoglobin sequences to generate tryptic peptide mass lists and theoretical MS/MS spectra. Following a comprehensive DIA analysis (50 Da isolation windows), we were able to detect and identify very specific tryptic peptides for all four targeted proteins for each animal species tested with observed m/z below 3 ppm compared to theoretical m/z. Additionally, high-resolution MS/MS spectra reveal b/y ions compatible with amino acid sequence of each targeted peptide (4 proteotypic peptides per species). The analyses were successfully performed with raw and cooked meat. Specifically, the method was capable of detecting trace amounts of pork or horse meat into a mixture before and after cooking (71°C internal temperature). The present method developed use specific pork and horse peptide biomarkers enabling the detection of contamination with a very limited amount of sample. Sequence, mass and MS/MS signature ions of species-specific peptides will be presented for targeted Myoglobin, Myosin-1, Myosin-2 and β-Haemoglobin tryptic peptides. Amino acids differing between the sequences of the selected four species will be outlined in order to present a detail analytical strategy to perform systematic meat speciation using high-resolution MS and a DIA approach for a better protein coverage and future data mining.

Keywords: meat adulteration, LC–HRMS, meat authenticity, orbitrap

P83 RAPID DETECTION OF SPICE & HERB ADULTERATION USING NEAR-INFRARED SPECTROSCOPY AND DSA-TOF MASS SPECTROMETRY

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High value commodities, such as spices, are prone to adulteration by unscrupulous suppliers. The adulteration, typically economically motivated can also have food safety implications depending on the nature of the adulterant discovered. Commonly adulterated spices include vanilla, black pepper, and cinnamon. Recently the spotlight has turned to herbs as well, with oregano also being a target for adulteration; despite its lower value. This paper will demonstrate how the application of Fourier Transform Near-Infrared Spectroscopy (FT-NIR) in conjunction with the innovative Adulterant Screen™ software, and Direct Sample Analysis (DSA) coupled to Time of Flight (TOF) mass spectrometry offer fast, sensitive methodologies for the confirmation of authenticity and identification of unknown adulterants.

Keywords: herbs and spices, adulteration, FT NIR, ambient mass spectrometry, DSA-TOF

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DETERMINATION OF ANISATIN IN BOTANICAL VARIETIES OF STAR ANISE USING QUPPE - METHOD AND LC-MS/MS**Sonja Masselter¹, Hermann Unterluggauer^{2*}, Roman Fischer³, Florian Kraller⁴**^{1,2,3} AGES - Austrian Agency for Health and Food Safety, Innsbruck, Austria⁴ MCI Management Center Innsbruck, Innsbruck, Austria

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Chinese star anise is the dried and, as the name already implies, star shaped fruit of the star anise tree (*Illicium verum*). The fruit is used primarily as spice in Asian cuisine, but also as flavoring agent to aromatize foodstuff like fruit jams and drinks. Star anise is also known as remedy to combat colic, asthma and bronchitis. Star anise contains essential oils - in particular anethole, but also shikimic acid, which is an intermediate for the synthesis of anti-influenza drug oseltamivir (Tamiflu) [1]. Japanese star anise (*Illicium anisatum*) is a different variety and highly toxic species. Its toxicity is derived from neurotoxic sesquiterpene lactones such as anisatin, neoanisatin, and pseudoanisatin. The botanical variety *Illicium anisatum* is also a star shaped fruit and differs only marginally from *Illicium verum* when applying micro- and macroscopic investigation. It is known from literature that even *Illicium verum* contains traces of Anisatin – however – these levels are roughly 100 to 1000 fold higher in *Illicium anisatum*, which makes this toxin a good candidate for distinction at molecular level. In previous years, several cases of intoxications have been reported, caused by adulteration or confusion from Chinese star anise with other varieties. In 2002 the European Commission decided to implement special conditions on the import of star anise from third countries, including the analysis of consignments [2]. Besides economical aspects there is of course the overall goal to prevent health concerns derived from food fraud. Recently a poisoning incident with suspicion of star anise occurred in Austria. Urgent solutions were needed to differentiate between the different varieties of star anise. The idea was to develop a fast and reliable method, based on chemical analytical methods, to distinguish between these varieties and to detect adulteration of Chinese star anise. In a short-term project this aim could be accomplished applying simple QuPpe–Method for sample extraction using acidified methanol, followed by LC–MS/MS analysis for identification and quantitation of anisatin levels in various *Illicium* varieties [3,4]. QuPpe Method stands for Quick Polar Pesticides Method and was originally developed for the analysis of highly polar pesticides. The applicability and consistency of this method has been confirmed using certified reference material (*Illicium verum*, *Illicium anisatum*). The developed method has been shown to be suitable and sensitive enough, knowing that even *Illicium verum* contains traces of Anisatin and a safety level of 1 mg/kg has been proposed by Lederer et al. [5].

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[4] Mathon C. et al., *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 30 (9) 2013[5] Lederer I. et al., *J Agric. Food Chem.*, 54 (6) 2006**Keywords:** star anise, food fraud, Anisatin, QuPpe – Method, LC-MS/MS, *Illicium verum*, *Illicium anisatum***Acknowledgement:** Dr. Reinhard Länger, AGES Medicines and Medical Devices Agency

P85 UNTARGETED DETECTION OF ADULTERANTS IN PAPRIKA

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Securing the food chains from primary production to consumer ready food against any kind of contaminations and/or adulterations is a prerequisite for food safety. Hereby a particular challenge is that fraudsters may add substances or mixtures of an unforeseen nature to a commodity and that approaches are necessary that allow the untargeted detection of such adulterations. Within the frame of the EU-project SPICED (Securing the Spices and Herbs Commodity Chains in Europe against Deliberate, Accidental or Natural Biological and Chemical Contamination, 7th framework program) non-targeted fingerprinting methods are evaluated and improved to progress the detection of chemical adulterations and to ensure authenticity of spices and herbs. A particular focus of the presented study is the untargeted detection of adulterants using various statistical approaches. In this study, a representative set of authentic as well as adulterated (spiked) paprika samples were investigated by nuclear magnetic resonance (¹H-NMR) spectroscopy and Fourier transform infrared (FT-IR) spectroscopy. The samples were spiked with various chemical agents ranging from azo dyes such as Sudan I and IV to inorganic compounds such as silicon dioxide. The authentic data set, which serves as basis for the determination of critical limits to identify potentially adulterated samples. A comparison of the statistical approaches to determine (unforeseen) chemical agents will be presented.

