Assuring the integrity of the food chain: Turning science into solutions

EDITORS
MICHELE SUMAN - ELENA MAESTRI - PAUL BRERETON
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ORGANIZED BY
Ensuring the Integrity of the European food chain (FoodIntegrity)

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

www.foodintegrity2017-parma.eu
https://www.facebook.com/foodintegrity2017parma/
#FoodIntegrityParma17
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Editorial realization by Sale in Zucca.

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(Authors of the single lecture/poster abstracts here published are jointly-responsible for their correspondent submitted content.)

ISBN 9788894106657
We are delighted to have here in Parma many representatives of funding bodies, food retailers, processing food industries, quality brands, public administrations, control laboratories, authorities, NGOs and the research community who work in the food authentication area, to attend the FoodIntegrity Conference 2017.

This conference, integrated within the activities of the European Funded FoodIntegrity project (www.foodintegrity.eu), will focus on the latest research outputs on developments and strategies in the field of FoodIntegrity - safety, quality, authenticity and traceability, from the project and beyond.

Italy and, of course, Parma, has a huge agricultural heritage to be preserved at world level, with the excellence of “Made in Italy” characterized by precise quality and safety but also strong authenticity connotations: all this makes FoodIntegrity a highly strategic project for our Country.

Therefore, it is an honour to host this fourth International Conference FoodIntegrity here. In particular, in this 2017 edition we will give emphasis to direct and indirect impacts on the industrial sector: that is why the event’s subtitle is “Turning science into solutions”!

The conference is organized with keynote lectures, oral and poster sessions, workshops, awards, tasting/instrumental demo sessions, commercial exhibitions and companies user meetings, social program (including a social dinner in an exclusive place, which recalls the tradition of music and the figure of the great Italian composer Giuseppe Verdi).

My best wishes to all the participants for the coming years: work together to make more concrete and applicable the “European/Worldwide toolbox” against food frauds and adulterations. In these next few days, the immediate wish is rather to exchange knowledge-science and friendship, get new ideas and establish new collaborations, enjoying Parma and its wonderful cultural and gastronomic aspects!

Michele Suman (FoodIntegrity 2017 Chair) and the Scientific/Organizing Committee
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www.sciex.com

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www.bruker.com
Elementar Analysensysteme is the world’s leading German manufacturer of analytical instruments for non-metallic elements like carbon, nitrogen, sulfur, hydrogen, oxygen or chlorine in all organic and the majority of inorganic substances. We provide complete solutions for all kind of stable isotope analysis of organic elements like CHNOS. Stable isotope analysis brings unique insight into the origin of an unknown sample and helps to understand for example food adulteration, future climate scenarios and biological processes. Furthermore, we are experienced specialists for CHNOS elemental analysis, Nitrogen/Protein analysis by Dumas combustion, and TOC / TNb analysis for micro and macro samples. Special digestion units and accessories complement the area of non-metallic elemental analysis. Our main application fields cover a wide range from fundamental research to highly specialized quality control.

www.elementar.de

The role of food testing laboratories has never been more critical. Safety and quality are of major concern to consumers, governments and food producers. These groups face a variety of challenges, including a growing number of food contaminants, more stringent regulations, protection of trade markets, preservation of brand image, and increased competition. Waters is a leader in developing food testing systems that integrate column & sample prep chemistries, analytical instrumentation and data management software. Waters comprehensive solutions enable food laboratories to identify diverse chemical compounds, meet compliance requirements, decrease operation costs, increase productivity, and, most importantly, help ensure public safety.

www.waters.com

Romer Labs is a leading global supplier of diagnostic solutions for food and feed safety. Founded in Washington, MO, in 1982, we became over the years a leading provider of diagnostic solutions for the agricultural, food and feed industry.

We offer a broad range of innovative testing solutions and services covering mycotoxins, food pathogens, food allergens, gluten, GMO, veterinary drug residues, and other food contaminants.

Furthermore, we operate 4 accredited, full-service laboratories in Austria, UK, US and Singapore. Using cutting-edge technology in the fields of chromatography and immunological analysis, our labs offer services for the analysis of mycotoxins, food allergens, meat speciation, VDR and GMO. Romer Labs is at the forefront of diagnostic technology and we are constantly expanding our product and service portfolio to meet your continuously evolving demands. Our key objective at Romer Labs is to provide scientifically sound, high-quality products and an exceptional service, in line with our mission – Making the World’s Food Safer®.

www.romerlabs.com
Isotopic Ratio Institute is the private Center of Applied Research on Traceability and develops chemiometric models through isotopic ratio mass spectrometry such as isotopic measurement of heavy elements (Pb, Sr, U).

The Institute is born from the collaboration between Massimo Peruzzo (Eurolab Analysis) and Marco Ferrante (Laboratori Nazionali del Gran Sasso, LNGS). Since 2015 IRI is located in Northern Italy.

OUR VISION: to become a point of reference in the development of innovative traceability models in Italy and in the rest of Europe. Our strength is our know-how of Isotope Ratio Mass Spectrometry. In LNGS (Gran Sasso National Laboratories) we worked on isotope chemistry of heavy elements (Sr and Pb) using instruments for Thermal Ionization Mass Spectrometry (TIMS). Nowadays IRI is collaborating with Ametek-Spectro for the using of Spectro MS instruments in isotopic ratio measurements.

Our strong know-how has made us the most reliable partner in the market of products’ traceability. We are developing projects with some of the main Italian and international companies belonging to food and fashion industries.

In the food market, our aim is to increase the value of raw materials such as Italian tomato, corn and cheese; in the fashion and luxury market we are tracking raw materials against commercial fraud.

www.en.isotopicratio.com

Ocean Optics is the inventor of the world's first miniature spectrometer and a global leader in UV-Vis, NIR and Raman spectroscopy for research, life sciences, food and agriculture, education and OEM applications. Ocean Optics’ extensive line of complementary technologies includes spectrometers, light sources, chemical sensors, optical fibers, thin film coatings, software and complete system integration.

Ocean Optics is focused on helping the food and beverage industry leverage spectroscopy to measure quality, ensure consistency and authenticate real vs. fraudulent products. Ocean Optics modular approach offers the advantages of small, robust portable equipment ideal for monitoring crops or measurements in challenging real world environments.

www.oceanoptics.com

The Food Authenticity Network is a free toolkit for the detection of food fraud that can help to fight food fraud and build a more resilient food supply chain.

The Food Authenticity Network is a UK Department for Environment, Food and Rural Affairs 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 stakeholders have 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.

Please visit our website and sign up (it’s very simple & free to join) to become a member.

www.foodauthenticity.uk
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Open-Co is leader in Italy and one of the first software houses in Europe, specialized in the development and implementation of LIMS systems.

The key factor for a successful LIMS implementation is INTEGRATION. Prolab.Q LIMS platform enables the creation of “strong integration Scenarios” between the different systems of the company IT ecosystem (ERP, MES, WM, etc.).

Open-Co is a company that is steadily growing thanks to great attention to market changes and continuous software development activities in order to create innovative solutions that support our customers in daily challenges.

www.openco.it

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www.ionbench.com

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FOOD & DRINK BUSINESS EUROPE is the senior management magazine, website and newsletter for food and drink manufacturers in the UK and Europe. Over 24,700 copies are circulated to key decision-makers with responsibility for purchasing capital equipment, engineering, filtration, raw materials, logistics, Information Technology, packaging services.

Established for over 20 years Food & Drink Business magazine, website and newsletter has built a reputation in the food and beverage manufacturing sector for providing indepth analysis of the key trends affecting our industry. Our editor, Mike Rohan, has access to the chief executives of the blue chip food and beverage manufacturers across Europe.

The key to the success of the publication’s circulation is the quality of the circulation and readership profile. Food & Drink Business has employed a team of dedicated researchers charged with identifying key decision makers responsible for capital investment, R&D, engineering and new product development.

Request a media pack: colin@prempub.com

www.prempub.com
Who we are: Sale in Zucca is a communication agency whose activities include paper-based publishing, web content design, corporate and institutional communication and scientific dissemination. We lead our clients (companies, public authorities, associations) through all the stages of their projects, from the idea to the realization.

What we do: Content creation, knowledge management, educational projects, website and social media design and updating, press releases, newsletters, house organs, magazines, brochures and any other kind of publication planning, writing and editing.

How we work: We always become passionate about our clients’ stories. No matter if they lead an international corporation or a small family business: we tell such stories with competence, creativity and that “grain of salt” that sometimes makes the difference!

[www.saleinzucca.it](http://www.saleinzucca.it)

SecuringIndustry.com is a free-to-access information service that covers the issues surrounding supply chain and brand security. Our aim is to provide practical advice and intelligence to help manufacturers define and pursue their own strategies for tackling crime including counterfeiting, product diversion, adulteration and theft.

We cover key developments in: coding and track-and-trace technologies; the evolution of data standards; overt, covert and forensic authentication systems; cargo thefts and shipment security; developments in the global regulatory environment; enforcement actions and case studies; and much more...

The site incorporates breaking news and features, researched and written by specialist industry journalists and guest writers, as well as a regularly updated feed of external editorial from the world's press, a comprehensive and intuitive directory of security-related goods, technologies and services, plus the latest market research and events in supply chain security across multiple industrial sectors.

Our editorial mission is to identify the most important and relevant information in supply chain security and bring it into one place for our community of readers, with the ultimate aim of helping to protect the rights and welfare of the public and the interests and reputations of commercial organisations.

[www.securingindustry.com](http://www.securingindustry.com)

Regularly examining the new technologies and developments within the food industry, New Food is the industry's leading bi-monthly magazine and essential reading for anyone involved in the sector.

For the past 15 years, we have been covering the major topics that impact on the sector, including new product development, food processing, analysis, safety, QC/QA and legislation. Here at New Food, we pride ourselves on our editorial integrity. This means we attract the leading industry experts to write on subjects that our readers need to know more about.

New Food is proud to be affiliated with EFFoST, the European Federation of Food Science & Technology, a non-profit association that federates food science and technology organisations throughout Europe. ABC audited, New Food is regularly distributed to 13,594 key decision-makers in the food industry predominantly across Europe.

[www.newfoodmagazine.com](http://www.newfoodmagazine.com)
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www.comune.parma.it

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www.pr.camcom.it

Societa Chimica Italiana Divisione di Spettrometria di Massa

www.spettrometriadimassa.it

ALMA

www.alma.scuolacucina.it
FULL PROGRAMME
# MAY 10\textsuperscript{TH}

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<thead>
<tr>
<th>TIME</th>
<th>TOPIC</th>
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<tr>
<td>07:45 - 08:30</td>
<td>Registration</td>
<td>Local Organizers</td>
</tr>
<tr>
<td>08:30 - 09:00</td>
<td>Official Opening and Welcome, illustration of the agenda</td>
<td>Local Organizers &amp; FoodIntegrity Coordinator, Authorities</td>
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<tr>
<td>09:00 - 09:25</td>
<td><strong>Introduction:</strong> Food Fraud – Stakeholder Implications and Mitigations</td>
<td>Chairs: Christophe Cavin (Nestlé) &amp; Peter Whelan (Food Safety Authority Ireland)</td>
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<tr>
<td>09:25 - 09:50</td>
<td>Food safety implications in the context of food authenticity – Keynote</td>
<td>Barbara Gallani (European Food Safety Authority)</td>
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<tr>
<td>09:50 - 10:15</td>
<td>Strategies of quality management system against food fraud – Keynote</td>
<td>Beatriz Torres (International Featured Standards)</td>
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<tr>
<td>10:30 - 11:00</td>
<td>Turning science into solutions-the role of the FoodIntegrity project</td>
<td>Paul Brereton (Fera Science Ltd)</td>
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<tr>
<td>11:00 - 11:25</td>
<td>New Solutions for Identifying Emerging Risks</td>
<td>Chairs: Lucy Foster (Department for Environment, Food and Rural Affairs UK) &amp; Niels Lucas Luijckx (TNO)</td>
</tr>
<tr>
<td>11:25 - 11:50</td>
<td>Economic aspects about FoodIntegrity &amp; challenges connected to e-commerce – Keynote</td>
<td>Stefano Vaccari (ICQRF/ Italian Ministry of Agriculture)</td>
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<td>11:25 - 11:50</td>
<td>The use of social media: identifying norovirus outbreaks &amp; other future directions – Keynote</td>
<td>Tim Johnston (Food Standards Agency UK)</td>
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<td>11:50 - 12:10</td>
<td>Results of a pan-European survey on fish mislabeling: a citizen science approach</td>
<td>Miguel Ángel Pardo González (AZTI Tecnalia)</td>
</tr>
<tr>
<td>12:10 - 12:30</td>
<td>Early warning system and food big data: from supply chain surveillance to fraud detection</td>
<td>Vahid Mojtahed (Fera Science Ltd)</td>
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<tr>
<td>12:30 - 12:50</td>
<td>Predicting food fraud- a Bayesian Network approach</td>
<td>Hans Marvin (RIKILT Wageningen UR)</td>
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“Capturing an FI2017 screenshot“: photo all together!”
12:50 - 14:30
Lunch break
Posters View
Demo Corner
Virgin olive oil tasting session: the nose – the most advanced analytical instrument
Vendor seminars

SESSION 3
Parallel Session (A): Rapid & Confirmatory Analytical Solutions

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<th>TIME</th>
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<tr>
<td>14:30 - 14:55</td>
<td>Rapid authentication of coffee blends: old problem, new methods - Keynote</td>
<td>Luciano Navarini (illycaffé)</td>
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<tr>
<td>14:55 - 15:15</td>
<td>What is the origin of this garlic? Metabolomic fingerprinting employing high resolution mass spectrometry may give a rapid answer</td>
<td>Jana Hajslova (University of Chemistry and Technology, Prague)</td>
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<tr>
<td>15:15 - 15:35</td>
<td>A rapid universal tool for colorimetric authentication of food combining nanotechnology with DNA barcoding</td>
<td>Paola Valentini (IIT)</td>
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</table>
15:35 - 15:55  Carotenoid profiling as tool for verifying egg production claims in the UK  Katharina Heinrich (Fera Science Ltd)

15:55 - 16:15  Targeted / untargeted approaches and data handling. How to support authenticity of food and raw materials  Caterina Durante (Chemstamp)

16:15 - 16:35  Physi-Trace: rapid origin traceability of Australian pork products  Garry Lee (Food Science Solutions Perth Australia)

16:35 - 16:55  Foodintegrity with Isotope Fingerprints: unlocking the truth  Christopher Brodie (Thermo Fisher Scientific)

**Parallel Session (B) - Workshop:**

**Good risk management requirements, trust in labels-claims & best practices**  Organized & Chaired by Federica Camin (Fondazione Mach)

**1st Part (14:30-15:30) – Short Talks**
- Fabio Del Bravo (Ismea)
- Rosa Vano (Castillo de Canena)
- Gerald A. Herrmann, (Organic Services)
- Marco Nocetti (Consorzio Parmigiano Reggiano)
- Massimo Sacco (Acetificio Ponti)
- Francois Guyon (Scl.Finances.Gouv)

**2nd Part (15:30-17:00) - Interactive Exercise (world cafe):**
- Three discussion tables will be organized (1. PDO foods; 2. PGI foods; 3 organic foods).
- People will be divided in 3 group who will visit each table for 20 minutes discussing each time a different issue (economic importance, promotion, protection)
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<tr>
<td>17:00 - 17:30</td>
<td>Coffee break</td>
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| 17:30 - 18:50 | **“Fast” Science** *(8’ speed presentations)* | Chairs: 
Elena Maestri (Siteia.Parma) & Jean Francois Morin (Eurofins) |

**First outcomes from new FoodIntegrity Work Packages projects launched in 2016:**

- A gradual step-by-step process for advancing knowledge on NIRS sensors technology for rapid, in situ and cost-effective authentication of Acorn Iberian Ham (Jamón iberico de Bellota)
- Fish Identification Software Hub (F.I.S.HUB) Classifier
- Other new WPs short presentations in potential addition

**Inspiring presentations from 6 young FoodIntegrity scientists (Young Researchers Award):**

- Non-target screening strategy based on GC-Q-TOF for Scotch whisky authentication
  - A novel approach to authenticate whole and refined grain durum wheat *(Triticum durum Desf.)* based on untargeted lipidomics
- Non-Targeted Methods guidance – Overview of draft USP guidance

Ana Garrido Varo  
(University of Córdoba)

Francesco Rossi  
(Politecnico di Torino)

Michal Stupak  
(University of Chemistry and Technology Prague)

Laura Righetti  
(University of Parma)