Keywords: non-targeted analysis, spices

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IN-SITU DETECTION OF FUNGICIDE ON FRUIT'S PEEL BY SURFACE-ENHANCED RAMAN SCATTERING

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Raman spectroscopy is perhaps the most powerful technique for molecular analysis among those currently available as it provides the so-called "molecular fingerprint" [1]. It is a fast, non-destructive and specific technique, that can be implemented by using roughened nanostructured metal surface, or metal colloids that, thanks to their plasmon activity, are able to amplify the signals of the adsorbed molecules on their surfaces [2]. This phenomenon, known as Surface-Enhanced Raman Scattering (SERS), allows to perform in-situ detection of surface contaminants, by deposition of metal nanoparticles directly onto the contaminated samples. Nevertheless, standardized methods of production and application of SERS systems are still needed in order to provide reproducible analytical methods, especially for in-situ applications in food analysis [3]. For this study, pyrimethanil was chosen as representative test material. It is a widely used fungicide and although it does not present toxicity to humans, it is a very harmful substance, especially to aquatic environments and, as a pesticide, in 2007 it has been included in Annex I COUNCIL DIRECTIVE (91/414 / EEC) concerning plant protection products. In order to identify the best nanostructure in terms of enhancement of the Raman signal, different types of gold nanoparticles have been synthesized and characterized [4; 5]. A model substrate that simulates the real case of pesticide in-situ detection has been prepared by testing different deposition methods of pyrimethanil and nanoparticles on silicon wafers. A calibration method for the quantitative measurement of pyrimethanil has been defined: the intensity of the Raman signal due to SERS effect has been correlated with the analyte concentration. However, the enhancement effect does not increase linearly with the pyrimethanil concentration but it depends on the amount of analyte and nanoparticles in the system. SERS effect is obtained when pyrimethanil molecules and gold nanoparticles are in a well-defined ratio. In order to detect low concentration of pyrimethanil, a calibration curve has been constructed by mixing the pesticide (at different concentrations) and the nanoparticles according to the optimal ratio. The efficiency of the developed method is verified by determining the concentration of pyrimethanil on fruit peel: a known concentration of the pesticide is deposited on the fruit surface, removed by washing, concentrated and mixed with a suitable amount of nanoparticles.

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Keywords: surface-enhanced Raman scattering (SERS), gold nanoparticles, pesticide, fruit peel, food analysis

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DETECTION OF HIGH TEMPERATURE STRESS OF PACKAGED BEER

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Beer content of 5-Hydroxymethylfurfural (5-HMF) and 2-furfural (2-F) and changes during storage provide a useful tool for the detection of high temperature stress of packaged beer, having also influence on its colour. This is a topic of particular relevance to beers produced for export, which may spend significant periods of time exposed to high temperature, either in transit or during warehouse storage, when beer is to be consumed in regions with hot climates. In this study, bottled Pilsner beers stored under controlled conditions were used to evaluate kinetics of 5-HMF and 2-F formation during storage at 30, 40 and 50°C. This type of beer was chosen since it is the most popular beer style in the world. Additionally, since 5-HMF and 2-F formation are often associated with colour development, and studies related with colour changes during beer storage are scarce, correlation between its formation during beer storage at different temperatures and CIE colour parameters was also studied. The HMF content of recently prepared Pilsener beers ranged between 3.64 and 3.94 mg/L, whereas 2-F content ranged between 0.1 and 0.14 mg/L. A linear increase of HMF and 2-F was observed during storage time, presenting higher values when higher storage temperatures were applied. Changes in 5-HMF and 2-F were explained using zero-order reaction kinetics and the dependence of the rate constant on temperature was described by the Arrhenius equation. The rate constants of HMF formation were 10 times higher in comparison to that of furfural formation. Calculated activation energy for 5-HMF and 2-F were 101.85 kJ mol⁻¹ and 86.05 kJ mol⁻¹, respectively. A strong negative correlation between CIE L* and a* (-0.718), L* and 5-HMF content ($r = -0.761^{**}$) and L* and 2-F values (-0.723) was observed, whereas positive correlations were found between a* and HMF ($r=0.937^{**}$) ($p<0.01$) and also between a* and 2-F ($r=0.896^{**}$) ($p<0.01$), which corroborate that higher temperature stress of packaged beer influenced its colour due to greater Maillard development.

The formation of 5-HMF and 2-F, calculated by the analysis of the beer and knowledge of the production date, may be used to estimate the average temperature at which a given beer sample has been stored, if the initial content has been characterized previously. Alternatively, it is possible to estimate the age of a beer if it is known at what temperature the beer has been stored. Moreover, measurements of colour parameters (L*, a*, b* and ΔE^*), can also be a useful tool for the detection of high temperature stress of bottled beer since a strong highly significant negative correlation was found between CIE L* and 5-HMF and 2-F contents and a highly significant positive correlation was found between a* and ΔE and HMF and also between a* and ΔE and 2-F.

Keywords: packaged beer, temperature stress, furanic aldehydes, zero-order reaction kinetics

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DERIVATIVE SPECTROSCOPIC DETERMINATION OF ENROFLOXACIN IN SOME NATURAL SAMPLES

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Antibiotic residues in foods pose a serious threat to public health. This is especially true of the fluoroquinolones, a class of broad-spectrum antibiotics which includes enrofloxacin. The use of fluoroquinolones in both humans and animals is restricted in many countries due to the quality and severity of potential adverse effects. These effects occur during or even long after use and include central nervous system toxicity, peripheral neuropathy, blood disorders, and brain, liver, endocrine, musculoskeletal and gastrointestinal dysfunction. The FDA has recommended black box warning for all fluoroquinolone antibiotics due to human fatalities. Enrofloxacin is used in the treatment of systemic infections including urinary tract, respiratory, gastrointestinal, and skin infections. Because of a very broad spectrum of activities against both Gram-negative and Gram-positive bacteria and lower side effects, Enrofloxacin has also been widely used for the treatment of some infectious diseases in pets and livestock. However, Enrofloxacin residues may persist in animal body and may result in the development of drug-resistant bacterial strains or allergies [1]. A new, simple, rapid, wide applicable range and reliable derivative spectrophotometric method has been developed for determination of enrofloxacin in some natural samples.

- [1] Hai-tang Z., Jin-qing J., Zi-liang W., Xin-yao C., Xing-you L., San-hu W., Kun Z., Jin-shan C. Development of an indirect competitive ELISA for simultaneous detection of enrofloxacin and ciprofloxacin. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*, 2011, 12(11):884-891.

Keywords: derivative, determination, enrofloxacin, meat

P89**QUALITY CONTROL OF EXTRA VIRGIN OLIVE OIL BY PROCESSING THE IMAGES OF OLIVES****Enrique S. Pariente¹, John C. Cancilla², Regina Aroca-Santos³, Gemma Matute⁴, José Torrecilla^{5*}**^{1, 2, 3, 4, 5} Complutense University of Madrid, Madrid, Spain

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The quality control in most producing industries is one of the most crucial points in world trade. This protection is even more important in the food sector, where not only the food quality must be controlled, but also the health of their consumers and prevention of any type of adulteration must be ensured. This is why legal regulations are commonly published around the world. This situation is even more important when the goods are characteristic of a country's idiosyncrasy, like the extra virgin olive oil (EVOO) from Mediterranean countries. Regarding the field of EVOO, a vital component of the Mediterranean diet, many research groups around the world are working on the development of methods to monitor its quality and prevent its loss. These groups are working on developing new equipment, applications, and/or powerful chemometric tools to detect any condition that causes a decrease of EVOO quality. Most of them are working with the oil itself, but here a new way based on the analysis of the olive images by supervised artificial neural network is proposed. The independent variables of the model have been taken from olive photographs to design a fast way to classify olives according to their quality. The percentage of misclassification of this tool is lower than 5%. These results offer a good tool to protect EVOO quality, as success in this regard would be compelling for the EVOO producers, as better olives are directly related to the production of higher-quality extra virgin olive oils.

Keywords: images, artificial intelligence, extra virgin olive oil, food quality control

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QUALITY ESTIMATION OF EXTRA VIRGIN OLIVE OIL DURING SHIPMENT AND STORAGE

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One of the main properties of extra virgin olive oil (EVOO) is that it possesses multiple advantages for human health, which turns the quality control of this functional food into a great necessity. In the present research, samples of three different Spanish EVOOs (Marqués de Valdeusa, Empeltre and As Pontis), which are currently being exported to USA, were held under three different temperature conditions (3°C, 40°C and room temperature) simulating some of the possible conditions that EVOO suffers during shipment and storage. The consequences of the different temperatures, as well as time, led to an alteration of the properties of the EVOO samples, which were studied by means of absorption visible spectroscopy and neural network (NN) modeling, which is a non-linear mathematical tool that has been used to relate the absorption with time and temperature. The absorption peaks representing the chlorophylls and carotenoids present in EVOO decrease with time and temperature. Generally, the results show that higher temperatures contribute more in the degradation of EVOO when compared to lower ones. The obtained information was used to create, design, and optimize a neural network, which is able to fairly distinguish the time and temperature conditions that EVOO samples underwent. This technique is fast, user-friendly, and non-destructive so it could be of great use for the real-time quality control of edible oils during, for example, their distribution chain, as ideal conditions could be potentially optimized.

Keywords: real time quality control, extra virgin olive oil, artificial intelligence, distribution chain

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THE ANALYSIS OF FATTY ACID METHYL ESTERS BY INNOVATIVE GAS CHROMATOGRAPHY - VACUUM ULTRAVIOLET ABSORPTION DETECTION

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Fatty acids and their corresponding methyl esters (FAMES) are important analytes for consideration in terms of food science, nutrition, and bio-based fuels. Typically, these are characterized by gas chromatography – mass spectrometry (GC–MS), but the complexity of the system, as well as many closely related isomers and isobars, can make complete speciation difficult. We have applied a new vacuum ultraviolet absorption detector for GC (GC–VUV) to demonstrate its superior capability for FAME characterization. GC–VUV measures the absorption of eluting compounds in the 115–240 nm range where all chemical species absorb. Each FAME and class of FAME have unique absorption features that enable both qualitative and quantitative analysis. FAMES present difficulties with regard to analysis by GC–MS. Specifically, cis-/trans-isomers and degree/position of unsaturated double bonds are difficult to determine. Spectral filters, creating selected chromatograms, can be applied for specification of compound classes. Here, saturated, monounsaturated, and polyunsaturated FAMES are well differentiated with the aid of spectral filters. Saturated FAMES absorb strongly in the range of 125–160 nm, while unsaturated absorb in the 170–200 nm range, such that absorption increases with the number of double bonds. Ratios of responses based on those spectral filters (170–200 nm/ 125–160 nm) provides clear delineation of degree of saturation for FAMES. Model and real food oil samples have been used to demonstrate the effectiveness of GC–VUV for FAMES analysis.

Keywords: vacuum ultra violet detection, gas chromatography, FAMES, cis-/trans isomers, saturated vs unsaturated FAMES

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EFFECTIVE QUECHERS CLEANUP AND QUANTITATION OF PLANAR PESTICIDES FROM GREEN FOOD USING A NOVEL GRAPHITIZED CARBON BLACK AND A ZIRCONIA-BASED ADSORBENT

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Pesticides with a planar structure, such as hexachlorobenzene and chlorothalonil, are among those commonly used during the cultivation of spinach and other green, leafy crops. Extraction and analysis of these residues is complicated by the presence of chlorophyll in the food matrices. Large pigment molecules like chlorophyll are deleterious to both GC–MS and LC–MS/MS analyses; accumulating in the inlet and degrading column performance in GC and contaminating the source in LC. Method EN15662 recommends the addition of Graphitized Carbon Black (GCB) to cleanup tubes to remove chlorophyll and other pigment interferences. In addition to removing pigment molecules, however, GCB also retains the planar pesticides because of π - π interactions between the graphitic carbon and the planar structures of the pesticides. Previous work shows a near linear inverse relationship between pigment removal and recovery of planar pesticides. Suggestions to overcome this problem include adding toluene to the extraction solvent to minimize binding to the GCB and using smaller amounts of GCB to balance recovery of planar pesticides with color removal. Data show that neither of these solutions is completely satisfactory. This poster, shows a QuEChERS cleanup mix, Supel QuE Verde that comprises a proprietary GCB and a zirconia-containing adsorbent, Z-Sep+, to remove chlorophyll while maintaining high recovery of planar as well as other pesticide residues in green food. Color removal was determined using a UV-Vis spectrophotometer. Planar pesticide recovery was determined using a GC–MS/MS Triple Quad. The effects of quantity and surface areas of GCB on the color removal and pesticide recovery will also be discussed.

Keywords: QuEChERS, pesticide residue analysis, GC–MS, green food

P93**ELISA TESTS RELIABILITY WITHIN RAW AND HEAT- TREATED COW MILK DETECTION IN SHEEP MILK AND CHEESE****Lucia Zeleňáková^{1*}, Alica Bobková², Martina Fikselová³**^{1,2,3} SUA, Nitra, Slovakia

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The aim of this study was to perform laboratory tests and to compare the reliability of two commercial ELISA tests (RC-bovino and RIDASCREEN CIS) within raw and heat treated cow milk detection in sheep milk and cheese in order to obtain a high-quality, reliable and economically beneficial method suitable for routine application in practice. Raw sheep milk, cow milk and heat-treated cow milk (pasteurisation at 72°C for 15 sec. or at 85°C for 3 sec.) were mixed in precisely defined proportions (0–100% cow milk in sheep milk). The milk mixtures were sampled to detect adulteration and subsequently cheese was made. Our results regarding the detection of raw cow milk in samples of sheep milk were comparable for both of the used ELISA tests. The reliability of ELISA kits were subsequently analysed for the detection of various additions of raw and heat-treated cow milk in sheep milk and cheeses. The differences between ELISA tests were identified in the detection of 0.5% raw milk in the sheep milk. While the ELISA test RC-bovino detected 0.197%, the ELISA test RIDASCREEN CIS was able to detect up to 0.448%. However, in a concentration range between 0–0.5%, quantification is more sensitive to imprecision. The decrease of cow milk amount by 53.53% and 59.34 % (at 5% low and high pasteurized cow milk) and by 62.64% and 66.56% (at 75% low and high pasteurized cow milk) was detected. In context with the above mentioned, the relationship between the real and detected amount of cow milk (%) in different production stages (milk, cheese) using a regression analysis was examined. However, lower reliability of the detection was indicated by R² values, which ranged from 0.5175 to 0.6346 (milk) and from 0.4058 to 0.4398 (cheese). In practice this means that although individual percentage of cow milk in the sample can be detected (%), but in the unknown sample can not be clearly confirmed whether the cow milk was raw or heat- treated. In this context, the results can be inaccurate and may not correspond to the real situation. As was noted above, one of the solution is to set a specific regression curves for each of the heat treatment of analysed milk. The values of determination coefficients were higher than 0.82, which assumes the conditions for the reliable determination of raw or heat-treated cow milk in sheep milk. The only limitation here is the knowledge of cow milk heat treatment. In conclusion, the analysis has shown that the ELISA tests identified the presence of cow milk, but quantification was not exact because of irreversible changes caused by the manufacturing process. Denaturation and fermentation process is known to affect the binding sites of the specific molecules of each type of protein. Despite this fact, both producers recommended ELISA tests for the detection of sheep milk and cheese adulteration by cow milk.