Kenny Xie  
(United States Pharmacopeia)
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<th>TIME</th>
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<tr>
<td>20.00 - 23.00</td>
<td>Conference Dinner / Social Event [Corale Verdi Restaurant - Parma]</td>
<td>Sara Erasmus (Stellenbosch University South Africa)</td>
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<td>Barbara Prandi (University of Parma)</td>
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<td>Telmo Fernandes (Universidade do Porto)</td>
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<td></td>
<td>• Volatile fingerprinting using PTR-MS paves the way for South African</td>
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<td></td>
<td>lamb to acquire PGI status</td>
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<td></td>
<td>• Food fraud detection through species-specific marker peptides</td>
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<td></td>
<td>• COIBar-HRM as a novel approach for the discrimination of hake species</td>
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<td>23.00 - 01.00</td>
<td>“Disco FoodIntegrity”: Young (&amp; “Less Young”!) Night Entertainment</td>
<td>Sara Erasmus (Stellenbosch University South Africa)</td>
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<td></td>
<td>Event [StarHotel - Parma]</td>
<td>Barbara Prandi (University of Parma)</td>
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<td>Telmo Fernandes (Universidade do Porto)</td>
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<td></td>
<td>(free entrance)</td>
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<td></td>
<td>Parallel Session (C) - Workshop: FoodIntegrity 2020 Challenges -</td>
<td>Organized &amp; Chaired by Saskia Van Ruth (Wageningen University and Research)</td>
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<td></td>
<td>Unresolved and Emerging gaps</td>
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### MAY 11TH

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<tr>
<th>TIME</th>
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<th>SPEAKER</th>
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| 9:00 - 11:00 | • This Workshop will be based on an interactive debate and online voting to identify the major unresolved and emerging gaps in assuring the integrity of the food chain  
• Procedure of the first FoodIntegrity gap analysis, gaps covered in the FoodIntegrity & other EU projects, continuing/unresolved gaps. | Introduction to online voting tool:  
Kristian Holst Laursen (University of Copenhagen)  
• The consumer perspective: Camille Perrin (BEUC – The European Consumer Organization)  
• The industry perspective: Christophe Cavin (Nestlé)  
• The authorities perspective: Chris Vansteenkiste (Europol)  
Panel discussion with: Chris Elliott (Queens University Belfast), Quincy Lissaur (SSAFE) and the audience moderated by Georg Melzer-Venturi (Eutema) and Francis Butler (University College Dublin) |

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#### SESSION 7

**Parallel Session (D) Consumer Issues: Quality vs Convenience – Satisfying Consumer Needs**

**Chairs:** Robert Home (FIBL) & Claudia Berti (Barilla)

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<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:00 - 09:25</td>
<td>Counterfeiting of food &amp; public health impact: priority for EU Law Enforcement Agencies - Keynote</td>
<td>Sergio Tirro (Carabinieri NAS)</td>
</tr>
<tr>
<td>09:25 - 10:05</td>
<td>“Journalistic Video-Shock-Talk” on food frauds &amp; food crime - Keynote</td>
<td>Luca Ponzi (RAI Radiotelevisione Italiana)</td>
</tr>
<tr>
<td>10:00 - 10:20</td>
<td>Food or Wildlife? Consumers’ poor knowledge of fish appearance hampers seafood market transparency and sustainability</td>
<td>Stefano Mariani (University of Salford, Manchester, UK)</td>
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<tr>
<td>10:05 - 10:25</td>
<td>Chinese consumer attitudes to European products</td>
<td>Lynn Frewer (Newcastle University)</td>
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<tr>
<td>10:25 - 10:45</td>
<td>Olive oil trade standards inside and outside EU: analysing differences</td>
<td>Diego Luis Garcia-Gonzalez (CSIC)</td>
</tr>
<tr>
<td>10:45 - 11:05</td>
<td>Gastronomy, Culture &amp; Integrity. Food identity: characteristics and evolutions</td>
<td>Luca Govoni (ALMA Scuola Internazionale di Cucina Italiana)</td>
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<tr>
<td>11:00 - 11:30</td>
<td>Coffee break</td>
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<tr>
<td>11:30 - 11:55</td>
<td>Foodintegrity: a global perspective - Keynote</td>
<td>Chris Elliott (Queen's University Belfast)</td>
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<tr>
<td>11:55 - 12:15</td>
<td>Overview of the EU challenges for food fraud</td>
<td>Eric Marin (DG SANTE European Commission)</td>
</tr>
<tr>
<td>12:15 - 12:35</td>
<td>Role of analytical testing for food fraud risk mitigation – how much is enough</td>
<td>Francis Butler (University College Dublin)</td>
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<tr>
<td>12:35 - 12:50</td>
<td>The EU H2020 OLEUM Project: state of play and first advancements</td>
<td>Tullia Gallina Toschi (University of Bologna)</td>
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<td>12:50 - 13:10</td>
<td>Poster Awards</td>
<td>Chairs of FI2017 &amp; FI Coordinator</td>
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<td>13:10 - 14:30</td>
<td>Lunch break</td>
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<td>14:30 - 17:30</td>
<td>Visit to Barilla Plant (only for registered participants in advance - limited number)</td>
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Invitation to Demo Corner
Demonstration of approaches developed by the FoodIntegrity for food authentication

10 & 11 May, 2017 • 13:30-14:30

What can you learn?

- FoodIntegrity Knowledge base: An information resource on food authenticity, description of the database and demonstration of its functionality
  
  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
  
  Oral explanation on-site from 13:30 to 14:00

- Industrial perspective of relevant food chains vulnerabilities vs Current analytical methods and technologies that can be applied
  
  Infographics in your mobile devices to bring together available data on industrially exploited analytical tools for detection of food fraud, and identify reliable indicators/markers

- Chinese consumer attitudes to food fraud, short description of the survey and its outcomes
  
  Video on examination of Chinese consumers’ attitudes and perceptions towards the safety and integrity of imported European foods

- Investigation of available and potential future technologies for authentication of branded spirits and/or categories
  
  “Hands on” demonstration of authentication of spirit drinks
  
  Sensory analysis of whisky

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

  Specific Virgin olive oil tasting session: The nose - the most advanced analytical instrument will be organised on 10th May, 13:45–14:15. In this session, the differences in the aroma of samples of virgin olive oils and their relation to quality and authenticity will be presented. It will be your opportunity to learn more about one of the most appreciated food products in the Mediterranean diet and put some authenticity and integrity issues in practice.

  Do you really think you know the fish you eat?

  Humans have eaten fish for millennia, but post-industrial urbanisation has weakened the cultural links between wild living resources and consumers.

  We argue that educating societies about the diversity of species that still underpin a significant portion of our foods is a key step to instil awareness in consumers and achieving seafood sustainability.

  Here you can test your own ability to recognise fish species that are mainstays of European markets.

- Do you wish to participate in the FoodIntegrity events?

- Do you wish to be trained in food authentication strategies?

  Join us at the Demo Corner and learn more on opportunities we offer!

How to get involved?

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

You may register on-site for the project COMMUNICATION or 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.

www.foodintegrity.eu

The project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.
WORKSHOPS
WORKSHOP: GOOD RISK MANAGEMENT REQUIREMENTS, TRUST IN LABEL CLAIMS & BEST PRACTICES

The workshop will deepen the themes of economic significance and importance of label claims, and will give to stakeholders and official bodies guidelines for promoting trust in premium products.

DATE & TIME
10th May
2.30 pm – 5.00 pm

PROGRAMME

1st PART (2.30-3.30 pm) – Short Talks [8-10 minutes]

Speakers:
- Fabio Del Bravo (Ismea, Italy):
  “Economic role, trend and evolution of PDO/PGI products in Italy and Europe”.
- Rosa Vano (Castillo de Canena, Spain):
  “Importance of labelling, consumer sensitivity and quality”.
- Gerald A. Herrmann (Organic Services GmbH, Germany):
  “Italy’s Organic Grain Sector comes together and implements an Integrity Management System that combats food fraud”.
- Marco Nocetti (Consorzio Parmigiano Reggiano, Italy):
  “Analytical tools for protecting the PDO cheese Parmigiano Reggiano”.
- Massimo Sacco (Acetificio Ponti, Italy):
  “Tools for protecting the IGP Aceto Balsamico di Modena”.
- Francois Guyon (scl.finances.gouv.fr):
  “Control of wine authenticity: a Label Story!”

2nd PART (3.30-4.30 pm) – Interactive Exercise (World Cafe)

The World Cafe is an easy-to-use method for creating a living network of collaborative dialogues. It is built on the assumption that “People already have within them the wisdom and creativity to confront even the most difficult challenges; that the answers we need are available to us; and that we are Wiser Together than we are alone”.

Three discussion tables will be organised:
1. PDO foods;
2. PGI foods;
3. Organic foods.

People will be divided in 3 groups who will visit each table for 20 minutes discussing each time a different issue (economic importance, promotion, protection).

Moderator-Animator: Federica Camin (FEM), and Simon Kelly (IAEA-FAO).

CONCLUSIONS (4.30-5.00 pm)

Each table leader give a short summary of the discussion.
WORKSHOP: FOODINTEGRITY 2020
CHALLENGES – UNRESOLVED
AND EMERGING GAPS

Interactive debate and online voting to identify the major unresolved and emerging gaps in assuring the integrity of the food chain.

DATE & TIME
11th May
9.00 am – 11.00 am

PROGRAMME

• **Introduction:** Procedure of the first FoodIntegrity gap analysis, initially identified gaps, gaps covered in the FoodIntegrity project and other EU projects, and continuing and unresolved gaps: **Saskia van Ruth** (Wageningen University and Research).

• **Introduction to online voting tool:** **Kristian Holst Laursen** (University of Copenhagen).

• **Keynote speakers – Continuing, unresolved, and emerging gaps**
  - The consumer perspective: **Camille Perrin** (BEUC – The European Consumer Organization).
  - The industry perspective: **Christophe Cavin** (Nestlé).
  - The authorities perspective: **Chris Vansteenkiste** (Europol).

• **Panel discussion** with Chris Elliot (Queens University Belfast), Quincy Lissaur (SSAFE) and the audience moderated by Georg Melzer-Venturi (Eutema) and Francis Butler (University College Dublin).

• **Close and wrap up of workshop findings:** **Saskia van Ruth** (Wageningen University and Research).
ORAL SESSIONS

L1 FOOD SAFETY IMPLICATIONS IN THE CONTEXT OF FOOD AUTHENTICITY
   B. Gallani

L2 STRATEGIES OF QUALITY MANAGEMENT SYSTEM AGAINST FOOD FRAUD
   B. Torres

L3 FOOD FRAUD: INTELLECTUAL PROPERTY ASPECTS
   AND BUILDING NATIONAL AWARENESS OF FOOD CRIME
   H. Watkins

L4 TURNING SCIENCE INTO SOLUTIONS: THE ROLE OF THE FOODINTEGRITY PROJECT
   P. Brereton

L5 ECONOMIC ASPECTS ABOUT FOODINTEGRITY & CHALLENGES CONNECTED TO E-COMMERCE
   S. Vaccari

L6 THE USE OF SOCIAL MEDIA:
   IDENTIFYING NOROVIRUS OUTBREAKS & OTHER FUTURE DIRECTIONS
   T. Johnston

L7 RESULTS OF A PAN-EUROPEAN SURVEY ON FISH MISLABELLING:
   A CITIZEN SCIENCE APPROACH
   M.A. Pardo, E. Jiménez, B. Pérez-Villareal, K. Ólafsson, G. Ólafsdottir, J.R. Viðarsson

L8 EARLY WARNING SYSTEM AND FOOD BIGDATA:
   FROM SUPPLY CHAIN SURVEILLANCE TO FRAUD DETECTION
   V. Mojtahed

L9 PREDICTING FOOD FRAUD - A BAYESIAN NETWORK APPROACH
   H. Marvin, Y. Bouzembrak

L10 RAPID AUTHENTICATION OF COFFEE BLENDS: OLD PROBLEM, NEW METHODS
   L. Navarini

L11 WHAT IS THE ORIGIN OF THIS GARLIC?
   METABOLOMIC FINGERPRINTING EMPLOYING HIGH RESOLUTION
   MASS SPECTROMETRY MAY GIVE A RAPID ANSWER
   J. Hajšlova, V. Hrbek, M. Rektorisova, H. Chmelarova, J. Ovesna

L12 A RAPID UNIVERSAL TOOL FOR COLORIMETRIC
   AUTHENTICATION OF FOOD COMBINING NANOTECHNOLOGY WITH DNA BARCODING
   P. Valentini, P.P. Pompa, A. Galimberti, V. Mezzasalma, M. Casiraghi, M. Labra, F. De Mattia

L13 CAROTENOID PROFILING AS TOOL FOR VERIFYING EGG PRODUCTION CLAIMS IN THE UK?
   K. Heinrich, C. Pye, J. Donarski

L14 TARGETED / UNTARGETED APPROACHES AND DATA HANDLING.
   HOW TO SUPPORT AUTHENTICITY OF FOOD AND RAW MATERIALS
   C. Durante, M. Cocchi, M. Li Vigni, D. Bertelli, A. Marchetti, P. Lambertini, S. Michelini
L15 PHYSI-TRACE: RAPID ORIGIN TRACEABILITY OF AUSTRALIAN PORK PRODUCTS
G. Lee, J. Watling

L16 FOODINTEGRITY WITH ISOTOPE FINGERPRINTS: UNLOCKING THE TRUTH
C. Brodie

L17 ECONOMIC ROLE, TREND AND EVOLUTION OF PDO/PGI PRODUCTS IN ITALY AND EUROPE
F. Bravo

L18 EXTRA VIRGIN OLIVE OIL LABELLING- “STORY TELLING”
R. Vaño

L19 CHECK ORGANIC: ENSURING THE INTEGRITY OF THE ORGANIC FOOD SUPPLY CHAIN
G. Herrmann

L20 ANALYTICAL TOOLS FOR PROTECTING THE PDO CHEESE PARMIGIANO REGGIANO
M. Nocetti

L21 SCIENTIFIC METHODS TO ASSURE INTEGRITY OF BALSAMIC VINEGAR
M. Sacco

L22 CONTROL OF WINE AUTHENTICITY: A LABEL STORY!

L23 A GRADUAL STEP-BY-STEP PROCESS FOR ADVANCING KNOWLEDGE ON NIRS SENSOR TECHNOLOGY FOR RAPID, IN SITU AND COST-EFFECTIVE AUTHENTICATION OF ACORN IBERIAN HAM (JAMÓN IBÉRICO DE BELLOTA)
A. Garrido-Varo, D.C. Peréz-Marín, C. Riccioli, E. De Pedro

L24 FISH IDENTIFICATION SOFTWARE HUB (F.I.S.HUB) CLASSIFIER
F. Rossi, A. Benso, C. Gkoumpili, I. Coscia, S. Mariani, V. Campia, P.L. Acutis, P. Ubaldi, L. Magnani

L25 NON TARGET SCREENING STRATEGY BASED ON GC-Q-TOF FOR SCOTCH WHISKY AUTHENTICATION
M. Stupak, M. Tomaniova, J. Hajslova, I. Goodall

L26 A NOVEL APPROACH TO AUTHENTICATE WHOLE AND REFINED GRAIN DURUM WHEAT (TRITICUM DURUM DESF.) BASED ON UNTARGETED LIPIDOMICS
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L27 NON TARGETED METHODS GUIDANCE - OVERVIEW OF DRAFT USP GUIDANCE
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L28 VOLATILE FINGERPRINTING USING PTR-MS PAVES THE WAY FOR SOUTH AFRICAN LAMB TO ACQUIRE PGI STATUS
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L31  COUNTERFEITING OF FOOD & PUBLIC HEALTH IMPACT: PRIORITY FOR EU LAW ENFORCEMENT AGENCIES  
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L32  CIBO CRIMINALE (CRIMINAL FOOD)  
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L33  FOOD OR WILDLIFE? CONSUMERS’ POOR KNOWLEDGE OF FISH APPEARANCE HAMPERS SEAFOOD MARKET TRANSPARENCY AND SUSTAINABILITY  

L34  CHINESE CONSUMER ATTITUDES TO EUROPEAN PRODUCTS  
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L35  OLIVE OIL TRADE STANDARDS INSIDE AD OUTSIDE EU: ANALYSING DIFFERENCES  

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L37  FOODINTEGRITY: A GLOBAL PERSPECTIVE  
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L38  OVERVIEW OF EU CHALLENGES FOR FOOD FRAUD  
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L39  ROLE OF ANALYTICAL TESTING FOR FOOD FRAUD RISK MITIGATION - HOW MUCH IS ENOUGH  
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L40  THE EU H2020 OLEUM PROJECT: STATE OF PLAY AND FIRST ADVANCEMENTS  
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ENSURING THE AUTHENTICITY & SAFETY OF FOOD WITH NEW ADVANCES IN LC-MS/MS WORKFLOWS – BRINGING ROUTINE CLOSER THAN EVER

ORGANIZED BY:

SCIEX

DATE & TIME
10th May
1.00 – 2.00 p.m.

SPEAKER
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ABSTRACT

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 or allergen free? 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 result 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. We will also highlight new developments from SCIEX in routine methods for the authenticity of meat and allergen detection in foodstuffs using LC-MS/MS.
RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY: AN EMERGING DISRUPTIVE TECHNOLOGY FOR FOOD CHARACTERISATION?

DATE & TIME
10th May
1.00 – 2.00 p.m.

SPEAKER
Dr Sara Stead
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ABSTRACT
Mass spectrometry has traditionally been one of the ‘last resorts’ for food quality and composition analysis. While gas chromatography-MS (GC-MS), GC isotope ratio-MS (GC IR-MS), and liquid chromatography-MS (LC-MS) are widely used for food and agricultural product analysis, MS methods (including these) are generally considered to be slow, expensive and not amenable for routine application, mostly due to laborious sample preparation procedures. The advent of ambient ionization mass spectrometric methods remove most of the constraints associated with sample preparation and opened new opportunities for point-of-control monitoring.
Since ambient ionization MS (AIMS) methods require minimal or no sample preparation, the use of internal standards (or even external calibrators) is often impossible, resulting in the lack of quantitative information provided by these methods. Nevertheless, the spectral profiles are highly characteristic of the type, origin, age, etc. of the sample, which makes these approaches excellent for rapid profiling analysis. In these cases the MS spectral information is used as a ‘fingerprint’ for the identification of critical attributes associated with both the genetic origin and environmental exposure of the sample.
Rapid Evaporative Ionization MS (REIMS) was originally developed as a direct combination of electrosurgery (surgical diathermy) and MS, for the intraoperative identification of cancerous tissue and surgical margin control. However, it has become clear from extensive collaborative studies with the food testing industry that the method can equally be used for the instantaneous characterisation of meat and fish as well as practically any water-containing food commodity and has potential for the development of an automated at-line testing platform.
Proof-of-principle applications have been developed addressing various food quality and composition testing requirements, e.g. detection of undeclared ingredients in processed foods and establishing authenticity of various products, e.g. Protected Designation of Origin (PDO) status dairy products, processed meats, farming production method (organic vs. conventional), geographical origin of pistachio nuts and botanical origin of monofloral honey.
SOCIAL PROGRAMME
CONFERENCE DINNER

DATE & TIME
10th May
20.00 p.m.

Parma is renown in the world for at least two reasons: good music and good food. We decided to bring them together for the Conference dinner, on May 10th, which will take place in Corale Verdi.

Corale Verdi is one of the most popular restaurants in town; it owes its fame to its traditional menu full of local delicacies (hand made stuffed pasta, cold cuts...), but also to the strong connection to the town's traditional appreciation for music.

Its name, that means Verdi's Choir, is due to the fact that the restaurant is also the seat of an actual Choir: an estimated musical institution founded in 1905 and well known for its deep knowledge and love of Giuseppe Verdi's works.

Walking beside the restaurant, it is not unusual to hear pleasant voices singing Opera arias! Also, the great conductor Arturo Toscanini (1867-1957) used to live just a few metres away from the Corale, and his birthplace is now a museum.

The building – which dates back to the early 19th century, when it was a Ducal property – includes a shaded, confortable yard where people love to gather to spend evenings playing games and enjoying a drink. The neighborhood of the restaurant is quite charming too: it lies right between the green meadows of Parco Ducale and the old district of Oltretorrente with its multi-coloured houses and lively, cozy alleys.
DISCO FOODINTEGRITY

**DATE & TIME**
10th May
23.00 p.m.

The Foodintegrity 2017 social event is more than a dinner! At about **11.00 pm, we will return back to the Starhotel for an unforgettable night**. Dj Davidino and his staff will create a **fantastic atmosphere** with lights, sounds and music: a party that you cannot miss and a good opportunity for chatting, drinking and... **dancing**.

The event is **open to all the registered people**, and the entrance is free. For a couple of hours, forget the conference formalism: we want you on the dance floor.

**Let the science move your body!**

VISIT TO BARILLA PLANT

**DATE & TIME**
11th May
03.00 - 05.30 p.m.

The Pedrignano district hosts Barilla headquarters, R&D and Pilot plants, three plants, a mill and the central warehouse.

The district employs 1,600 people and it is the biggest pasta plant since 1969.

The organizers will give to FoodIntegrity 2017 Conference participants and accompanying persons the opportunity of a guided tour.

Those who want to join the visit need to subscribe.

**Please note:** the visit is reserved to FoodIntegrity 2017 Conference participants and accompanying persons. Registration will close once the maximum number of participants (100) is reached.
ORAL SESSIONS
FOOD SAFETY IMPLICATIONS IN THE CONTEXT OF FOOD AUTHENTICITY

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Keywords: food, safety, authenticity, risk.
Whilst food authenticity is fundamentally a risk management problem, it does impact on risk assessment through the potential consumer health risks that it can raise. These can be direct risks, through the substitution of food grade materials by cheaper non-food grade materials, or indirectly by substituting one food ingredient by another (whilst specifically not declaring this), which may lead to changes in exposure to hazards as well as loss of traceability and increase in associated risks.
Motivation for fraudulent behaviours are many, but are mostly driven by considerations of cost and profit. These considerations come to the fore when costs can be transformed into profit, for example, as was the case in the Chinese melamine incident of 2008.
Specifically concerning EFSA, the main activity in this area is through the Emerging Risks identification process where it is recognised that fraudulent behaviour is an important driver for introducing new risks into the food chain. Here, EFSA maintains a system based on information exchange networks to anticipate the introduction of novel risks into the food chain.
In addition to food authenticity per se, the same concerns can be applied to all material used in the production of food, including packaging and other food contact materials as well as other substances such as pesticides. In all these cases, the use of non-authentic material can introduce significant risks into the foodchain.
In the wider context, concerns are not only limited to consumer safety but such fraudulent behaviours can also impact on animal and plant welfare and the environment, as is the case for adulterated pesticides, and potentially for mis-labelled fish species or mis-labelled organic/free range products.
Whilst addressing food authenticity is clearly a risk management responsibility, EFSA has in place a process to ensure it is prepared to support the risk manager on addressing both well-known and emerging risks to human, plant, animal and environmental risks coming from such activities.
STRATEGIES OF QUALITY MANAGEMENT SYSTEM AGAINST FOOD FRAUD

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Keywords: authenticity, standards, food safety management, supply chain.

IFS began in 2003 under the name International Food Standard. The focus at the time was to ensure food safety and process optimization. IFS has remained true to these principles and have since expanded its portfolio which now comprises seven different standards and two programs. The aim of these Standards is to help industry prevent food outbreaks and product recalls, optimize processes and facilitate the sale of compliant products. IFS is applicable in the whole supply chain (with the exception of pre-farm gates), covering the fields of food processing, logistics, trade, packaging as well as the production of household and personal care products. Currently, there are almost 20,000 certified companies worldwide against one of the seven standards. IFS is responsible for providing a global tool for the assessment and certification of the safety and quality of products. IFS offers all companies that are part of the supply chain a clear-cut certification of all common quality and safety aspects of their product ranges and/or services. Nine out of ten of the largest European food retailers, many other retailers worldwide, accept the IFS standards. IFS Food version 6 already contains requirements related to food authenticity.

The food industry needs guidance to control these threats and what’s more to avoid making further and costly mistakes in their own facilities. The most recently updated standard “Wholesale / Cash & Carry” includes requirements directly related to Food Fraud and product authenticity, intended to strengthen the company’s product safety management system. The vulnerability assessment enables the evaluation of risks considering different aspects of the purchased products, the supply chain and the food treatment. One of the main objectives is also to place the focus of the companies on the point that they have the responsibility to check the authenticity (incl. fraud) of their raw materials and/or semi-finished products and/or finished products in order to minimize the risk of operating sites purchasing fraudulent or adulterated raw materials. For the next version of IFS Food the topic of food fraud/authenticity will be even more strengthen.
FOOD FRAUD: INTELLECTUAL PROPERTY ASPECTS AND BUILDING NATIONAL AWARENESS OF FOOD CRIME

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Keywords: Food Crime, Fraud, Collaboration, Awareness.

The UK has one of the safest food industries in the world. This session will consider how improving the awareness of the public, increasing industry engagement, promoting the use of academic experts and moving towards a collaborative approach can improve consumer safety. Huw Watkins, a former senior police detective, joined the UK Government department responsible for intellectual property. He will consider how the events of 2012, the Czech methanol scandal, the 2013 ‘Horsegate’ case, and subsequent reports and changes in the UK led to a greater awareness of the problem of food fraud involving counterfeit or substandard food and drink.
TURNING SCIENCE INTO SOLUTIONS: THE ROLE OF THE FOODINTEGRITY PROJECT

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Keywords: Food fraud, food authenticity, EU RTD, Seventh Framework Programme.

FoodIntegrity is a large pan-European research and networking activity sponsored primarily by the European Commission through its Seventh Framework Programme. The €12M (€9M EU) interdisciplinary project aims to develop improved networks, systems and tools for assuring the integrity of the food supply. Comprising 60 participants from Europe, Asia and South America, the project that started in 2014 is currently 2/3 through its 5 year programme. Key outputs to date are:

• The development of a European Network of >230 members from 43 countries
• The production of an interactive Knowledge Base that end users can interrogate to identify food authenticity issues mapped against the methods and databases that can be used to detect them
• An extensive analysis of the current gaps in the current state of the art for detecting and preventing food fraud
• An inventory of the key issues, methods and standards concerned with the authentication of olive oil and spirits
• Case studies of potential implementation of testing methods in the food industry
• A study looking at Chinese consumer attitudes to European food products
• A “Citizen Science” study looking at fish mislabeling in the restaurant sector
• Eight new research activities that started in 2016
• 1 scientific opinion, 4 international conferences and 4 international workshops

In the remaining 20 months of the project FoodIntegrity will deliver:

• State of the art Early Warning Systems
• A protocol on validating non targeted methods of analysis
• Methods for analyzing complex foods
• Food authentication using mobile phone technology
• Feasibility studies on transparency along the food chain
• Implementation of rapid methodology in the pork sector
• 6 more Scientific Opinions on key FoodIntegrity issues
• White paper on verification of FoodIntegrity in cases where analytical methods are not applicable
• Recommendations on future research
• Extensive dissemination and knowledge transfer activities

Acknowledgments: The author would like to acknowledge the 20 workpackage leaders and the large number of scientists that contribute to FoodIntegrity from the 60 organisations that participate in the project.
ECONOMIC ASPECTS ABOUT FOODINTEGRITY & CHALLENGES CONNECTED TO E-COMMERCE

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**Keywords:** ICQRF, EU Heritage, Protection, web Control.

The concept of “food” has evolved in new ways in the last few years. The goal of “safe food” from the health point was accompanied by that of “food “identity “, capable, as stated in the Milan paper, to play an important role in defining each person’s identity and is a cultural component that describes and gives value to a territory and its inhabitants. A relevant indication of this perspective is the reg. EU 1151/12, which defines geographical indications’ union cultural heritage “.

Protecting the integrity of the food is thus the protection of all components, both tangible and intangible, of the food. In economic terms this shifts the focus of increasing producers’ ability to protect its intellectual property rights, including those of IG.

The ICQRF, one of the largest European authorities for fraud prevention and detection in food, has played a powerful action in recent years to the protection of “Food identity”. With more than 1,700 actions of protection across national borders and on the web it was built a series of actions that has few comparisons in the world.

Protecting the integrity of the food web implemented dall’ICQRF gave interesting and unique results in Europe: European platforms Ebay and Alibaba are now very low levels of irregularities to the Italian quality products and just as you are doing with Amazon.

Cooperation with the relevant authorities in individual European countries made it possible to block products that evoked Italian protected food quickly and effectively.

ICQRF is working to extend the protection to web platforms in the Chinese language, despite language difficulties.

Upcoming developments are the actions on social networks and increasingly careful action of web marketing channels located outside Europe.
L6
THE USE OF SOCIAL MEDIA: IDENTIFYING NOROVIRUS OUTBREAKS & OTHER FUTURE DIRECTIONS

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Keywords: Norovirus, Social Media, Twitter.

Norovirus is a gastro-intestinal virus that causes diarrhoea and vomiting. It lasts for 2 days, with an infectious period for up to 48 hours after that. It is transmitted person-to-person, person-to-food, and food-to-person.

Public Health England publish laboratory case volumes, but with a time lag. By the time the data is available, the spike was likely to have passed, so the impact of any intervention would be reduced. We wanted to be able to pre-empt and prepare for any rise in cases.

The Food Standards Agency discovered a correlation between Tweets containing words relating to Norovirus symptoms, and developed a model to highlight when an outbreak is unfolding. The project started as a Masters project with a student from the London School of Economics and Political Sciences. The Model uses logistic regression, taking the changes in the volumes of Tweets containing words relating to Norovirus symptoms to predict the probability of an outbreak occurring or not. It is run every week over the winter months. The language used on Twitter means there is a lot of ‘noise’ within the system, so part of the requirement of the model is to filter out, as far as possible, any Tweets that have links to being ill that are unrelated to Norovirus – such as those related to alcohol!

If an outbreak is detected, a joint intervention is initiated within the Food Standards Agency, and the National Health Service choices website, to give patients advice about action to take to help prevent further spread.
RESULTS OF A PAN-EUROPEAN SURVEY ON FISH MISLABELLING: A CITIZEN SCIENCE APPROACH

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Keywords: seafood, mislabeling, fraud, Citizen Science

Introduction
In the European Union the identification of seafood is mandatory, 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 seafood labels indicate the complete scientific name of the species (i.e. genus and species, Latin binomial nomenclature) without inducing errors and in order to ensure a high level of protection for human health. Recent studies have stated that the average percentage of reported misdescription is 20-30%, but the vast majority of studies have focused at retail level and studies focused on restaurants are poorly represented [1]. As a matter of fact, the purpose of this study was to identify the % of restaurants serving fish that does not fit which is detailed in the menu in Europe. For that reason a specific survey was focused on HORECA sector (restaurants, hotels, catering, self-services, bar, pub, take away, etc...) all over Europe.

Methodology
Fish survey was performed from 2015 to 2016. Sampling plan was designed at random stratified according to % of fish consumption (kg/capita/year) in 23 countries. Taking advantage of citizen science, we have involved more than 100 amateur or nonprofessional scientists and scientists as collectors of fish samples from European restaurants. These samples were collected following a detailed protocol following previous and similar fish surveys [2, 3]. All the confidence intervals (α = 0.05) were calculated using Wilson’s method [2]. Samples were managed following the directive UNE-EN-ISO 9001:2000 and subsequently analyzed with standard protocols, based on DNA sequencing of mitochondrial cytochrome oxidase subunit I (COI) gene with COI universal primers validated in different European and international projects (LABELFISH, Barcode of Life) and for several peer reviewed scientific articles [1, 2, 4-7]. The software Sequencher v5.2.4 (Gene Codes Corporation) was used to align the forward and the reverse sequences for each sample and the consensus sequence was species identified by using BOLD database (BARCODE OF LIFE DATA SYSTEMS). In some cases, NCBI-blast also used to verify the species identity. Samples that were not species identified as the restaurant had claimed they were, were isolated again and the whole method repeated. In the case of tunas, the species identification was complemented with
the analysis of the sequence of the mitochondrial cytochrome b (CYTB) gene by sequencing of with a molecular method accredited under the directive UNE-EN-ISO 17025:2005. Mislabeling determination of each sample was assessed attending to the list of admissible species that can be sold under the commercial name indicated on the menu. Different sources were checked depending of the official list of commercial designations, and respective scientific species authorized, in each country and the global biodiversity information system on finfishes database (www.fishbase.org).

Results
The results of this survey will be presented and the citizen science approach discussed.

Acknowledgments: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007e2013) under grant agreement (FOODINTEGRITY nº 613688) and the Basque Government (Ekonomiaren Garapen eta Azpiegitura Saila - Departamento de Desarrollo Económico e Infraestructuras, Nekazaritza, Arrantza eta Eli. Politika sail - Vice. de Agricultura, Pesca y Políticas Alimentarias, Dirección de Calidad e Industrias Alimentarias)

References
5. Fish Barcode of Life (FISH-BOL).
Emerging risks are developing issues that are perceived to be potentially significant threats but they may not be fully understood. Food fraud, for instance, is an example of emerging risk as its nature and origin is constantly changing thus rendering reliance on historical data. Frauds, in general, are often the consequence of sudden changes in relevant socio-economic, climatic, regulatory drivers. These drivers, individually or collectively, form an information landscape that we call it Food Bigdata and we are able to extract valuable intelligence about emerging risks at the horizon.

Early warning system (EWS) is a collective system for surveilling food supply chain and identifying anomalies as signs of food fraud. They are proactive and cost-effective measures to predict or prevent food frauds compared to reactive analytical testing that costs a lot for a food business operator that uses hundreds to thousands of different ingredients for producing its products. EWS composes of three main components i) systematically collecting and combining high Volume, high Velocity, high variety data sets (known as the three V’s of Food Bigdata); ii) processing data using advanced analytics such as machine learning and statistical theory to identify anomalies; iii) communicating warnings regarding time, type, and origin of commodity to food safety authorities or food business operators.