Keywords: RIDASCREEN, ELISA, adulteration, milk, cheese

P94 USE OF HEADSPACE SOLID PHASE MICROEXTRACTION AND GC-MS FOR ANALYSIS OF TERPENE PROFILES OF HOPS

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Terpenes are small molecules synthesized by some plants. The name “terpene” is derived from turpentine, which contains high concentrations of these compounds. Terpene molecules are constructed from the joining of isoprene units in a “head-to-tail” configuration. Classification is then done according to the number of these isoprene units in the structure. The configurations of terpenes can be cyclic or open, and can include double bonds, and hydroxyl, carbonyl or other functional groups. Terpenes are contained in the essential oils derived from plants, and often impart characteristic aromas to the plant and/or oil. For example, dl-limonene, which is found in lemon, orange, caraway and other plant oils, has a lemon-like odor. In this application, headspace-solid phase microextraction (HS-SPME) using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, combined with GC/MS, was used to profile some of the predominant terpenes present in common hops. A simple headspace extraction was performed on three different hop samples; (1) dried hop flowers of unknown variety (2) pelletized Cascade hops (3) pelletized US Goldings hops. Chromatographic separation was performed on a 60 m nonpolar Equity-1 capillary GC column, and identification was achieved using retention indices and spectral library match. Terpene profiling was able to differentiate old, oxidized hops from samples in good condition. In addition, identification of the presence of specific terpenes was used to differentiate one hop variety from another.

Keywords: terpene, hops, SPME, aroma and headspace extraction

P95**FROM GRAPE TO WINE: INFLUENCE OF WINEMAKING ON PHENOLIC PROFILE AND IN VIVO ANTIOXIDANT ACTIVITY****Mariana Lingua¹, María Fabani², Daniel Wunderlin³, María Baroni^{4*}**^{1,2,3,4} CONICET-ICYTAC, Córdoba, Argentina

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Phenolic are components of wine with a great impact on its sensorial characteristics, especially colour and flavour. They have also shown beneficial effects on human health, protecting against cardiovascular and degenerative diseases. Several factors, including grape variety, grape ripeness, environmental factors and technological procedures used during winemaking, can qualitatively and quantitatively affect the phenolic composition of the grape and wine and, therefore, their nutritional and quality properties. In this sense our main goal was to assess the effects of the winemaking technology on phenolic profile of grapes and wines and the influence of these changes on the in vivo antioxidant activity (AC), considering three main varieties produced in Argentina: Merlot, Syrah and Cabernet Sauvignon. Grapes and wines samples were collected from the same vineyard in the Province of San Juan, Argentina. In vivo antioxidant activity was determined using the yeast *Saccharomyces cerevisiae* exposed to H₂O₂ as an oxidizing agent. The antioxidant activity was evaluated by measuring the survival rates of yeast and changes in antioxidant enzymes: Glutathione Reductase (GR) and Glutathione Peroxidase (GPX). Forty-five compounds, including anthocyanins, flavonols, flavanols, phenolic acids and stilbens, were identified by HPLC–PDA–ESI–MS/MS. Results show that phenolic composition and antioxidant activity vary along the winemaking process and between varieties. In vivo AC demonstrated that yeast previously incubated with grape and wine polyphenols showed enhanced resistance to H₂O₂. Furthermore, GR and GPx activities were positively correlated with the chemopreventive effect observed. Data obtained was analysed by multiple regression analysis (MRA) in order to evaluate the relationship between phenolic profile and AC of grapes and wines. Results from MRA analysis showed that Syrah had the highest AC, because of their phenolic profile characterised by the highest content in anthocyanin compounds, while the content of trans-resveratrol in Merlot grapes and wine probably contributes to its lower activity. Furthermore, changes in the phenolic profile from grape to wine, because of the winemaking process, affected the AC. In this sense, grapes were characterised by higher content of kaempferol-3-glucoside and fertaric acid that may contribute positively to its higher in vivo AC. On the other hand, ethyl gallate contributed negatively to the AC of wines. Results presented in this study underline differences in phenolic profile and in vivo antioxidant activity of products involved in the winemaking process: grape as a raw material, wine as a final product. Furthermore, our results suggest that the AC of wine depends upon the grape variety used, since it largely determines the qualitative and quantitative composition in polyphenolic compounds.

Keywords: grape, pomace, wine, polyphenols, antioxidant**Acknowledgement:** CONICET and Universidad Nacional de Córdoba (Argentina)

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USE OF DEFATTED CHIA SEEDS TO ENHANCE THE NUTRITIONAL QUALITY OF WHEAT PASTA

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Nutritional quality and food authenticity are issues of great interest in the food industry, which concerns not only to consumers, but also to producers and distributors. Nowadays, there is a widespread preference to the consumption of foods that, in addition to its nutritional properties, provide components that could reduce the risk of certain diseases. One of the most studied functions corresponds to the antioxidant capacity, since it is involved in protection against oxidative damage of cells and tissues, having an important role in the prevention of numerous diseases related to the overproduction of free radicals, such as cancer, diabetes, cardiovascular disorders, etc. The antioxidant property is generally associated with high content of phenolic compounds. Chia (*Salvia hispanica* L.) is an annual species belonging to the Lamiaceae plant family, traditionally grown in pre-Colombian Central America. Chia seeds possess an interesting nutritional value due to the presence of polyunsaturated fatty acids (ω -3/ ω -6), vitamins, minerals and antioxidant). In particular, Chia seeds have the highest percentage of alpha linolenic acid (~60 %) among the described natural sources. Therefore, Chia is considered an important nutraceutical product being a good alternative for enriching foods, producing functional foods and gluten-free products. Recently, the European Parliament and the European Council (2009/827/UE; 2014/890/UE) approved its use as a novel food ingredient. Within this framework, the aim of the present work was to assess the antioxidant capacity of pasta produced with partial substitution of wheat flour with different proportions of chia defatted flour (0%, 2.5%, 5% and 10%), in order to exploit this residual by-product, obtained after oil extraction, as an ingredient in the manufacture of a supplemented food product. The evaluation of different quality indicators was performed, together with the extraction of phenolic compounds. Antioxidant capacity of the extracts was measured by means of classical chemical methods, including: Folin-Ciocalteu, FRAP, TEAC and DPPH. Our results show that the increasing content of chia flour improves the antioxidant properties of the produced pasta, demonstrating that defatted chia seeds can be used as an ingredient to enhance the nutritional quality of foods.

Keywords: chia, pasta, antioxidant, functional foods, polyphenols

Acknowledgement: CONICET and Universidad Nacional de Córdoba (Argentina)

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TOWARDS STANDARDISATION OF MICROBIOLOGICAL SAFETY AND QUALITY OF BEE POLLEN

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Bees gather pollen during the process of plant pollination. Collected pollen grains agglutinate with salivary secretions, nectar or honey and compress it into two pollen baskets on their hind legs. In this way they form two “pallets” of pollen and bring it into beehive. Beside high proportion of water bee pollen contains proteins, essential amino acids, sugars, fibres, vitamins, beta-carotenes, minerals, enzymes, fatty acids and bioactive compounds like phenols. Pollen has an important role in the health and survival of the bee colony, as it is their only source of protein. Since the benefits of bee pollen for humans are recognized, beekeepers have started to collect the bee pollen with specialized traps. Today, we can use bee pollen as a supplement or as a food by itself. The process of bee pollen collection, processing and storage is fairly new and, although there are some tips and instructions for it, strict guidelines have not yet been established and/or standardized. Bee pollen is a natural food product that can be greatly contaminated by microorganisms, which can influence its safety, quality and durability. In our research we have assessed the important stages of bee pollen collection, processing and storage on the product’s quality and safety. The first step of bee pollen processing is the collection of bee pollen in bee pollen traps. We investigated the influence of the bee pollen trap materials and the time and frequency of its collection on the microbial load of bee pollen. We found that different bee pollen trap materials had little influence on the microbial count and composition of bee pollen. The appropriate time and frequency of bee pollen collection was once or, if the quantity of the bee pollen required it, more times within 24 hours. Furthermore, we investigated the influence of storage conditions and thermal treatment on the microbial indicators of the quality and safety of bee pollen. Fresh bee pollen is a quickly perishable foodstuff, durable only for a few days at room temperature, and should therefore not be stored in these conditions before processing. Refrigeration at 4°C somewhat expands its durability. The best condition for storage of unprocessed bee pollen was freezing it at -20°C, as this had little influence on its sensory characteristics and microbial count. The optimal way of preserving bee pollen appeared to be drying it at 35°C for 48 hours. This way of processing bee pollen ensured its durability, as it suspended mainly yeast spoilage, which was confirmed as the most critical for shortening the shelf-life of product. Drying bee pollen at optimized conditions keep the sensory properties and does not deteriorate the texture of bee pollen and the processed product can be stored at room temperature for an extended period of time.

Keywords: bee pollen, processing, safety and quality, durability, microbiological indicators

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BATTLING FOOD FRAUD BY QUANTITATIVE INGREDIENT PROFILING - APPLICATION OF NMR TO OLIVE OIL AND HONEY

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Economically motivated food fraud, which includes declaration of a false variety or geographic origin or dilution with low quality components, is on the rise. Since most kinds of adulterations do not pose an immediate health risk for the consumer, development of proper testing methods had no priority in the past. However, a loss of consumer confidence associated with cases of food fraud causes significant economic damage, even for producers that comply with formalities. Quantitative ¹H-NMR profiles provide a powerful means to verify authenticity of products and to simultaneously test for the quality of goods. We here present an application to honey and olive oil. In particular, we show that ¹H-NMR profiles of polyfloral honeys provide fingerprints which are characteristic for the geographic origin. Further cases of honey-laundry can thus be prevented, which at the same time adds value to domestic products. Furthermore, we show that NMR profiling of the highly valued Manuka honey is suited to confirm the variety as well as the potential to discriminate Manuka honey from New Zealand and Australia. Regarding olive oil, we demonstrate that NMR has the potential to identify unlawful treatments of products which are then sold as extra virgin olive oils (EVOO). Examples are olive oils with sensory defects that are softly deodorized and EVOO which are blended with other vegetable oils as well as mineral oil.

Keywords: NMR-profiling, honey, Manuka, olive oil, multi-parameter-screening

P99**STANDARDIZATION OF SLOVENIAN ROYAL JELLY: THE FIRST DATA ON ITS COMPOSITION****Jasna Bertonec¹, Tomaž Polak², Nives Ogrinc³, Mojca Korošec^{4*}**^{1,2} University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia³ Jožef Stefan Institute, Ljubljana, Slovenia⁴ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

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Royal jelly (RJ) is a secretion from the glands of working honey bees and it is used in the nutrition of bee larvae. Because of the many positive attributes also for human health RJ is considered as a functional food. It is widely used in food supplements. Since the production of RJ is very time consuming and expensive, this honey bee product is not safe from adulteration, which presents a serious threat to its quality. Due to the increasing demand for RJ abuse of cheap sugar syrups and protein sources is most common in the adulteration practice. Data on composition of RJ are important for determining the standard composition of this bee product, as well as to evaluate its quality and authenticity. The most commonly used criteria of quality and authenticity are the composition of sugars, water content, protein content, and the content of 10-hydroxy-2-decenoic acid, a compound unique to RJ. Standard composition of RJ was so far determined in only few countries. The aim of our research was to determine quality parameters of Slovenian RJ. Twenty-one samples of fresh RJ were included, 18 from the main Slovenian RJ producers. The contents of water, ash, free acids, fat, protein, 10-hydroxy-2-decenoic acid, pH value and stable carbon and nitrogen isotopes were determined by chemical analyses. All analyzed parameters were statistically evaluated with different methods. Results of analyses were compared with the quality standards for fresh RJ, defined by the International Honey Commission, and with the available data from literature. All samples of Slovenian RJ met the established quality criteria. The content of 10-hydroxy-2-decenoic acid was in all analyzed samples above defined value. The results of analyzed Slovenian RJ were comparable with data obtained by other researchers; greater differences were found only in fat content and $\delta^{15}\text{N}$ value. Slovenian RJ contained significantly more water, fat and 10-hydroxy-2-decenoic acid, and less protein. The results of the analyzed parameters provide a foundation for definition of the standard composition of Slovenian RJ. These parameters could serve for determination of the quality and authenticity of the Slovenian RJ and could assist in monitoring the presence of RJ of foreign origin on the Slovenian market.