We present a prototype of our EWS that can potentially monitor the global food supply chain in real time for more than 3,000 commodities under food, feed, and drink categories. We demonstrate the application of EWS for various commodities and in particular show its predictability for horsemeat scandal in 2013.
Food supply chains are complex and many factors influence directly or indirectly the occurrence of food fraud which makes it difficult to be detected and prevented. Prediction models for food fraud would be useful to help directing control systems. To be successful such models should be based on a holistic approach that takes into account factors that may lead or are linked to a fraudulent action. Bayesian Network (BN) would allow such holistic approach and therefore we have explored its potential for this purpose.

To this end, food fraud incidents published in the databases i) Rapid Alert for Food and Feed (RASFF) in EU and ii) the Economically Motivated Adulteration incidents database (EMA) in USA published in the period 2000-2015 were retrieved and linked to 15 other data sources expected to be related to food fraud. This additional data include among others: prices of the fraudulent product at the time of detection, trade volumes of the product between the country of detection and country of origin, the supply chain index of the country of origin, price spike of the fraudulent product around the period of fraud detection, etc.

The BN model was built with 80% of collected data and validated with the remaining 20%. The constructed BN model had a predictive accuracy of 91.5% for the fraud type and it was shown that the BN models are very useful in scenario studies.

The developed BN model demonstrates how expert knowledge and data can be combined within a model to assist risk managers to better understand the factors and their interrelationships.
RAPID AUTHENTICATION OF COFFEE BLENDS: OLD PROBLEM, NEW METHODS

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Keywords: coffee, authentication, commercial frauds.

Roasted coffee can be subjected to commercial frauds, when high-quality *Coffea arabica* species, described as “100% Arabica”, “pure Arabica” or “Highland coffee”, is intentionally mixed with the less expensive *Coffea canephora* var. *robusta*. The detection and quantification of such fraudulent undeclared blending in commercial samples is therefore important to protect consumers. Green coffee beans are relatively simple to be identified on the basis of their color, shape and size; in the case of roasted whole beans, a morphological discrimination may be attempted as well. However, the detection of *C. canephora* var. *robusta* in roasted and ground coffee blends, is more challenging and when the sensory analyses fail, chemical means have to be adopted. In this framework, new methods to detected this old but still practiced fraud will be presented and discussed.
WHAT IS THE ORIGIN OF THIS GARLIC?
METABOLOMOMIC FINGERPRINTING
EMPLOYING HIGH RESOLUTION MASS SPECTROMETRY MAY GIVE A RAPID ANSWER

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Background of Study
Garlic (Allium sativum L.) is a highly popular food ingredient in various gastronomies worldwide. In addition to uses as a seasoning, garlic finds multiple applications in traditional folk medicine, both for protective and curative purposes. The taste, shape and color of various garlic cultivars may largely differ, nevertheless, all of them contain a unique set of bioactive secondary metabolites, among them organo-sulphur compounds, S-alk(en)yl-L-cysteine sulphoxides such as alliin, and γ glutamylcysteines are the most important. Desintegration of garlic tissue results in a release of aliinase (EC 4.4.1.4, alliin alkenyl-sulfenate lyase, or alliin C-S lyase), the enzyme specific for Allium genus catalyses rapid conversion of alliin to pyruvate, ammonia, alk(en)ylsulfenic acid and subsequently are produced dialk(en)ylthiosulfinates, compound responsible for characteristic aroma of freshly chopped garlic. However, dialk(en)ylthiosulfinates are unstable, they are converted into a variety of breakdown products including thiosulfonates, disulfides, trisulfides, etc. which are associated with aroma of culinary processed garlic.

Annually, more than 60,000 tons of garlic are imported into the European Union (EU), mainly from China, Argentina and other countries. Garlics grown in the Czech republic, are highly preferred by consumers due to intensive and rich aroma, while the sensorial properties of imported garlics are classified as rather drab. In the recent years, fraudulent practices consisting in an intentional substitution of traditional ´Czech garlic´ by a cheaper brands have been encountered. In this context, not surprisingly, the demand for authentication of garlic varieties as well as geographic origin has become very urgent. Although molecular biology-based tests for a variety identification, is a feasible option, its cost, labour demand and speed are limiting factors. Several studies concerned with ´chemical´ methods enabling characterization of various pre-selected garlic quality parameters (e.g. content of alliin, profile of volatiles, minerals...) have been published, nevertheless, the generic applicability of these targeted strategies was shown to be rather limited.
Recently, metabolomics (i.e. a comprehensive analysis of the metabolome, focused on the broadest possible range of small molecules, <1200 Da, without a particular bias to specific groups of metabolites) has become an efficient authentication strategy for various applications in food analysis. While metabolomic fingerprinting has been highly efficient for authentication of various food commodities, as regards garlic, only few papers employing this non-target screening have been published until now, the most comprehensive was based on 1H high resolution NMR.

Objectives
In our study, we have critically assessed three mass spectrometry-based metabolomic approaches with regards to their potential to generate data enabling to characterize garlic sets of different origin – Czech Republic, Spain and China. Following instrumental platforms were used: (i) ambient mass spectrometry utilizing direct analysis in real time (DART) ion source coupled to mass spectrometer with an orbitrap mass analyser; (ii) electrospray ion source (ESI) - mass spectrometer with time of the flight (TOF) mass analyser, direct infusion (DI) of sample into ion source; (iii) high performance liquid chromatography (HPLC) – ESI source - mass spectrometer with a TOF mass analyser. Advanced chemometric strategies, represented by principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), were employed for interpretation of the acquired data set.

Results
When considering the instrumental tools applicable for garlic authentication based on metabolomics, the use of HRMS platform for fingerprinting purpose seemed us to be challenging, and, until now not fully explored option. In preliminary experiments, aqueous methanol was found as the most suitable solvent mixture for isolation of the broadest possible number of metabolites occurring in garlic bulbs. To avoid (uncontrolled) origination of biotransformation products from cysteine-derived sulfoxides due to alliin lyase action, this enzyme had to be chemically inhibited.

Chemometric analysis of the generated data showed, that the HPLC-HRMS technique provided the best separation of the garlic samples according to the country of origin. This is clearly due to the higher number of obtained mass spectral ‘features’ in comparison to the other two high throughput techniques, DART-HRMS and DI-HRMS. Excellent prediction ability, up to 100%, for the OPLS-DA models could be achieved. Alliin, phosphatidylcholine (16:0/18:2), arginine, dehydroalanine, phosphatidyl ethanolamine (16:0/22:6), L-γ-Glutamyl-S-allyl-L-cysteine, choline glycerol phosphate, were identified as the compounds contributing most to correct classification of the samples. The example of data processing by chemometric analysis, and selection procedure for markers will be presented.

In any case, this study has shown the potential investigated approach to enable geographical origin of tested garlics, it should be mentioned, however, that fingerprints database has to be continuously expanded to obtain robust classification model.

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A RAPID UNIVERSAL TOOL FOR COLORIMETRIC AUTHENTICATION OF FOOD COMBINING NANOTECHNOLOGY WITH DNA BARCODING

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Keywords: biosensors, nanoparticles, DNA, food authentication.

Food frauds are a widespread public health issue, raising both toxicity and nutritional concerns, due to the adulteration of food items with undeclared, cheaper ingredients. Moreover, food counterfeiting is responsible for a huge economic burden, which impacts the global food market with yearly losses of $10 billion. Increasing consumers’ awareness and governmental regulations claim for a more controlled food market, although a cost-effective method to assess the authenticity of food is still lacking. Alteration is particularly relevant for certain market segments, such as seafood, and herbal products, where the final items are often processed in the form of slices or powder before sale, thus becoming morphologically unidentifiable. One of the gold standard molecular techniques to assess the authenticity of food items is DNA barcoding, which uses sequencing of standardized genetic markers to achieve unequivocal identification. However, this approach is strongly limited by analytical constraints and timeframes, making it not suitable for the needs of the food market, especially in case of perishable items, leading to largely unmet products assessment before commercialization. To address such issues, we propose an innovative, low-cost (<1 Euro/test), rapid, and universal test, named NanoTracer, to assess the genetic authenticity of food products and ingredients, employing limited instrumentation and cost-effective reagents. The principle of NanoTracer is to simplify all the analytical steps required for genetic authentication, in order to make the test sequencing-free and portable outside specialized large-scale facilities. NanoTracer is designed to be used along the food supply chain, it can be directly applied to raw and complex food matrices, and requires minimal instrumentation and a simple and rapid processing (2-3 hours), compatible with the routine control of perishable food items. In particular, it is based on the combination of a rapid and robust DNA extraction from raw food matrices, simplified barcodes target, and universal DNA-functionalized gold nanoparticle probes. The test result can be read in few minutes, as a red-to-violet color change visible by the naked eye, allowing colorimetric food authentication. NanoTracer proved to be highly versatile, with possible uses ranging from the authentication of frequently substituted species, to the detection of new unregulated species, which may raise toxicity and public health concerns. We demonstrated that it can efficiently discriminate even low percentages of contaminants from genetically related species, thus strikingly outperforming the more complex instrumental analyses based on DNA barcoding. Therefore, NanoTracer has truly the potential to become a standardized test for the genetic identification of any food, filling the current gap in the offer of affordable technologies for molecular testing of food.

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CAROTENOID PROFILING AS TOOL FOR VERIFYING EGG PRODUCTION CLAIMS IN THE UK?

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Keywords: eggs, carotenoids, production claims, HPLC-UV/VIS.

We developed and validated a method for the determination of production origin for eggs by (i) developing a carotenoid profiling method for hen eggs (and feed) and (ii) analysing hen eggs and feed from different productions methods [enriched caged (C), barn (B), free range (F) and organic (O)] and regions in the UK [England (E), Northern Ireland (NI), Scotland (S) and Wales (W)].

As part of the Defra project, FA0159, 18 eggs and a feed sample were collected by poultry officers from each of 16 different UK farms. On arrival at Fera Science Ltd, the egg samples were stored at 3 conditions (+4, +19 and +23 °C) for a 5 week period, generating 6 time points (week 0, 1, 2, 3, 4, 5). Individual eggs were taken from each farming system for week 0, 3 and 5, when stored at +4°C. In addition, at week 5, eggs stored at +19°C and +23°C were also sampled in order to give an indication of whether carotenoid profiles are affected by elevated temperatures. A total of 78 egg samples and 14 feed samples were analysed for carotenoids (capsorubin, capsanthin, lutein, zeaxanthin, citranaxanthin, β-cryptoxanthin, ethyl-8'apo-β-carotene-8'-oate, β-apo-8'-carotenal, β-carotene and canthaxanthin) by HPLC-UV/VIS; see Figure 1 - carotenoids affecting colour of yolks.

Figure 1. Picture of cracked eggs from different English production sites and measured total carotenoid concentrations (mg kg-1). The longer the wavelength the more red the carotenoid. Yolks of sample E-O were much lighter in colour, as the total carotenoid concentration consisted mainly of lutein/zeaxanthin, while E-F and E-B contained a mixture of carotenoids of similar overall concentrations; whilst E-C contained the highest amount of citranaxanthin.
Capsorubin and canthaxanthin were not detected in any of the egg samples, whereas capsanthin, lutein/zeaxanthin and β-carotene were detected in all samples; citranaxanthin and β-8'-apo-carotenal were absent in organic ones. Only lutein/zeaxanthin were present (≤ 1 mg kg⁻¹) in all feed samples. This suggests that the carotenoids are accumulated within the egg yolk, as the concentrations ranged from 0.8 to 12.7 mg kg⁻¹ for week 0 samples. The absence of citranaxanthin and β-8'-apo-carotenal in organic feed samples was also confirmed. Differentiation of organic farming practice using the procedure reported by van Ruth et al (2011) was not possible due to lack of the canthaxanthin marker in UK feed. But the presence of citranaxanthin and β-8'-apo-carotenal in non-organic egg samples might be used, as a marker in UK eggs, to differentiate them from organic ones.

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TARGETED / UNTARGETED APPROACHES AND DATA HANDLING. HOW TO SUPPORT AUTHENTICITY OF FOOD AND RAW MATERIALS

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Keywords: food authenticity, data analysis, untargeted approach, target approach.

The confirmation of foodstuffs and their ingredients quality is a matter of primary importance in order to enhance consumer confidence in food products; at the same time, it represents a powerful tool to ensure fair marketing competitiveness for the different food producers. Food authentication verifies the degree of accordance of a product with its description on the label and law requirements and it could be considered a further assurance of quality and safety. In the last decades, the advances in the technologies, used to analyze and measure the different chemical and physical properties of food, have revolutionized the analytical approaches used in this context. At present time, targeted and untargeted approaches are easily supported by high sensitivity advanced analytical techniques (GC-MS, LC-MS/MS, NMR etc.) as well as non-destructive and fast methodologies (NIR, MIR, RAMAN etc.). However, the data obtained by these analyses must be processed in order to obtain useful information and real answers, and this step (data handling) sometimes comes out as the “bottleneck” of the development of ‘authenticity tools’ due to its complexity. In this context, the synergistic use of instrumental analytical techniques and chemometrics represents a proficient and practical way to obtain prompt and trustworthy results. This contribution is focused on highlighting the potentialities of chemometrics tools and the different ‘systematic approaches’ to be used in resolving some real issues related to the development of food authenticity models using both targeted and untargeted approaches.

The first case of study aims to develop an innovative, simple and fast tool for the evaluation of authenticity of grated Protected Designation of Origin (PDO) Parmigiano Reggiano cheese combining fingerprinting signals, obtained by RAMAN spectroscopy, with chemometrics techniques (untargeted approach). A fast (maximum 5 second of acquisition time) handheld Raman spectrometer (barely larger
than a smart phone) was used to analyze a total of 100 cheese samples, both of authentic Parmigiano Reggiano cheese (80 samples) and of competitors with different geographical origins (20 samples). The raw RAMAN signals (evaluated “as is”), Figure 1, do not allow obtaining immediate results due to the presence of several different irrelevant sources of variation. The use of a systematic chemometrics framework corrects this spurious variability (signal pre-processing) and enhances the information naturally present in the data.

![RAMAN spectra of investigated cheese samples](image)

Soft Independent Modeling of Class Analogy (SIMCA) analysis was used to build a one-class model and to classify the different samples. SIMCA analysis correctly classified all the investigated samples (Non Error Rate, NER: 100%) showing that RAMAN spectroscopy combined with multivariate and statistical approaches can be suitable for a sensitive, non-destructive, rapid and inexpensive classification/discrimination of grated PR cheese authenticity.

The second case of study consists in a targeted approach to develop geographical traceability models of Lambrusco of Modena PDO wines. In particular, primary (i.e. isotope ratios of radiogenic and light elements and metals content) and secondary indicators (NMR spectra and polyphenols fingerprint) have been monitored on different samples. The innovation of the adopted strategy lies in the use of a systematic approach for developing geographical traceability model, which requires a deep knowledge of the whole matrices, which characterize the investigated systems (soils, branches, grape juices, intermediate products and wines). For these reasons, a representative sampling for each investigated matrix (soils, branches and wines) has been performed by means of Design of Experiments techniques and different indicators were monitored in all the analyzed matrices. A Data-Fusion approach was used to merge in the most significant way the information obtained by different analytical techniques on the investigated matrices, and the use of chemometrics tools produced useful information about the differences/similarity among the samples, which contributed to build a unique geographical link between the investigated wines and their territory of provenance.

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L15

PHYSI-TRACE: RAPID ORIGIN TRACEABILITY OF AUSTRALIAN PORK PRODUCTS

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Keywords: traceability, Country of origin, elemental fingerprint, Physi-Trace.