Keywords: Slovenian royal jelly, characterization, standard composition, quality, authenticity

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DNA AND ISOTOPIC FINGERPRINTS HELP ANALYZING GEOGRAPHIC ORIGIN AND AUTHENTICITY OF SAFFRON SPICE

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Saffron spice, due to economic reasons, is often subject to fraud, especially with respect to denomination of origin. In this pilot study of saffron spice samples labelled as originating from various countries have been analyzed for their H-, C- and N- isotope ratios and for their genetic fingerprint using organellar as well as nuclear DNA markers. Regional variations in the stable isotope patterns evidence differences in the respective ambient environment and agricultural practices. Interpretation of the isotope data with respect to geographic origin leads to isotope fingerprints enabling the discrimination of geographic origin of most of the investigated samples. DNA based analysis of genetic diversity in the saffron samples evidences extremely limited variation present in *C.sativus*. The latter method thus is an extremely potent tool for identification of fraudulent presence of other biogenic material either as admixture with or as replacement of true saffron.

Keywords: geographic origin, authenticity, *Crocus sativus*, stable isotopes, genetic markers

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P101 RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS) FOR FOOD AUTHENTICITY TESTING

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The quality, safety and authenticity of food are of principle interest for society and are regulated by legislation. Food fraud is used to encompass deliberate and intentional substitution, addition, tampering, or misrepresentation of food, ingredients, packaging or false statements for economic gain. Due to their high market value, meat and fish products are often targets for species substitution, adulteration, mislabelling and questions raised about geographic origin or means of production. Absence of a declared species or presence of an undeclared species would raise doubts about the claimed provenance of the product and some cuts of meat are more valuable than others. Testing food is one of the key ways of checking whether food businesses are complying with food law. Current methods used for determination of species and adulteration (e.g. ELISA, genomics, chromatography, spectroscopy or mass spectrometry) are time consuming, can be costly and typically located in a laboratory some distance from the producer and retailer. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) combined with multivariate statistical analysis (Principal Component Analysis and Linear Discriminate Analysis) is an emerging technique for near real time characterization of tissues with no requirement for sample preparation [1,2]. Samples are analysed by direct cutting of the surface of the sample using hand-held sampling devices powered by an electrosurgical RF-generator; a monopolar cutting electrode (the iKnife) or bipolar forceps. The resulting "smoke" or aerosol generated is transferred to the mass spectrometer via a Venturi air jet pump-based ion transfer apparatus mounted in the orthogonal position relative to the atmospheric interface of a quadrupole time of flight mass spectrometer. Although mass spectra acquired from food samples, including a range of different fish and meat species or from different cuts of meat, look similar, the profile of the lipid components has been shown to be useful for classification purposes using multivariate statistical methods. Using these spectra, training samples were used to classify the reference groups to build PCA/LDA models. The models were verified with cross-validation and independent test sets. We present data that demonstrates the potential capability of the REIMS technique to accurately discriminate meat muscle samples from different species and for detection of offal in processed meat products. Results are provided immediately using prototype "real-time recogniser" software, which will be launched at ASMS in June 2016.

[1] J. Balog et al., *Analytical Chemistry*, 82, 7343-50 (2010)

[2] J. Balog et al., *Science Translational Medicine*, 5, 194ra93 (2013)

Keywords: Rapid Evaporative Ionisation Mass Spectrometry (REIMS), food fraud, speciation, Xevo G2-XS QToF, multivariate statistical analysis

P102 UNTARGETED GLYCOSYLATED SIMPLE PHENOL PROFILING IN OENOLOGICAL TANNINS BY HIGH RESOLUTION MASS ANALYTICAL METHOD (SPE-LC-Q-ORBITRAP)

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Oenological tannins, highly reactive phenolic compounds, are extracted from different botanical sources, including grape, quebracho, oak, chestnut, tara and galla, and are authorised by the Organisation Internationale de la Vigne et du Vin (OIV) as clarifiers of musts and wines due to their affinity to bind proteins. Tannin addition is accepted in Europe, South Africa, USA, Australia and New Zealand, even though with some differences between different countries. Phenolic compounds are ubiquitous in natural kingdom and contribute to the antioxidant intake of many plant foods. Free simple phenols, present both in free and glycosidically bound forms, have the simplest chemical structure of all phenols, but their same antioxidant and other biological properties. Despite there are a large number of commercial tannins, there is little information about their glycosidically bound simple phenolic content. Combining an on-line SPE-UHPLC method with high resolution mass spectrometry (Q-Orbitrap), a new untargeted approach for a detailed description of the bound phenol profiles in oenological tannins was developed. On-line SPE clean-up was performed on a HyperSepTM Retain PEP SPE cartridge, while the chromatographic separation on an Acquity UPLC BEH C18 analytical column. Identification and quantification of glycosylated phenolic compounds were performed acquiring mass spectra in full MS-data dependent MS/MS analysis at mass resolving power of 140.000, in negative ion mode and with a heated electrospray. The untargeted approach, validated using expressly synthesized glycosidic precursors, let to characterize 88 monoglycosides (42 hexoside precursors, 46 pentoside) and 63 diglycosides (22 hexoside-hexoside precursor, 14 hexoside-pentoside, 16 pentoside-hexoside, 11 pentoside-pentoside). The proposed method provided a new approach to characterized oenological tannins on the basis of their botanical origin: oak tannins are characterized by the exclusive presence of coniferyl alcohol-pent, isopropiovanillone-pent, orcinol-pent, phenol-pent, coniferaldehyde-hex, vanillyl ethyl ether-hex, gallic acid-hex-pent, coniferaldehyde-hex-hex; marc tannins by scopoletin-pent, p-carboxyphenol-pent-pent, homovanillic alcohol-hex, vanillin-hex-pent; grape skin tannins by phenol-hex, 4-vinylphenol-pent-pent and isopropiosiringone-hex-hex; blueberry tannins by 4-vinylguayacol-pent, p-carboxyphenol-hex-pent and vanillic acid-pent-pent; citrus tannins by acetosyringone-hex, syringaldehyde-hex-hex and guaiacol-pent-pent; quebracho tannins by pyrocatechol-hex-hex and tyrosol-hex-hex; tea tannins by aesculatin-pent and syringol-hex; green tea tannins by isopropiosyringone-pent and salicylic acid-hex; chestnut tannins by eugenol-hex; tara tannins by gentisic acid-pent-pent; acacia tannins by catechin-pent; gambier acacia tannins by caffeic acid-hex-pent; brazilian acacia tannins by aesculatin-hex-pent; mimosa tannins by homovanillic alcohol-hex-hex.