Fraudulent behavior involving food for financial gain or more sinister motives is as old as time itself. While the true extent of the problem will never be known, in Australia it can be estimated that at least 10% of the food supply is not what it is claimed. The problem is potentially greater for Australia’s export sector. Australian produce typically command a premium internationally because horticultural and agricultural practices within the country are of an extremely high standard and the product has developed an international reputation for consistent quality. Over the past decade, there have been several reported cases of fraudulent labelling of Australian produce (overseas product being sold as Australian produced), from wine to milk to pork and beef. There is growing concern that with an increasing incidence of fraudulent labelling, that potentially Australian food exporters market access could be jeopardised should such product pose a food safety or related threat. Australian exporters are also losing sales through these activities. Of equal if not greater importance is the negative impact that such an incidence would have on the “Australian” brand. Without the ability to quickly prove country of origin through either a creditable traceability system and/or forensic food technologies, Australian food exporters are potentially exposed. Whilst Australia does have expertise and some food forensic technologies developed over the last 10 years, the suite of technologies and databases required to rapidly respond to a food fraud and safety incidence requiring proof of origin is less than adequate. Here we present a robust, rapid and low cost traceability system (Physi-Trace) developed with the Australian pork industry that can be used to validate the integrity of Australian pork and provide rapid trace back in the event of a food safety related incident. Physi-Trace is based on chemical fingerprinting and currently provides protection to approximately 85% of all pig producers in Australia. Under the Physi-Trace project, over 5000 samples have been analysed. It is generally accepted that elemental fingerprinting of animal based foods is problematic - composition of animal based foods need to be treated with caution as animals are usually reared in very different geolocations, moved around and fed with feed sourced from multiple regions. However, multivariate analysis techniques that generate discriminant models have allowed the classification of the pork meat into user-defined groups of interests, for example, countries, states and farms. The result of the statistical analysis indicates that it is possible to use the trace metal composition of fresh pork meat to classify the pork meat back to its region of origin. The cost of trace element analysis is also a problem as the cost is prohibitive...
for traceability applications. The majority of the cost is usually associated with the construction of comprehensive databases. The Physi-Trace project has developed a methodology where comparison with established databases is no longer necessary leading to a cost-effective system. The program also extends to processed meats and pig livers without the need for separate analysis programs. Pig livers are an increasingly important export commodity for Australian pig processors. In 2016, several food-related events involving pig liver both internationally and nationally highlighted the need for rapid traceability back to farm of origin. In response to these incidents, Australian pork commissioned an investigation to establish if pork liver elemental signatures could be compared to raw meat elemental signatures. The results indicate that due to differential bioaccumulation properties of liver, one could not compare elemental signatures of liver with raw meat. However, on further investigation, mathematical corrections of the elemental liver data could be conducted to facilitate the simultaneous provenancing of multiple tissue types within a single set of analyses. Such chemical traceability enables a rapid and cost-effective means of provenancing pork liver. Due to the difficulty in falsifying such a signature it also provides a robust means of trace back validation of pork livers. The analytical and interpretational protocols developed through the course of the Physi-Trace research project have been successfully applied in over six real-life cases which required the validation of the country or farm of origin.
L16

FOODINTEGRITY WITH ISOTOPE FINGERPRINTS: UNLOCKING THE TRUTH

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Fraud in food and beverage products include misrepresentation or tampering with packaging and labelling; adulteration, normally replacing a higher quality, original material with one of lesser quality one or extending a product by adding an adulterant; and misrepresentation of product origin. Increased complexity in the food and beverage supply chain has provided greater opportunity for economically motivated food and beverage fraud. Consequently, legislation has been enacted globally to protect food and beverage products with respect to production processes and product labelling. The combination of legislation and food fraud practices demand a reliable, high throughput and cost effective analytical technique that can identify food and beverage products that are not what they are claimed to be. Detecting food and beverage fraud can be achieved using stable isotope fingerprints because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. We show data for carbon, nitrogen, sulfur, hydrogen and oxygen isotopes and demonstrate how isotope fingerprints offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products.
“What’s more American than Parmesan cheese?” is a famous – at least in Italy – title of The Wall Street Journal Europe of March 2014.
The article showed the difficulties of Americans to understand the difference between a trademark and a “quality scheme” such as PDO and PGI European quality schemes.

Suppose a group of Parmesan cheese producers in Parma wanted to advertise that they all adhere to certain standards in the production of their cheese, – said the writer – they could create a logo, trademark it and require cheesemaker who want to use the logo to get certified.

Where is the weakness of this thought? It seems apparently perfect except that the way that cheese is produced - and obviously its name - is a “public heritage” of that specific territory and not a private trademark of one or of a little group of producers.

This thought is going to be the guideline of the brief analysis conducted about the situation in Italy and the entire EU of PDO/PGI products, through some question such as: what is a typical product? The quality schemes involve only few countries or are an opportunity for a relevant part of EU? Are the PDO/PGI products able to meet the mainstream of consumers? What is the actual situation of PDO/PGI products in EU? Finally, what are the main trends?
There is lack of order and classification under the “Extra Virgin Olive Oil” category. The offer has multiplied and is infinitely more complex...consumer knowledge and demands are bigger. Labels are stuck, because there have been no changes for years. Compare to other oils, extra virgin olive oil labelling restrictions are more severe. No health claim or value can be included. On the other side there is also lack of control and quite a few EVOS claim something without scientific support, affecting negatively those that follow the low. The story telling on the label could be sometime mislead using values unapproachable and in many cases uncertain. A deep review is needed.
CHECK ORGANIC: ENSURING THE INTEGRITY OF THE ORGANIC FOOD SUPPLY CHAIN

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Keywords: Supply chain integrity, Traceability, Real-time verification, Organic food fraud, Industry based solution.

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.
About 135,000 tons of Parmigiano Reggiano P.D.O. Cheese are produced every year, 20% of which is sold as grated cheese. The risk of fraud is considerable being the price of authentic Parmigiano Reggiano cheese three/fourfold the price of many competitors. So, together with documental controls inside the factories, Consortium of Parmigiano Reggiano cheese regularly controls the product on the market by analytical tools developed in the years in partnership with many external research centers. Presence/absence of specific tracers (lysozyme, CPFA, copper), indicator of age (free amminoacids on total protein) or of origin (isotopic and miner profile), metabolomic (LC-HRMS, MALDI-TOF), phisical (NIR or Raman spectroscopy) or sensory (electronic nose) approaches are used to recognise if the grated cheese is authentic or not.
L21

SCIENTIFIC METHODS TO ASSURE INTEGRITY OF BALSAMIC VINEGAR

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The aceto balsamico di Modena is an important part of the history of the gastronomic science. The modern history of this product began in Middle Age and moves across the subsequent seven centuries. It was a market niche in 70s and in 2009 the EU rule recognized the balsamico vinegar of Modena as a product with the European denomination of origin. So another product became a name to add to the list of foodstuffs with Italian sounding. The increasing adulterations and frauds of it are requesting a big effort for vinegar producers to find the scientific methods to reduce the economic damage related with false product in the world. The use of isotopic ratio and other very advanced analysis (molecular and peptides patterns with NMR or MALDI-TOF Mass Spectrometry) applied on the finished product, coupled with a proper monitoring work on the raw materials, shall be the better instruments to reduce false products on the market.
CONTROL OF WINE AUTHENTICITY: A LABEL STORY!

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Although the label does not make the wine, the wine must reflect the information written on the label, or inversely!
As a result, the whole authenticity control of a wine is based on the confrontation between analytical results obtained on the beverage and the information provided on the label. This presentation is an overview of technical aspects of wine control: from the examination of the label (visual or by Raman spectroscopy) to other sophisticated analysis (ultra-low radioactivity, stable isotopes quantification, HPLC-co-IRMS, 1H-NMR…) without forgetting the fundamental classical analysis (alcoholic strength, volatile acidity, organic acid concentration…).
L23

A GRADUAL STEP-BY STEP PROCESS FOR ADVANCING KNOWLEDGE ON NIRS SENSORS TECHNOLOGY FOR RAPID, IN SITU AND COST-EFFECTIVE AUTHENTICATION OF ACORN IBERIAN HAM (JAMÓN IBÉRICO DE BELLOTA)

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Keywords: NIRS, in situ analysis, Iberian pig authentication, non-destructive technology.

Acorn Iberian pig ham (“Jamón Ibérico de Bellota”) is one of the most expensive luxury food products produced in Europe. Since January 2014, a new system of labelling for Iberian ham products came into force in Spain, aiming to avoid mislabelling and fraud in the market, where prices for a cured leg of Iberian ham range from hundreds to thousands of euros. Spanish legislation classifies Iberian pig products into different categories mainly depending on the feeding regime and the race involved. Currently, the official quality-control systems used for determining the feeding regime of Iberian pigs are just based on on-farm inspections, while in the past also laboratory analysis of the fatty-acid composition of melted subcutaneous fat using gas chromatography (GC) was used. Nevertheless, GC is still used at industry level for production self-control and producer payment. Acorn Iberian hams (i.e. free-range animals fed on grass and acorns and 100% Iberian blood, with both parents pure-bred) should be identified with a “black label” and they are the best category and therefore the more expensive. The fatty acid profile of the adipose tissue from Iberian Pig fed with acorns is most appropriate from a technological and commercial point of view (higher concentration of oleic acid and lower of palmitic, stearic and linoleic acids). More than twenty years of research undertaken by the authors’ group have demonstrated the feasibility of Near Infrared Spectroscopy (NIRS) for an accurate prediction of the percentage of the four main fatty acids (palmitic, esteraic, oleic and linoleic) of Iberian pig, either using spectra of melted fat or of intact adipose tissue.

The piece of research presented here has been undertaken as part of the work to do during the first 6 months of the involvement of the authors in the FI Project (WP19 and WP21). The aim is to design a system of "voluntary labelling" based on Near Infrared Spectroscopy (NIRS) in combination with information and communication technologies (ICTs) to be used in the Iberian Pig industry sector for ensuring correct labelling and detection of frauds.
The specific goal of this paper is to show the step-by-step given I) to obtain a large and well-authenticated spectral NIR database -using an at-line research grade monochromator NIR instrument (Fig 1)- for the prediction of the main four fatty acids - present in the IP tissue II) to compare the accuracy and precision of those models, with the obtained with the same data set recorded on a NIR portable instrument (Fig 2), after transferring the initial database using a mathematic cloning procedure at a laboratory scale. Finally, it will be shown other key steps in the process of improvement of the predictive models obtained for the portable MicroNIR instrument at laboratory scale for its implementation for in situ analysis at the slaughterhouse.

Table 1. Comparison between accuracy and precision statistics for the best models for predicting C16:0, C18:0, C18:1 and C18:2 using the “at-line” FOSSNIRSystems-6500 and the MicroNIR portable instrument. N (cal) =460, N (val) =56.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Model</th>
<th>RMSEC</th>
<th>RMSECV</th>
<th>RMSEP</th>
<th>R²p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>FNS6500</td>
<td>0.72</td>
<td>0.73</td>
<td>0.53</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>MN1700</td>
<td></td>
<td></td>
<td>0.77</td>
<td>0.94</td>
</tr>
<tr>
<td>Stearic</td>
<td>FNS6500</td>
<td>0.42</td>
<td>0.43</td>
<td>0.31</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>MN1700</td>
<td></td>
<td></td>
<td>0.61</td>
<td>0.95</td>
</tr>
<tr>
<td>Oleic</td>
<td>FNS6500</td>
<td>1.12</td>
<td>1.13</td>
<td>0.83</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>MN1700</td>
<td></td>
<td></td>
<td>2.58</td>
<td>0.94</td>
</tr>
<tr>
<td>Linoleic</td>
<td>FNS6500</td>
<td>0.36</td>
<td>0.37</td>
<td>0.33</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>MN1700</td>
<td></td>
<td></td>
<td>0.56</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Acknowledgement:** The research undertaken was funded by EU FP7 FOODINTEGRITY project (no: Project No 613688). FI04 – Topic 4, Rapid, on-site, cost-effective methods for feed/food fraud detection.
Fish and seafood are among the top ten commodities considered by EU as the most at risk for frauds. Mislabelling, and in particular species substitution, i.e. selling a species different from that declared on the label, are the most frequent frauds in seafood. Currently the two most used countermeasures are visual inspection by experts and DNA analysis. Although the first method has the advantage of being performed directly at selling points, it requires experienced personnel and it heavily relies on a subjective judgement. DNA analysis, on the other hand, is a very accurate method for species identification, but it is expensive and can interfere with the production line.

The objective of the Fish Identification Software Hub (F.I.S.Hub) project (WP15 of the Foodintegrity project) is to overcome the limitations of both methods by developing a software framework to be used on the field, by both professionals and lay people, to detect species substitution. The F.I.S.HUB software will be able to identify the species of a fish from its picture; it will be based on a photo database and a machine-learning server for image analysis and classification, and will be accessible through a user-friendly app for mobile phones and other portable devices. Here we present an update on the development of the project.

A list of species was selected by the partners to create the data base (DB) according to commercial importance and likelihood of substitution. More species and families may be later added to the DB. The selected species belong to three different order: Clupeiformes, Gadiformes and Pleuronectiformes, and are into seven families: Clupeidae, Engraulidae, Gadidae, Merluccidae, Soleidae, Pleuronectidae and Scophthalmidae. Many similarities are shared among species on an intra-family level.

Two photo protocols were established, shared and applied by the partners to collect pictures in a standardised way. A cloud mass storage service was created to store the collected photographs.
The F.I.S.HUB classifier is based on a Deep Learning Neural Network able to analyse the pictures stored in the DB and to find the association between each fish picture and its related species. The classifier mainly consist of a so called Convolutional Neural Network and it can auto-generate the features useful for discriminating the characteristics of the different fish species/family/order. Preliminary result have been calculated over the DB, with a global accuracy by order is 96.9% and the global accuracy per-species is 90.5%. Although a few species have not yet been fully covered, the database is already usable to train the deep network and the obtained results are promising. Hierarchical classification seems to be the correct procedure and is currently under development. In particular, in the next months of the project, with additional pictures and species, the classifier will also be trained to perform on-shot validation for unknown species identification.

Acknowledgments: The project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.
NON TARGET SCREENING STRATEGY BASED ON GC-Q-TOF FOR SCOTCH WHISKY AUTHENTICATION

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Keywords: whisky, authentication, GC-Q-TOF, screening

Whisky is one of the most popular spirit drinks in the world. Unfortunately, this high valued commodity is vulnerable to fraud. To detect fraudulent practices and document quality parameters, a number of laboratory tests based on various principles including chromatography and spectroscopy has been developed. In most cases, the analytical methods are based on targeted screening strategies. In our study, to isolate and pre-concentrate as much as possible volatile and semi-volatile compounds, ethyl-acetate extraction was used. In the next step, non-target approach, fingerprinting of whisky components based on gas chromatography coupled with tandem mass spectrometry (Q-TOF mass analyzer) was employed. The data obtained by analysis of a unique set of 180 authentic whisky samples (differing in region of origin, maturation in various cask and age) provided by the Scotch Whisky Research Institute were assessed by advanced chemometric methods. Principal component analysis (PCA), partial least squares discriminant analysis (PLS–DA) and orthogonal PLS–DA were applied for classification model construction. Very good separation according maturation casks (bourbon, sherry, red, white and port wine) was achieved (see Figure 1A), significant markers most contributing to the clustering were identified (e.g. diethyl tartrate or vanillin acetate). Selected markers enabled separation not only between samples aged in ‘wine’ and/or ‘bourbon’ casks and even the samples of Highland origin, as shown at Figure 1B. In the next phase, 20 fake samples and 24 ‘unknown’ samples provided again by Scotch Whisky Research Institute were analyzed and the data processed in the same way. As documented in Figure 2, some differences could be observed in volatiles profiles of authentic and fake samples.
Employing the chemometric model developed for this purpose, marker compounds enabling distinguishing fake samples were found. Based on their mass spectra, several food additives (e.g. triacetine - E 1518) were identified. These ‘new’ markers might be added on the target analytes list for a routine control.

Acknowledgement: This work was supported by the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and by the “National Program of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.
A NOVEL APPROACH TO AUTHENTICATE WHOLE AND REFINED GRAIN DURUM WHEAT (TRITICUM DURUM DESF.) BASED ON UNTARGETED LIPIDOMICS

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Keywords: Authenticity, Common wheat, Durum wheat, Untargeted Lipidomics.

Pasta constitutes a dominant portion of a standard Mediterranean diet, supplying a large fraction of the needs for energy-rich materials, such as considerable amounts of carbohydrates, proteins, fiber or minerals. As a result, 14.3 million tons of pasta are produced worldwide according to the survey carried out by the Associations of Pasta Manufacturers of the European Union (UN.A.F.P.A, 2015). 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. Nowadays, wheat is mainly authenticated by genomics and proteomics approaches. This fraud has a huge impact both 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 the present work the possibility of using an untargeted metabolomics strategy was explored in order to discriminate between common and durum wheat lipidome. A first study was conducted by analyzing 52 samples from two durum and common wheat varieties. Afterwards, an extended and independent sample set (172 samples and five varieties) was used for as a confirmatory study to verify the stability and consistency of the models obtained. Among the metabolites resulted statistically significant in both preliminary and confirmatory study, alkylresorcinols, and in particular heptadecyl-resorcinol (AR 17:0), could be further used for the discrimination of common and durum whole grain flour, being present in the outer layer of the kernel and thus lost during refine process. By contrast, digalactosyl diglyceride...
(DGDG 36:4), an abundant membrane-forming lipid mainly concentrated in the common wheat inner layer, may be distinguish durum wheat adulteration also in refined flours. Putatively identified markers were evaluated applying The receiver operator characteristic (ROC) curves analysis resulting in individual marker AUC >90% both in preliminary and confirmatory study. In addition, the untargeted analysis was shown to be an effective approach differentiating between authentic durum wheat and its adulterated admixture down to 3% adulteration level, which is the maximum contamination level allowed by Italian legislation. The results demonstrate that untargeted lipidomics, in conjunction with chemometric tools has potential as a screening tool for the detection of wheat fraud.

<table>
<thead>
<tr>
<th>Marker</th>
<th>DGDG 36:4</th>
<th>AR 17:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary set</td>
<td>95.7 (90.3-100)</td>
<td>100 (100-100)</td>
</tr>
<tr>
<td>Confirmatory set</td>
<td>96.7 (93.8-99.5)</td>
<td>98.0 (94.5-100)</td>
</tr>
</tbody>
</table>

Figure 1. Receiver operating characteristic (ROC) curves of heptadecyl-resorcinol (AR 17:0) and digalactosyl diglyceride (DGDG 36:4) in the training and validation sets.
Keywords: non-targeted, chemometrics, food fraud, economically motivated adulteration.

Non-targeted testing for potential adulterants in foods and food ingredients is becoming a more common approach to identify products and determining whether or not more specific analytical testing for adulteration might be advised. A non-targeted method for detecting adulteration is one which models the properties of the authentic material rather than the properties of the adulterants or any of the adulterant’s characteristics. Confusion on terminology and lack of guidance on procedures to develop and validate a non-targeted method for food fraud detection has limited the wider application of these methods. US Pharmacopeia has developed a guidance document to address this need. The document covers all aspects of non-targeted testing, from the collection and analysis of reference samples, through development of the non-targeted testing statistical models, to monitoring and maintenance, as well as advice on abnormal samples handling. The overview of the draft guidance will be presented. Advantages and challenges of using non-targeted methods will also be discussed.
VOLATILE FINGERPRINTING USING PTR-MS PAVES THE WAY FOR SOUTH AFRICAN LAMB TO ACQUIRE PGI STATUS

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Keywords: extensive grazing, geographical origin, lamb fat, lamb meat.

Globally, increased attention is being given to the labelling and branding of specific sheep meat such as the Northumbrian lamb (UK), Ronaldsdale lamb (Scotland), Texel Lambsham (Netherlands), Ternasco de Aragon (Spain), Patagonian lamb (Argentina) and Agnello della Maremma (Italy). In South Africa, lamb typically produced in the Northern parts (the Karoo region) of the country is known as Karoo lamb. The meat is appreciated and valued by consumers for its unique sensory quality (e.g. herbaceous aroma and flavour) due to the diet of the sheep. Indigenous, herbaceous Karoo bushes and shrubs are key components of the diet and believed to function as a natural herb/spice. Due to the quality and value associated with Karoo lamb, fraudulent activities may occur. For instance, lamb may be sold as Karoo lamb when in actual fact it has been produced in a feedlot or different region. Similar to Karoo lamb, other characteristic sheep production sites also exist in South Africa where the typical diet of the sheep, associated with the region and traditional farming practices, lends the lamb meat unique sensory qualities. However, scientific evidence is required to authenticate the meat and prevent fraudulence. This was achieved by measuring the volatile fingerprints of South African lamb meat and fat using proton transfer reaction-mass spectrometry (PTR-MS). Meat and fat of the Longissimus lumborum (LL) of lambs from six different regions were assessed. Analysis showed that the volatile fingerprints were affected by the origin of the meat. A distinct fingerprint mass spectrum for lamb fat of the six different regions was developed (Fig. 1). The Karoo samples contained the ions m/z 77, 97, 135, 137 and 138, and had highest concentration of the ions m/z 41, 43, 59, 71, 81, 83 and 89. The monoterpenes mass ratios m/z 81, 135 and 137 were particularly useful for indicating dietary differences. The fat had a higher concentration of monoterpenes, validating the direct link with the herbaceous plant samples, which could serve as markers for future classification purposes. The classification of the origin of the lamb was achieved by examining the calculated and recorded fingerprints in combination with chemometrics. Four different partial least square discriminant analysis (PLS-DA) models were fitted to the data to
classify lamb meat and fat samples into “region of origin” (six different regions) and “origin” (Karoo vs. Non-Karoo). Performance of the models was assessed by external validation. The estimation models classified samples 100% correctly. However, validation of the first two models gave only 42% (fat) and 58% (meat) correct classification of region. Whereas, the validation results of the second two models were better with 92% (fat) and 83% (meat) correct classification of origin. The separation between the Karoo and Non-Karoo regions was clearly distinguishable (Fig. 2). Overall the significant differences between the Karoo and Non-Karoo samples indicated the typicality of Karoo lamb with the result being promising in the view of development of an authentication test required to strengthen its PGI status.

**Figure 1** Mean fingerprint mass spectrum for lamb fat of the different regions generated by PTR-MS.

**Figure 2** PLS-DA scores plot on axes 1 and 2 of the mass spectral data of lamb meat (upper) and fat (lower) determined by PTR-MS for origin (Karoo vs. Non-Karoo) classification. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

**Acknowledgements:** Financial support from the National Research Foundation, South African Research Chairs Initiative (SARChI), Meat Industry Trust (MIT) and Foundation Study Fund for South African Students (SSF) is acknowledged. Griekwaland-Wes Korporatief (GWK) for provision of the lamb samples. The help of staff from the Department of Food Science and Animal Sciences (Stellenbosch University) and RIKILT (Wageningen University and Research) is appreciated.
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FOOD FRAUD DETECTION THROUGH SPECIES-SPECIFIC MARKER PEPTIDES

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Keywords: food authenticity, mass spectrometry, meat products, wheat flour.

Food frauds are on the rise in the last years, with a 3fold increase from 2007 to 2013: only in Italy, during 2013, adulterated foodstuff was seized for a value of 441 million €. Meat derived products constituted most of the confiscated items (25%), followed by flour, bread and pasta (15% together), milk and derived product (9%) and wine and other alcoholic beverages (7%). The adulteration is more easily detected when occurs in raw materials, while it is more insidious when it is at processed product level. The replacement of a declared ingredient with a cheaper one involves the false declaration of geographic, species, botanical, or varietal origin. For what concerns the substitution of a certain species with a lower value one, different analytical tools are available. DNA based techniques are for sure the most species-specific, relying on the genome of the analysed organism. Also immunoassays to detect specific proteins are diffused in the field of food analysis, being quite fast and user friendly. Anyway both these approaches suffer of several problems after food processing, due to DNA and protein degradation/denaturation. Also peptides (naturally present or generated by means of enzymatic cleavage) might allow discriminating between two different species. The LC/MS analysis of species-specific peptides has the advantage that short peptide sequence more easily survive degradation due to thermal treatments and extreme pH, which can be found in processed foods. In this work we focused on two of the most commonly adulterated food items (meat and flour), demonstrating that marker peptides are a valuable tool for food fraud detection. In the first case, we developed a MS method able to discriminate between pork and beef meat (Prandi et al., 2017, Food Control), using two collagen derived peptides obtained by tryptic digestion. The method was applicable also on a highly processed and complex multi ingredient product such as ragout and an accurate quantification of beef and pork meat was achieved, while ELISA assays failed to detect any animal species. The method is currently being extended to other meat species. For what concern durum wheat flours, a diffused adulteration is the partial or total replacement of durum wheat semolina with the cheaper soft wheat flour in dry pasta production, leading to a strong decrease in texture quality and sensorial properties. A marker peptide whose sequence is encoded by DD genome (present only in the hexaploid T. aestivum) was identified. The developed method enables to detect durum wheat adulteration with common wheat in an accurate quantitative way (Prandi et al., 2012, Anal Bioanal Chem).
COIBAR-HRM AS A NOVEL APPROACH FOR THE DISCRIMINATION OF HAKE SPECIES

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Keywords: HRM, hake, Real-time PCR, species discrimination.

*Merluccius* genus represents an important group of fish commonly sold all over the world. *Merluccius merluccius* (European hake) is the most appreciated species within the *Merluccius* genus and its market value is higher than other species, such as *M. productus* (North Pacific hake), *M. hubbsi* (Argentine hake), *M. capensis* (Whiting) and *M. paradoxus* (Deepwater hake) [1, 2]. Mislabelling and fraud issues usually occur in commercially available hake products, which arises the need of developing reliable and cost-effective tools for authenticity purposes. The aim of this work was to evaluate the use of a DNA barcode of cytochrome oxidase I (COI) gene as a potential molecular marker for the differentiation of five genetically related hake species combined with high resolution melting (HRM) analysis. An *in silico* analysis was performed for the design of universal primers targeting a COI region of the selected species. Hake samples and other fish, crustacean and mollusc species, along with meat and plant species, commonly used as food, were used for specificity testing. DNA was extracted with the NucleoSpin Food kit, with yield and purity being evaluated by UV spectrophotometry in a micro-volume plate accessory. Specificity and sensitivity of the designed primers were assessed by qualitative PCR and the fragments were further sequenced. A real-time PCR assay using EvaGreen dye coupled to HRM analysis was then successfully developed. The method was shown to be efficient at detecting European hake (*M. merluccius*) down to the level of 0.2 pg, as well as discriminating it from the other referred four hake species with a level of confidence above 99%. The feasibility of the assay was tested in 45 fish-containing foods, showing that a level of 10% of mislabelling was observed among the positive samples with the specific designed primers. It can be conclude that a novel method for the detection of adulterations in *Merluccius* spp. was successfully developed and applied to different food matrices, enabling tracing low levels of hake well as a full discrimination of the selected five species.

Acknowledgements: This work was supported by FCT grants UID/QUI/50006/2013, NORTE-07-0124-FEDER-000069 and FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688). This work was supported by FCT grant no. UID/QUI/50006/2013. T. Fernandes and J. Costa are grateful to FCT grants (SFRH/BD/93711/2013 and SFRH/BPD/102404/2014, respectively) financed by POPH-QREN (subsidised by FSE and MCTES).

References:
Crime analysis results have confirmed that food crime is a trans-national phenomenon that has developed differently in countries with different legal systems. This is a barrier, sometimes insurmountable, for police forces and enforcement agencies. Counterfeit food products cause significant harm to the health and safety of consumers and even cause fatalities. The presentation describes both the organization and the point of view of a specialized police department operating in the food sector and reports briefly some recent cases carried out at EU level. Organized Crime Groups exploit legislative loopholes and are able to quickly identify, react to and even anticipate changes in legislation.

In 2010, the EU established a multi-annual policy cycle to ensure effective cooperation between Member States’ law enforcement agencies, EU Institutions, EU Agencies and relevant third parties in the fight against serious international and organised crime. Based on the Europol’s Serious and Organised Crime Threat Assessments (SOCTAs), the Council agreed a restricted number of crime priorities for 2013-2017. These were translated into strategic goals, and EMPACT projects (European Multidisciplinary Platform against Criminal Threats) were launched to coordinate ongoing actions by Member States and EU organisations against the identified threats.

Italy, namely Carabinieri NAS has been designated Driver of the crime priority called “counterfeit goods” aiming to disrupt the OCGs involved in the production and distribution of counterfeit goods violating health, safety and food regulations and those producing sub-standard goods”.

At the end of the presentation, a brief overview of the most significant EU police operational actions launched to tackle counterfeit products threatening public health will be presented.
CIBO CRIMINALE (CRIMINAL FOOD)

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Keywords: mafia, Italian sounding, trafficking.

Don Domenico Clericuzio is a boss of the Italian-American mafia, a fictional character created by Mario Puzo, the author of “The Godfather”, in 1966.

The scene is set in 1965. The baptism of two nephews of the boss is being celebrated in the garden, and the boss delivers this sentence to his sons. The author anticipates what the biggest problem of the mafias around the world will be: to reinvest the billions of euros derived from the drugs business, weapons and human trafficking. In 2009 the UN estimated a mafia turnover of 710 billion euro.

The 2015 Transcrime report indicates a mafia turnover of 110 billion euro in Europe, equivalent to 1% of Europe’s total Pil, and uncovers a surprising fact about how this figure originated. Firstly, a big part of it – almost 43 billion – is generated from the “Made in Italy” counterfeit (from fashion to food), but it also affects countries like France and Spain. In second place, with just under 30 billion, there are the Community frauds. And finally the drug trade ranks only third, with a turnover of 28 billion euro. This is the link between the mafia and the topics we are dealing with.
For some time in Italy we are often hearing a term, Italian sounding, which in my view is a rather effective way to define products that evoke Italian food culture and our excellent food industry, but that in fact have nothing to do with Italy.

According to Coldiretti, one of Italy’s farmers associations, 97% of provolone cheese and pasta sauces sold in supermarket in USA are falsely Italian. They have names like Pulcinella, Sole Mio, have labels that bear the Italian flag, the Vesuvius or the Milan cathedral. But in reality they are manufactured in New Zealand and in Asia, with local raw materials. Italian sounding numbers, calculated by Eurispes, are impressive: while Italian food export turnover in 2014 was 34.3 billion, Italian Sounding turnover was almost double with 60 billion euro annually. Which is 164 million euro per day.

The food sector is also being targeted by the organised crime for investing purposes, for a number of reasons. It is composed by many small companies, often family-run, that in the present market conditions and credit crunch can be more easily blackmailed, in addition to potentially be interested in injections of capitals of dubious provenance.

These companies are becoming impoverished. The products of the land, which are so expensive in shops and supermarkets, often are not remunerative enough for the farmers.

According to a calculation, even if biased as it is done by the Coldiretti, in Italy for 1 Euro spent in the supermarket, 60 cents go to the distribution, 23 to the transformation industry and only 17 cents go to the farmer.

There is another factor that makes the sector very attractive: we eat every day, it is a primary need and we need to eat even in time of crises, and we must pay immediately, in cash. This means that the large amount of money produced circulates very quickly, and fast circulation is of paramount importance during money-laundering operations. Trafficking in spoiled food or forged trademarks is much less risky than other illegal businesses. Almost no one goes to jail for a fake Parmesan cheese, unlike what happens when trafficking in drugs or weapons. And the gains are more or less the same.

Mafia wants to control the food sector “from the land to the table”.

Black work in the fields, products transportation, counterfeiting and international trafficking of adulterants and fake aliments, distribution.

To win the challenge we must form an alliance between the institutions, the consumers and the food industry, as is it clear that the problem does not only concern the small companies/producers, the niches. But it also clear that in case of defeat we will not only lose our heritage of flavors, traditions, taste and food excellence. We will also lose in health, and in the end we will see our righteous companies be replaced by the mafia ones.
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FOOD OR WILDLIFE? CONSUMERS’ POOR KNOWLEDGE OF FISH APPEARANCE HAMPERS SEAFOOD MARKET TRANSPARENCY AND SUSTAINABILITY

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Keywords: Fisheries, Seafood, Public Understanding, Consumers, Fish Diversity.

Seafood has represented a mainstay of human consumption for millennia. Since the industrial revolution, food supply chains have changed dramatically, loosening the links between living resources and consumers.

To date, there is little more than anecdotal information on the degree to which human societies in the developed world appreciate the diversity and the appearance of the fish species that underpin our foods. To fill this gap, we interviewed 720 European citizens from Belgium, Greece, Ireland, Italy, Spain and the United Kingdom, and measured their ability to recognize six commonly marketed European fish species: cod (Gadus morhua), Atlantic salmon (Salmo salar), anchovy (Engraulis encrasicolus), mackerel (Scomber scombrus), sea bass (Dicentrarchus labrax) and sole (Solea vulgaris).

We found that the overall people's accuracy was less than 30%; cod and salmon were significantly more recognized in Northern countries, and sole, seabass and anchovy, more accurately detected in Italy, Greece and Spain.

Overall differences were observed among countries and could in part be explained by age group and background of the interviewees.

We note that such a stark disconnection between the population and the diversity of life at the basis of key elements of people's diet creates a fertile ground for non-compliant operation in the seafood industry. In the context of the global challenge of food security, we stress the importance of revisiting current education practices in European countries and beyond.
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CHINESE CONSUMER ATTITUDES TO EUROPEAN PRODUCTS

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A potentially important outcome of authenticity assurances associated with food and drink is to reduce consumer concerns about food safety associated with 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 may play in relieving those concerns. Qualitative research was used to explore and understand 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 qualitative 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 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. Authenticity cues, including in association with European products, reduced food safety concerns. Different types of authenticity cues had differential effects in terms of alleviation of concerns. 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.
ORAL SESSIONS

L35

OLIVE OIL TRADE STANDARDS INSIDE AND OUTSIDE EU: ANALYSING DIFFERENCES

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Keywords: olive oil authenticity, regulation, harmonization, legal limits.

In order to enhance food quality and authenticity one of the requirements is to harmonize all the international regulations. In some foods, where the required analytical control is complex, such as olive oil, this task requires a previous analysis of the differences found in the trade standards inside and outside the EU. Different national and international normatives define the olive oil categories according to their chemical composition and the limits established for a series of chemical, physico-chemical and sensory parameters. Within FoodIntegrity project (WP4), the current standards from different national and international bodies have been cross-tabulated and compared with the assistance of stakeholders for a better comprehension.