Keywords: solid phase extraction, LC-MS, bound phenols, glycosilphenols

P103**AUTHENTICITY ASSESSMENT OF LINGONBERRIES (*VACCINIUM VITIS-IDAEA*) BASED PRODUCTS****Kamila Hurkova^{1*}, Josep Rubert², Milena Stranska-Zachariasova³, Vladimir Kocourek⁴, Jana Hajslova⁵**^{1, 2, 3, 4, 5} Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic

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Lingonberries (*Vaccinium vitis-idaea*) are one of the most popular berries in Central and Northern Europe and Baltic states. They represent a valuable source of bioactive compounds, especially polyphenols. The phytochemicals contained in lingonberries have been shown to have a positive influence on human health due to their significant antioxidant anti-inflammatory effects. For this reason, lingonberries are used in a number of different forms in the human diet: fresh, frozen, dried or in the form of tea or food supplements for Urinary Tract Infection (UTI) prevention. Recently, fraud suspicion on lingonberries-based products has been reported; *Vaccinium vitis-idaea* berries are partially or even totally replaced by less valued Cranberries (*Vaccinium macrocarpon*) grown mainly in North America. Although PCR methods may provide solution, these are not available in routine laboratories focused on food analysis. On this account, alternative analytical strategies have to be searched. In this study, metabolomic fingerprinting employing instrumental platform consisting of ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (HPLC–HRMS/MS) has been investigated. For this purpose, polar (methanolic) and non-polar (hexane:isopropyl alcohol) extracts of fresh, frozen and dried authentic berries of *Vaccinium vitis-idaea* and *Vaccinium macrocarpon* together with their admixtures were prepared and analyzed by U-HPLC–HRMS/MS. Subsequently, chemometric evaluation was performed to assess the differences between the samples and to identify significant markers, which are present exclusively or more significantly in one of the fruit species. In parallel, accurate mass of ions in MS and MS/MS spectra, various software packages and online libraries were employed for tentative marker identification. The unsupervised pattern recognition techniques, PCA and supervised pattern recognition techniques, PLS-DA and OPLS-DA, revealed significant differences between components in prepared extracts of lingonberries and cranberries. Clear clustering was found for both polar and non-polar extracts in positive and negative ionization modes. Anthocyanins, especially glycosylated peonidins (peonidin 3-O-glucoside, peonidin pentose) and phosphatidylcholines were identified as markers responsible for lingonberries and cranberries differentiation.

Keywords: cranberries, lingonberries; authenticity, metabolic fingerprinting, U-HPLC–HRMS/MS**Acknowledgement:** This work was supported by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272)

P104

OREGANO QUALITY AND AUTHENTICITY ASSESSMENT EMPLOYING SPME-GC-TOFMS

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The Oregano genus has a number of species and subspecies, where *Origanum vulgare* L., *Origanum heracleoticum* L., *Origanum dictamnus* L. and *Origanum onites* L. are the most common. Because of its unique flavour, oregano finds a wide range of culinary uses, moreover, isolated essential oil is a prized as a constituent of perfumes and cosmetic products, it is also used to improve storage stability of foods or as a functional ingredient; this is due to remarkable antioxidant and antibacterial properties.

The recent survey of oregano spices collected at the British market showed that more than 25% of this commodity is adulterated, mainly by foreign botanical material such as olive and myrtle leaves. To enable screening of oregano available at the Czech market, a rapid authentication strategy based on volatile metabolites profiling has been developed. Solid phase microextraction (SPME) was used for volatiles headspace sampling, gas chromatography mass spectrometry employing time of flight (TOFMS) mass analyser was used for their separation and detection. Large differences between the tested samples were and their clustering was found. For authenticity classification based on volatiles profiling, the variability of authentic oregano species has to be specified.

Keywords: oregano, authenticity, volatiles profiling, SPME–GC–MS

P105 ISOTOPE AND CHEMICAL PROFILING IN FOOD AUTHENTICATION

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Product Authentication Laboratory, a new laboratory located in Lodz, is mostly involved in the food control by employing isotope profiling and NMR profiling of organic compounds. In the case of isotope profiling there are two methods of the isotope ratio measurement: isotope ratio mass spectrometry (IRMS) and nuclear magnetic resonance spectroscopy (NMR). Our laboratory is equipped with isotope ratio mass spectrometer (MAT 253) with elemental analyser (Flash 2000 HT) and nuclear magnetic resonance spectrometer (Bruker 500 MHz) for liquid analysis (one probe with ¹⁹F lock channel is dedicated to the deuteron measurement). Currently we are working on the implementation of isotopic methods enabling quality control and detection of frauds in alcoholic beverages. We develop an innovative control method of rye spirits in order to ensure the quality of Polish vodka. Our research interests are also in the application of NMR for metabolomics, and metabolic profiling in a food analysis. We employ ¹H and ¹³C NMR combined with statistical analysis to examine the authenticity of food samples like honey, juice, oil etc. NMR fingerprints enable the identification of metabolites, the classification of samples according to their botanical and geographical origin and the detection of adulteration. Moreover, we conduct qualitative and quantitative analysis of diet supplements containing omega-3 fatty acids, fish oil and vitamins.

Keywords: food control, isotope profiling, chemical profiling, IRMS, NMR

P106

INTRODUCING THE FOOD FRAUD INITIAL SCREENING MODEL (FFIS)

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Food Fraud is illegal deception for economic gain using food. There are many types of fraud including adulterant-substances, tampering, theft, diversion and gray marketing, simulations, misbranded, and intellectual property rights product counterfeiting. The concept is beginning to be addressed by laws, regulations, standards, and certifications. Regardless of the presence of an actual health hazard, Food Fraud incidents can: negatively impact sales, brand equity, market capitalization; violate regulations such as Sarbanes-Oxley; and even lead to the criminal prosecution of corporate leaders. Emerging regulations and industry standards are requiring risk and vulnerability assessments of Food Fraud as a prerequisite to countermeasures and decision-making systems. These assessments and risk management systems are not familiar food safety tools. It is effective and efficient to utilize an enterprise risk management (ERM) framework, such as developed by the Committee of the Sponsoring Companies of the Treadway Commission (COSO). ERM risk assessment occurs into two stages: (1) a qualitative initial screening followed by (2) a more detailed quantitative assessment. All types of Food Fraud can result in enterprise-wide risks so an enterprise risk management system must cover all types of vulnerabilities. The model developed in this paper addresses the unmet need of the first stage referred to here as the Food Fraud Initial Screening (FFIS).

Keywords: food fraud, economically motivated adulteration, enterprise risk management, food crime, adulteration

P107

HOW CAN LC-HR-MS/MS BE USED TO ANALYZE THE AUTHENTICITY OF YOUR WINE?

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Liquid Chromatography coupled to High Resolution tandem Mass Spectrometry (LC–HR–MS/MS) was used for authenticity analysis of wine. LC–HR–MS/MS enables the profiling of target marker compounds but also non-target (unknown) screening capabilities. HR–MS/MS data acquired in information dependent acquisition (IDA) mode of SWATH[®] MS/MS(ALL) mode contain all information needed to identify unknown markers compounds and quantify them to authenticate food or beverages. Several red and white wines were directly analyzed after dilution using a SCIEX TripleTOF[®] system. The complexity of LC–HR–MS/MS data requires powerful software tools for data mining. The acquired data were analyzed with XCMSplus, an offline version of the most popular software for metabolomic profiling. This software is a powerful tool for statistical data processing. Additionally, the software is connected to a metabolite database which can be used to identify unknown marker compounds.