The conceptual definitions of olive oil categories are common for all the international regulatory bodies even literally. However, in regards to chemical definition (parameter limits), significant differences are found, which lead to a non-easy work for exporters and importers. Thus, differences have a great impact on the qualification of virgin olive oil categories up to the point that a sample qualified as EVOO might be qualified as another category depending on the trade standard. Seven international and national bodies have been considered since they are directly involved in the production of trade standards for olive oil categories although all of them are based on IOC trade standards, in greater or lesser proportion. The main international bodies are listed below.

International Olive Council (IOC): It can be considered the reference regulatory body because it is the only intergovernmental organization in the world (set up in 1959 under the auspices of the United Nations) that brings together most of olive oil producing and consuming stakeholders. The latest trade standard was published in July 2016 (COI/T.15/NC No 3/Rev. 11 - Trade standard applying to olive oils and olive-pomace oils). In addition, each parameter is associated to a standard method that is regularly updated.

Codex Alimentarius: The Codex Alimentarius or “Food Code” was established by FAO and the World Health Organization in 1963 to develop harmonised international food standards, and it includes specific chemical limits for olive oil. However, this organization is slower than IOC and EU in making changes to get adapted to new situations. The last regulation is Codex Standard for Olive Oils and Olive Pomace Oils (CODEX STAN 33-1981).
European Union: The European Union regulation includes a complete norm on olive oil including limits and associated methods (CEE No 2568/91 and subsequent amendments). Like IOC, The European Union has an expert group that discusses about methods and limits. This group is under Directorate-General for Agriculture and Rural Development (Sub-group Olive Oil). The 2568/91 regulation was amended by a series of updates. For example, Regulation 1348/2013 of 16 December 2013 amended the Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, updated by Commission Delegated Regulation (EU) 2015/1830 of 8 July 2015 and Commission Implementing Regulation (EU) 2015/1833 of 12 October 2015. In addition, more recent updates are published (e.g. EU, 2016/2095).

In addition to these international regulations, it is important to consider national norms that has important role in discussion, such as the norms from The United States Department of Agriculture, The State of California (Californian Department of Food and Agriculture, CDFA), and the Australian Standards (AS 5264—2011).

The reason of these disagreements between norms is diverse. One of the factors affecting the disagreements between international regulatory bodies is the climate differences between countries, that lead to different composition in the case of some locations and chemical parameters (e.g. some fatty acids). The spread of olive tree orchards all over the world when only a few decades ago they were circumscribed to Mediterranean countries is on the basis of these disagreements. On the other hand, the olive oil market, consumption, and export/import patterns are changing in the last years, and many regulatory bodies are being more active in protecting olive oil authenticity.

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GAstronomy, Culture & Integrity
Food Identity: Characteristics and Evolutions

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Keywords: history, culture, identity.

The French semiotician Roland Barthes called food as a system of communications, a system of images, a protocol of situations, of actions, a language, and as other languages has allowed and allows people to express themselves and interact with other people, with other societies. It seems evident that every society express their cultural identity through the production and consumption of food and that this model can have multiple expression systems. Even the sociologist Pierre Bourdieu, remember us, in his A Social Critique of the Judgement of Taste, that food is a hallmark of the differences between groups and cultures, and this property helps to reinforce the same group identity, to separate and distinguish “we” from “others.”

By applying the principles of structural linguistics in various different communication's systems, Barthes showed how it was possible to associate the words to food, and then divided them into syllables, nutrients, and letters and / or sounds, or chemical elements. The exclusionary practices, the opposition, then the opposite value flavours, the food association and the formation of user protocols constitute the rules of a gastronomic logical analysis that allow the reading of a complex system signs. However, if the dialects are a linguistic system of a specific geographical or cultural area, the theory of Barthes and then that of Bourdieu, allow to imagine how many linguistic and culinary concepts could coexist in a culture and how complex is the observation or the analysis of one of these ways of expression. Keywords or significant key concepts were extrapolated from food cultures with the idea to create some macro-categories, that could describe the motivational choices behind a preparation, without forgetting geography and cultural questions.
FOODINTEGRITY: A GLOBAL PERSPECTIVE

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The global food supply system is a highly complex one. It was evolved over many hundreds of years but due to World Trade Organisation agreements it have greatly enlarged in terms of size and complexity over the past 20 years. This enlargement of food supply has continued as the demand for food by the world’s ever growing population increases on a daily basis. The demand for low cost non seasonal food has also served to drive the global trade.

With the increased complexity comes a substantial increase in vulnerability. Our food system is vulnerable to both accidental and deliberate contamination as well as substantial levels of counterfeiting. With this fraud comes opportunities for organised crime to become involved and make large amounts of money. The consequences to the consumer are twofold; paying for lower quality food than they believe they are purchasing and risks in terms of food poisoning due to contamination with dangerous materials. Overall consumers have much less trust in the food they purchase than ever before.

There are many ways that food industries and govern agencies attempt to mitigate against food fraud. An overview of these will be given in terms of a systems based approach to combating the growing menace of food fraud and food crime.
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OVERVIEW OF EU CHALLENGES FOR FOOD FRAUD

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A number of initiatives have been taken to improve the capability of Member States’ competent authorities of identifying as early as possible those violations of food law which are motivated by the intention to obtain an undue benefit.
The main initiatives intended to enhance the EU control system as a whole for detecting and countering frauds in the food chain have been as follows:

• Creation of an EU Food Fraud Network, which includes representatives from the Commission and the 28 Member States, for a more efficient cross-border administrative assistance and cooperation;
• Development of a dedicated IT tool, the Administrative Assistance and Cooperation System, to enable the members of the network to rapidly exchange of information on potential cases of cross-border fraud;
• Organisation of specialised training for food inspectors, police and customs officers and judicial authorities of the Member States, concerning new investigation/control techniques related to food fraud;
• Coordinated controls at EU level to detect whether fraudulent practices are present in other areas than beef. Fish (substitution of species) and honey (adulteration with sugar) have been targeted by a coordinated control plan carried out in 2015 by the 28 Member States, Switzerland and Norway. The results have been published in December 2015, showing that efforts should be continued to prevent and deter food fraud.

The EU Food Fraud Network
The horsemeat scandal showed that one of the weaknesses of the current system of enforcement along the food chain was the difficulty for Member States' competent authorities to communicate efficiently with their counterparts in other Member States for the purposes of ensuring enforcement in cases of violations having cross-border impact.
The Commission decided therefore to activate a dedicated network of administrative assistance liaison bodies that would handle specific requests for cross-border cooperation in cases of “food fraud”. The dedicated liaison bodies are referred to as “Food Fraud Contact Points” (FFCP). They act, as all administrative assistance liaison bodies, within the legal framework provided in Title IV of Regulation (EC) No 882/2004. The group of FFCPs is collectively referred to as the “Food Fraud Network” or FFN. By engaging in their Administrative Assistance and Cooperation duties, the FFCP and the FFN help to improve the capability of competent authorities to:
• detect and prevent violations of food chain rules, also across borders and in potential cases of “food fraud”;
• collect the information which is needed (in accordance with applicable national rules) to further refer a case to investigation/prosecution.
Since its creation in July 2013, more than 300 cases have been exchanged through the Network.
The AAC system
In November 2015, the Commission launched a dedicated IT tool for the handling of cases that require administrative assistance to be deployed – the Administrative Assistance and Cooperation (AAC) system. This system is used by the Food Fraud Contact Points which compose the Food Fraud Network (FFN).

In a typical case, a competent authority in one Member State sends a request for assistance to another Member State, which in turn provides a feedback to this initial request. If needed, a follow up to this feedback may occur. The requested competent authority may need, for example, to carry out an inspection at the premises of a food business operator that may have exported fraudulent products to the requesting competent authority. The requested competent authority then notifies the requesting competent authority of the results of such inspection and the follow up actions which were taken.

Future
The Commission will continue its work to
• strengthen the exchange of information via members of the Food Fraud Network.
• coordinate action in case of cases which have a EU dimension;
• coordinate EU-wide control plans in sectors where there are indications of possible widespread malpractices;
• provide dedicated training to food inspectors, police and customs officers on new investigation/control techniques related to food fraud;
• In general strengthen the capability of the control system as a whole to assess at an early stage the potential vulnerability to fraud of the different parts of the food supply chain.

Challenges
• Cooperation with non-EU Member States;
• Enhancement of EU-coordinated cases;
• IT systems : data sharing/data mining
• Cooperation with stakeholders.
ROLE OF ANALYTICAL TESTING FOR FOOD FRAUD RISK MITIGATION – HOW MUCH IS ENOUGH

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Keywords: food fraud, testing, Sampling.

Food fraud is of high concern to the food industry. The practice is widespread and the nature of the fraud is varied and fraud can potentially happen at all stages in the food chain. A multitude of analytical technologies exist to detect fraud including chromatography, spectroscopy, DNA analysis, etc. However, in many cases the testing is expensive and some forms of fraud, such as some labelling changes, may not be detectable by analytical techniques. When analytical techniques are employed, the question immediately arises as to the extent and frequency of testing required. In this opinion paper, several aspects relating to the role of analytical testing for food fraud risk mitigation are explored. In the first instance, available databases detailing fraud occurrences were systematically examined to determine how frequently analytical testing triggered fraud detection. In many cases, analytical testing was not the trigger to detect fraud. This work was complimented by a structured survey of industry stakeholders to determine their experience of how successful has analytical detection been to detect fraud. In addition the paper considers a framework for deciding when to implement testing programmes for fraud and a framework to consider the economic considerations in fraud detection. Current regulatory issues relating to food fraud detection are explored as well as some of the main factors associated with statistical sampling for fraud detection. The occurrence of fraudulent product in the supply chain is typically not randomly distributed. Potentially this impacts on the success of the sampling scheme set up to detect fraud.
THE EU H2020 OLEUM PROJECT: STATE OF PLAY AND FIRST ADVANCEMENTS

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Keywords: quality, authenticity, olive oil, Horizon 2020.

The EU H2020 OLEUM project aims to better guarantee olive oil quality and authenticity by empowering the detection and fostering the prevention of olive oil fraud. OLEUM started on 1st September 2016 and will run for four years. The project is coordinated by Prof. Tullia Gallina Toschi of the Department of Agricultural and Food Sciences of the University of Bologna, Italy. Twenty partners covering fifteen countries, bring together competences from food analysis, food legislation, industrial equipment engineering, bioinformatics, communication and knowledge exchange. The OLEUM project will develop new and/or improved analytical methods for assuring the quality and authenticity of olive oil. Technology transfer will be ensured through a wide community of relevant stakeholders, institutions and laboratories involved in quality control (OLEUM Network). Moreover, an online integrated database of olive oil analytical methods and data related to chemical and organoleptic characteristics will be established (OLEUM Databank). The project results will be aimed to boost consumer confidence and ultimately enhance the competitiveness of the EU olive oil market (Figure 1). The EU is currently the largest producer of olive oil; nevertheless, non-EU countries are expanding their domestic production, thus increasing the competitiveness of the global market. This fact, together with the high value of olive oil, its reputation as a healthy source of dietary, and a lack of efficient and harmonised analytical methods for detecting fraud has increased significantly the vulnerability of extra virgin olive oil that can be exploited by counterfeiters. In fact, olive oil is one of the most popular targets for adulteration; in particular it can be subjected to illegal blending with other vegetable oils...
or low quality olive oils (e.g. soft-deodorized) and, more in general, to deliberate mislabelling of less expensive commercial categories of olive oil or of oils from different origin. The consortium has identified four main areas that need to be improved through research and development in the olive oil sector: legislative and regulatory, analytical, harmonization/coordination and consumer/market confidence. This presentation will describe the advancements and deliverables that have been achieved during the first nine months of the project. In particular, actions devoted to the identification of gaps in the normative framework and the analytical methods drawbacks will be shown (WP2). Moreover, a description of the whole sampling procedure, experimental design and objective focused on the development of innovative and revised analytical solutions addressing olive oil quality (WP3), and authenticity (WP4) issues, including the development of a Quantitative Panel Test (Figure 2), will also be presented. Advancements on the establishment of the OLEUM Databank (WP5) and of the OLEUM Network (WP6) will be discussed. Finally the strategy to maximize the impact and increase visibility of the project via targeted communication and dissemination activities to a wide audience of relevant stakeholders will be presented (WP7).

This work was developed in the context of the project OLEUM “Advanced solutions for assuring authenticity and quality of olive oil at global scale” funded by the European Commission within the Horizon 2020 Programme (2014–2020, grant agreement no. 635690).

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P1

A DATA-DEPENDENT WORKFLOW FOR SELECTION OF PEPTIDE TARGETS FOR ROBUST DETECTION OF ALLERGENS IN DIFFICULT FOOD MATRICES

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Keywords: allergens, bioinformatics, peptides.

Introduction
Management of food allergens during food production requires analytical methods allowing for the specific detection of food allergens in complex and processed food ingredients. The current paradigm for selecting peptides to act as surrogates for food allergens is to use untargeted MS data from the allergenic food in combination with bioinformatic analyses. Candidate target peptides are then used to generate targeted methods which are trialled in complex foods that are incurred or spiked with the allergenic food. This workflow is, in many cases, inefficient and can result in methods that do not function well in the presence of a food matrix. We therefore explored an alternative workflow for peptide target selection for food allergen detection.

Methods
Replicated (3 extracts, 3 analysis) data-dependent experiments were performed on raw and roasted peanut, and these materials spiked into cumin and garlic powders, using a Thermo Scientific QE+. Following label-free quantitation we analyzed data so that peptides which were both abundant and, most importantly, robust throughout processing and food matrices were identified. We selected 20 peptides with a range of recovery characteristics for parallel-reaction monitoring (PRM) experiments to examine the correlation of label-free quantitation and targeted quantitation methods in food matrices. Peptide targets which were most robust (occurring reproducibly in all conditions) were used for the development of a PRM method which was applied to raw and roasted peanut spiked into cumin and garlic at concentrations of 2-200,000 mg peanut.kg-1.
**Preliminary Data**
We examined the utility of untargeted analysis and subsequent label-free quantitation of food allergen peptides within a complex food matrix for target peptide selection. We designed a workflow for target selection based on initial data-dependent analysis of unprocessed and processed peanut in the absence or presence of cumin and garlic, two food matrices with recent issues with peanut contamination. The output from this workflow allows for the selection of peptide candidates based on their quantitative recovery from allergenic foods within a food matrix and after processing. We demonstrate that the performance of peptide targets in our label-free quantitation correlates with performance using parallel-reaction monitoring (PRM), a targeted approach. This workflow therefore allows for the identification of target peptides that are specific to the targeted food allergen, allow sensitive and robust detection in foods that have been thermally processed and are in the presence of a problematic food matrix. The advantages of this workflow over currently favored techniques are discussed. We furthermore demonstrate the use of a targeted method (PRM) based on selected robust peanut peptides for the detection of peanut in a spice (cumin and garlic) background and describe performance characteristics. As far as the authors are aware, this is the first evaluation of a PRM method for the detection of allergens in foods.

**Novel Aspect**
A novel workflow for discovery of robust peptide targets of allergens that are robust after processing and in problematic food matrices.
RAPID EVALUATION OF POLYPHENOLS ANTIOXIDANT CAPACITY BASED ON AU AND AG NANOPARTICLES PLASMONIC ASSAY

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Keywords: Au nanoparticles, Ag nanoparticles, Surface Plasmonic Resonance, Polyphenols, Flavonoids.

Food quality control is essential for the consumer protection and also for the food industry. Hence accessibility of analytical methods for the quality assurance and process control is highly required. In addition to classical instrumental analytical protocols, there is the need for complementary and rapid techniques able to detect key parameters, useful for evaluation of Foodintegrity. Short analysis times should be necessarily coupled to ease of procedure and low cost of the analysis.

Indeed, polyphenols are a class of chemical compounds of considerable interest in the food industry and for their nutritional and health proprieties [1-3]. For this reason, the determination of polyphenolic compounds and the evaluation of their intrinsic antioxidant capacity continues to attract considerable research efforts. This work exploits the ability of polyphenols to drive the formation of metal nanoparticles for analytical purposes. Nanoparticles based colorimetric assays have received considerable attention in the analytical field because of their simplicity and low cost since they do not require any expensive or complex instrumentation [4-6]. Localized surface plasmon resonance (LSPR) is one of the most remarkable features of gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs). Due to these inherent optical properties, colloidal solutions of Au and Ag NPs have high extinction coefficients and different colour in the visible region of the spectrum [6]. These recent works have demonstrated the correlation of the formation of AuNPs with total phenol content and antioxidant capacity of food, in extraction free assays also for foods with high fat content as olive oil and chocolate.

The investigation reported in here exploit the class selective formation of AuNPs and AgNPs mediated by polyphenols (Fig. 1); the ‘reaction’ (MNPs synthesis) can be described by a sigmoidal curve, obtained simply by measuring the absorption peak of the plasmonic band after few minutes of reaction. The curve is correlated to the phenols class and their antioxidant capacity.