Keywords: wine, authenticity, LC–MS/MS, high resolution, HR–MS

P108 NON-TARGET AND UNKNOWN SCREENING OF BEER SAMPLES USING LC-HR-MS/MS

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LC-MS/MS is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers limits the number of compound to quantify and identify. In addition there is an increasing demand for retrospective non-target (unknown) data analysis to identify unexpected food residues and contaminants. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run. Here, diluted beer samples were analyzed by LC-HR-MS/MS using the SCIEX QTOF system operated in high resolution accurate mass MS and MS/MS mode. Acquired data were compared against existing pesticide and mycotoxin libraries to identify potential chemical residues or contaminants. In addition, non-target peak finding was used to compare different samples to identify marker compounds characteristic for the brewing and lagering process. Tools built into SCIEX OS software allowed empirical formula finding based on accurate mass MS and MS/MS information. Data processing in SCIEX OS software is quick and allows intuitive data review.

Keywords: beer, authenticity, LC-MS/MS

P109

ARE PORK RESIDUES PRESENT IN MY GUMMY BEARS? GELATIN SPECIATION BY LC-MS/MS

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Following the Food Standards Agency (FSA)'s announcement in January that horse and pig DNA had been identified in beef products sold by several supermarket chains, further testing across Europe and beyond has revealed widespread incidences of such food contamination. This intended adulteration for financial gain or careless false declaration of meat products is a severe problem for consumers who have ethical or religious concerns about the consumption of pork or horse, more specifically the Muslim or Jewish communities who represent about 23% of the worldwide population. As the tolerance level for porcine and equine content in foods is 0%, for religious reasons, the limit of detection (LOD) needs to be as low as possible and so the continued development of more sensitive methods is necessary. However, pork based products are not only used as the meat but can also be found in gelling agents in food (for example in candy, ice cream, and marshmallows) as well as in the cosmetic and pharmaceutical industry in the form of gelatin. Gelatin is made from collagen, a protein, which has been extracted from the skin, bones, and connective tissues of animals such as cows, chicken, pigs, and fish. Here we present the results from the initial development of an LC-MS/MS method utilizing AB SCIEX TripleTOF® 5600 and QTRAP® 4500 LC-MS/MS systems for the determination of the origin of gelatin used in food products and also pharmaceutical capsules.

Keywords: meat speciation, LC-MS/MS, authenticity, pork, horse

P110 DETECTION OF FRAUDULENT BLENDS IN OLIVE OILS BY TRIACYLGLYCEROL FINGERPRINTING

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The main objective was to develop models based on acylglycerol composition to detect fraudulent blends in olive oil. To achieve it, samples were collected and analyzed, and models were developed using chemometrics. Twelve authentic samples of virgin olive oil (VOO), extra virgin olive oil (EVOO) and lampant olive oil (LOO) and 4 different soybean oils, sunflower oils and hazelnut oils, were used to prepare blends mixtures at 5% and 10%, and a latin square experimental design was applied. Oil samples were analyzed by direct electrospray-Ultra high resolution mass spectrometry (ESI-UHRMS) and high performance liquid chromatography-refractive index detector (HPLC-RID). For HPLC-RID, chromatograms were first aligned to correct deviations in the retention time using the icosift algorithm, and then, several data processing strategies were evaluated. PLS regression was developed for each data set (UHRMS and HPLC-RID) and the % of olive oil adulteration, and internally validated by leave-10%-out cross-validation, using SIMCA software (v13.0, Umetrics AB, Umea, Sweden). Root Mean Squared Error of Cross Validation (RMSEcv) was used as criteria to optimize and select models. Finally, ROC curves were calculated with adulteration values predicted by the PLS regression models to establish a decision criteria on the detection of adulteration (IBM SPSS Statistics v 20.0). The best results were obtained by developing independent models for high linoleic seed oils (sunflower and soybean oils) and for high oleic seed oil (hazelnut oil). For sunflower-soybean, all adulterated samples were unequivocally identified as adulterated by the model. Samples providing prediction results above 3.6% would be considered as adulterated, and samples providing values below 3.6% would be considered not-adulterated. For the hazelnut model, predicted values in the range between 2.7 and 6.6% could correspond both to genuine olive oils and to adulterated samples. Therefore, within this range, a decision on sample adulteration could not be raised. However, predicted values above 6.6% could be interpreted as olive oil samples adulterated with hazelnut oil (at least at 10%), and predicted values below 2.7% could be interpreted as genuine olive oils (or oils adulterated below 5%). For the HPLC-RID data, a similar decision criterion was established. In this case, threshold for the detection of samples adulterated with (at least 5% of) sunflower and soybean oil was set at 2.2%. For the detection of hazelnut oil, there was also an uncertainty range from 2.8% to 3.8%.

Keywords: olive oil, PLS regression, Adulteration, UHRMS, triacylglycerol

Acknowledgement: This project was funded by the INSA-UB (Institute of Nutrition and Food Safety)

P111**AUTHENTICATION OF IBERIAN PIG FEEDING SYSTEM BASED ON TRIACYLGLYCEROL PROFILE BY HRMS AND CHEMOMETRICS****Stefania Vichi¹, Alba Tres^{2*}, Juan Maria García-Casco³, Josep Caixach⁴, Francesc Guardiola⁵**^{1 2 5} University of Barcelona, Barcelona, Spain³ INIA-Centro de I+D en Cerdo Ibérico, Zafra (Badajoz), Spain⁴ CSIC-IDAEA, Barcelona, Spain

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The Iberian pigs are raised under different rearing systems and some of their dry-cured products are classified according to that by the recent Spanish regulation in three commercial categories with different quality characteristics (named Cebo, Cebo de campo, and Bellota). The high economic value of these products makes them susceptible to fraudulent commercial practices. To prevent them, analytical determinations capable to differentiate these categories would be very supportive. State-of-the-art strategies in food authentication rely on finding an analytical pattern characteristic for the authentic product. This unique pattern is used as a fingerprint of the authentic product to distinguish it from non-authentic products. In this approach to Iberian ham authentication, the performance of triacylglycerol (TAG) profiling by Ultra High Resolution Mass Spectrometry (UHRMS) was evaluated. Eighty subcutaneous fat samples from Iberian pigs from the different rearing systems were analysed by direct ESI-UHRMS using an Orbitrap-Exacte HCD. Chemometrics was then applied to develop classification models based on Partial Least Squares-Discriminant Analysis (PLS-DA), allowing to successfully discriminate Iberian pigs fed with or without acorns, as well as those reared indoor or outdoor. These results indicate that TAG profiling by direct ESI-UHRMS coupled to chemometric analysis is a promising tool for the authentication of Iberian products. Summary: Triacylglycerol profiling by direct electrospray-ultrahigh resolution mass spectrometry combined with chemometrics was successfully applied to authenticate fat samples from Iberian pigs from different categories according to the rearing systems (named Cebo, Cebo de campo and Bellota).

Keywords: triacylglycerol, profiling, feeding system, chemometrics

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