To evaluate the reliability and the suitability of the nanoparticles based proposed assay, the reactivity of sixteen polyphenols (belonging to different chemical classes) have been explored. The reactivity of phenols and their ability to form/stabilize the NPs of different metals, can be related to the antioxidant capacity and is able to discriminate the chemical class reactivity. Satisfactory analytical performances with good sensitivity and repeatability has been achieved. Data on different foods confirms the selectivity of the assay. Considering all the mentioned analytical behaviour, the difference in reaction of the polyphenols and the possibility to develop hand-held devices with optical detection (e.g. mobile phones), this approach can represent a valuable tool to have a rapid picture of polyphenolic pattern of foods and its fate during the entire food chain.
Acknowledgment: Authors acknowledge the financial contribution of the Italian Ministry of Foreign Affairs for the Project “Materiali nanostrutturati per sistemi (bio)chimici sensibili ai pesticidi”.

References

Figure 1. AuNPs (A) and AgNPs (B) formation using epicatechin as reducing agent. Coloured suspensions for different amounts are reported on top. AuNPs (A) and AgNPs (B) spectra have been obtained in the 25-100 µM concentration range.
DETERMINATION OF THE NITRATE, NITRITE AND POLYPHOSPHATES RESIDUES IN ICED AQUATIC PRODUCTS BY OPTIMIZATION ION CHROMATOGRAPHY METHOD

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Keywords: Optimized Ion Chromatography method, Nitrate & Nitrite, Polyphosphates, frozen Aquatic products.

This method is intended to determine the residues levels of Nitrate, Nitrite and polyphosphates in frozen Aquatic products by Optimized ion chromatography. Enzymes that may degrade polyphosphates during analysis are denatured by using ultrasonic and 75 degree centigrade. The result for phosphate (PO43-) should be treated with caution as some fish may contain free water soluble phosphate. The results showed 5 points as the following:

1) The time-saving using hydrophilic anion exchange column Dionex IonPacTM AS16 compared with AS11-HC.
2) Not needed put Acetocaustin into the extraction water owing to effect the retention time and only 75 degree centigrade hot water used for protein sediment to purge and prohibited the Poly phosphate enzyme to let stable the polyphosphate.
3) The separation was achieved through gradient elution using potassium hydroxide as the elutant. The determination coefficients of the optimized linear equations for these analyses were in the range of 0.9951–0.9986, and the recoveries of spiked samples ranged from 83.2% to 97.6% with RSD less than 7.7%.
4) (With successfully applied this method, Nitrate, Nitrite, Tripolyphosphate (P3O105-), Trimetaphosphate (P3O93-), pyrophosphate (P2O74-), phosphate (PO43-) and hexametaphosphate (P03 6-) were determined in different kinds of frozen aquatic products in Zhejiang, Fujian province and shanghai super marketing and whole sale and the results given a wide variation in the contents of nitrate, nitrite and polyphosphates existed among different frozen aquatic products, and all residual contents met the national maximum residue levels limited except that polyphosphates in two sample were more than 5 g/kg.
5) Testing method has been Optimized for Nitrate, Nitrite, Tripolyphosphate (P3O105-), Trimetaphosphate (P3O93-), Pyrophosphate (P2O74-), Phosphate (PO43-) and Hexametaphosphate (P03 6-) as Phosphate (PO43-) based on GB 2760-2014,etc.
6) Further did the degradation rate testing, given the obvious effect was obtained by using the distilled water immersion but no effective with boiled and steamed fish and peeled shrimps.
CHEMICAL PROFILE OF PHENOLIC COMPOUNDS IN WHEAT PASTA SUPPLEMENTED WITH CHIA

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Keywords: chia, pasta, phenolic compounds, HPLC-MS.

Chia seeds (*Salvia hispanica L.*) are considered as a good alternative to produce supplemented foods in order to improve its nutritional value. In our previous work, presented at Foodintegrity 2016, we showed the analysis of quality parameters and antioxidant activity of pasta produced with partial substitution of wheat flour with different proportions of chia defatted flour (0%, 2.5%, 5% and 10%). We have demonstrated that the increasing content of chia flour improves the antioxidant properties of the produced pasta.

Generally, the antioxidant activity is associated with the content of phenolic compounds. Considering that pasta is consumed after cooking and that the chemical profile of these components can be affected by high temperatures, our next goals were to assess the antioxidant capacity of raw and boiled pasta and to analyze the chemical profile of phenolic compounds through HPLC-MS. Initially, we performed the HPLC-MS/MS identification of the components of the acetone:water (80:20) extract obtained from chia defatted flour, determining the presence of 14 phenolic compounds, among which 12 of them can be considered structurally related to hydroxycinnamic acids such as caffeic acid, one was identified as quinic acid, and one corresponds to a flavone structure named hispidulin with very low abundance (<1μg/g). The extract is characterized by the prominent abundance of two main compounds identified as rosmarinic acid and its glycoside. Both components together with other 9 compounds, all of them related to caffeic acid, have been reported in other Salvia species, but none of them had been previously reported in chia apart from rosmarinic acid.

Subsequently, we evaluated the extracts of raw and boiled pasta using HPLC-MS quantitative analysis of the phenolic compounds detected in chia. All the polyphenols quantified in the extracts showed an increasing concentration correlated with the increased content of chia in raw and boiled pasta. On the other hand, the boiling process affected quantitatively the phenolic compounds profile. Caffeic acid, caffeic acid hexoside and danshensu showed higher concentration in boiled pasta than in raw pasta. However, rosmarinic acid glycoside, one of the most abundant components in raw pasta, was not detected after boiling. This drastic change indicates that chemical modifications are occurring...
which could explain the elevated content of the 3 other molecules mentioned. On the other hand, the levels of rosmarinic acid are not significantly affected by the boiling process, contributing to preserve the antioxidant capacity of pasta supplemented with chia at the moment of intake. The results show that the abundance of the main components is correlated with the antioxidant capacity measured by chemical methods; therefore, the increased antioxidant activity of supplemented pasta compared with control wheat pasta is explained by the presence of phenolic compounds provided by chia, particularly rosmarinic acid and other caffeic acid derivatives.

This work represents an interesting contribution in the field, considering that it is the first report with a detailed profile of phenolic compounds of defatted chia seeds and a derivative food, using HPLC-MS/MS methodology.

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P5

DEVELOPMENT OF A RAPID METHOD FOR THE UNTARGETED DETECTION OF CONTAMINANTS IN MILK USING VIBRATIONAL SPECTROSCOPY AND CHEMOMETRICS: THE EXAMPLE OF MELAMINE

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Keywords: untarget analysis; spectroscopy; Chemometrics; milk.

In this study, a rapid method for the characterization of a typical and important agro-food product, the milk, is proposed using vibrational spectroscopy (mid-infrared - MIR) and Chemometrics. The objective is to exploit the huge amount of information contained in the data generated by such techniques, which could support the concept of data-driven discovery or untargeted analysis. In contrast to targeted analysis, which uses information from known possible unusual ingredients, an untargeted experiment registers all information within a certain correlation/similarity, including data from new products. Untargeted detection methods are therefore required for screening products for a range of known and unknown adulterants. Untargeted analysis will mean alerts can be given more rapidly and fraud detected more easily. Until now, untargeted analysis has been associated mainly with direct analysis techniques, such as mass spectrometric-based metabolomics or isotope-assisted methods. Only a few studies have linked untargeted analysis with vibrational spectroscopic methods.

In this study, vibrational spectroscopic techniques combines with new concepts in multivariate analysis for characterizing liquid UHT milk samples spiked with varying levels of melamine. Melamine has been illegally added to food/feed to artificially elevate the protein content value of products. Since the discovery of melamine contamination in infant milk formula in China, strict regulations have been enforced throughout the world and many papers have been published on the use of such methods as wet chemistry, chromatography, mass spectrometry and vibrational spectroscopy to detect melamine in both raw and powdered milk. In this study, liquid ultra-high temperature (UHT) milk was contaminated with melamine at various levels ranging from 0.01% to 1% (100-10,000 ppm) and measured using Fourier Transform Mid-Infrared (FT-MIR) spectrometry in order to test the performance of the new chemometric method and determine its limits of detection.
P6

BOTTOM-UP APPROACHES FOR THE DETECTION OF LTPS IN ALMOND AND PISTACHIO

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Keywords: food allergy, lipid transfer proteins, tree nuts.

Food allergy is an issue of major concern for human health, as it can lead to life threatening symptoms and affects about 6-8% of young children and 3-4% of the global adult population. Tree nuts are among the foods which are most frequently responsible for IgE-mediated reactions, often associated with acute clinical symptoms and even anaphylactic shock, so the detection and the characterization of the allergens responsible for these reactions, are of crucial importance. Almond and pistachio are largely used in sweet bakery products as ingredients and pistachio is as well employed in the production of food of animal origin (e.g. mortadella). Like other nuts, almond and pistachio are currently ingredients that have to be mandatorily declared and highlighted in label, accomplishing to EU Regulation 1169/2011 and following amendments. Beside allergens that are major seed-storage proteins, such as Legumins, Vicilins and 2S Albumins, also species that are only minor constituents of the protein fraction are increasingly being recognized as relevant allergens in tree nuts. This latter group includes non-specific lipid transfer proteins (nsLTPs). The molecular features of nsLTPs, such as the presence of eight cysteine residues forming four disulfide bridges, confer a compact structure, decreasing the probability of degradation due to cooking or digestion, thereby increasing the chance of systemic absorption and severe allergic reactions. Few studies on LTP-induced allergies regarding almond and pistachio are available in the literature; for both, genes coding for LTPs were sequenced, but yet no protein belonging to this family has been isolated and characterized at protein level. In the present work, we describe for the first time the extraction and purification of an almond LTP, achieving its full characterization by using liquid chromatography and exact mass spectrometry; the full sequence was identified by means of LC-ESI-Orbitrap™-MS applying a bottom-up approach. The characterized protein consists of 92 amino acids and has a calculated exact MW of 9579.0. The presence of four disulfide bridges was confirmed after reduction, as shown by a mass increment of 8 Da. Finally, its potential allergenicity was confirmed via an in silico approach. The same experimental procedure used to isolate the novel almond LTP was applied to pistachio, but any attempt to detect the presence of an LTP in these nuts did not yield the expected results, indicating that this kind of protein is probably absent or present only in traces in pistachios. The results presented here demonstrate the enormous potential of advanced MS techniques for obtaining high-quality structural and functional data of allergenic proteins in a short time. The project “Developing innovative methods for detecting emerging food allergens and evaluation of their impact on consumer health: an integrated approach (INTEGRALL)” is gratefully acknowledged for funding.
**P7**

**DEVELOPMENT OF AN EASY PROTOCOL TO RAPIDLY DISCRIMINATE EXTRAVIRGIN OLIVE OIL FROM OLIVE AND SEED OIL BY MEANS OF DIRECT SAMPLE ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY**

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**Keywords:** oil, food frauds, mass spectrometry.

Olive oil is considered one of the most Italian agricultural commodities and represents an important product in terms of well-being and economy in Europe as well as around the world for its sensory, nutritional property, and for health. With the Regulation (EC) n. 2568/91 (1), the European Commission defines the characteristics and parameters for olive oil and the methods to differentiate oils in order to detect adulteration. Extravirgin oil is one of the most expensive edible oils, which makes it a preferred target for fraudsters. Despite the effort of regulatory and control bodies investigating the fraud, there are still some types of potential frauds that are very difficult to detect at present. In order to protect consumers from commercial frauds an easy and fast method to detect the real composition of oil is requested. In this study we analysed the fingerprints of oil to identify the most informative compounds in order to find out a marker capable to distinguish between extravirgin olive oil, refined olive and seed oil by Direct Sampling Analysis (DSA) coupled with a High Resolution Mass Spectrometer (AxION2 TOF - Perkin Elmer).

Samples of extravirgin oil, olive oil, sunflower seeds, corn seeds, peanuts seeds, soy and rape seeds were bought at supermarkets and stored at room temperature before extraction. Sample dilution was obtained by mixing 10 ul of oil with 1 ml of isopropanol and ammonium acetate 10 mM, vortexed for 5 minutes and then centrifuged for 2 minutes at 2000 x g. Ten microliters of each supernatant were pipetted directly onto the stainless mesh of the AxION DSA for N2 ionisation and analysis. Measurement was run in negative ionization mode with flight voltage of 10KV, corona current 4µA, T= 300 ° C, capillary exit -120 V, gas flow 3 L/min. Mass spectra were acquired in a range of m/z 50-1200 at an acquisition rate of 1 spectra/s. All samples were analysed within 30 seconds. Data acquisition, peak identification and error in mass accuracy were performed by using the software Axion (Perkin Elmer®).

The fatty acids (FA) composition was studied and the FA adduct [M-H]- searched in the mass spectrum was 281.2480 for oleic acid, 255.2324 for palmitic acid, 279.2324 for linoleic acid, 277.2167 for linolenic acid and 283.2637 for stearic acid. An applicant adduct corresponding to a mass/charge ratio of 563.7 (X compound) was systematically registered in all spectra. The data obtained were analysed and the ratio between oleic acid/X compound adduct was found to be a useful marker to distinguish extravirgin olive oil (E) from refined olive (O) and seed oil (S). In all E samples analysed the ratio of oleic acid adduct and the X adduct was in the range of 0-6, in S samples was 8-24, while in 80% of O samples was in the range of 7-22. Data obtained in this preliminary study showed the ratio of oleic acid adduct and the X adduct as a good strategy to discriminate different types of oils and therefore to protect consumers from commercial fraud. Further analysis are in progress to obtain a better discrimination between extravirgin and olive oil.

DEVELOPMENT OF AN EASY AND FAST PROTOCOL TO RAPIDLY DISCRIMINATE WILD FROM FARMED FISH BY MEANS OF DIRECT SAMPLE ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY

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Keywords: farmed, wild, fish.

The nutritional and quality attributes of fish make it an excellent product recommended for human health. However, as the world wild fish stocks are limited and the market demand increased over recent decade, fish farming developed in order to offer an alternative and to reduce costs for consumers. The sale of farmed as wild fish is a fraudulent practice, thus requiring the development of reliable analytical methods, based on sensitive, rapid and low cost techniques, in order to protect consumers from commercial frauds and to ensure food traceability (1). Studies have confirmed that the fatty acid composition of farmed and wild fish are different and diet is the main reason for these differences (2). Consumers often do not have instruments or competence to visually discriminate if a product corresponds to what is indicated on the label, therefore a specific control to protect them from commercial fraud is desirable. The aim of the present study was to develop an easy and fast method to extract and simultaneously detect fatty acids (FA) in wild and farmed fish by Direct Sampling Analysis (DSA) coupled with a Time of Flight Mass Spectrometer (AxION2 TOF). The FA investigated were Docosahexanoic acid (DHA), Eicosapentaenoic acid (EPA), Linoleic acid (LA) and Arachidonic acid (AA).

Samples from Mediterranean sea of wild and farmed Sea bream (Sparus aurata) and Sea Bass (Dicentrarchus labrax) were bought and stored at 4°C before FA extraction. Extraction consisted in mixing 5 g of homogenized muscle with 10 ml of n-hexane; samples were vortexed for 5 minutes and centrifuged for 1 minute at 2000 x g. Ten microliters of each supernatant were pipetted directly onto the stainless mesh of DSA; MS-TOF analysis were performed by using atmospheric pressure chemical ionization APCI source in negative mode. The optimised experimental parameters were: corona current -4µA, DSA source temperature 300 °C, flight voltage 10kV, capillary exit -120 V, drying gas flow rate 3 L/min.
Mass spectra were acquired in a scan range of 20-2000 (m/z) at an acquisition rate of 1 spectrum/sec. Calibration solution was infused at 10 µl/min. All samples were analysed within 30 seconds. Data acquisition, peak identification and error in mass accuracy were performed by using the software Axion (Perkin Elmer®). The adducts [M-H]- searched in the mass spectrum were 279.2324, 301.2167, 303.2324 and 327.2324 for [LA -H]-, [EPA-H]-, [AA-H]-, and [DHA-H]-, respectively. All the four analytes were correctly identified in the extracted samples; the most abundant peak (m/z) observed in the mass spectrum was [DHA-H]-, while [AA-H]- was the least abundant (Figure 1). In order to compensate species variability and obtain a discrimination between wild and farmed fish, we used the ratio between the most abundant FA recorded in the spectrum and the lowest one. We highlighted a consistent difference between [DHA-H]-/[AA-H]-: it was ≥ 8 for farmed species and ≤ 4 for wild species. The [EPA-H]-/[AA-H]- ratio was able to reveal a difference and it was higher than ≥ 5.3 in farmed species and ≤ 4.5 for wild species.

Figure 1: Example of mass spectrum of FA adducts extracted from a sample of Sea Bass

In this work we developed a simple, rapid and useful method to detect FA in fishes and the results are promising; data obtained showed the FA ratio as a good strategy to discriminate farmed from wild fishes and therefore to protect consumer from commercial fraud. Further analysis, increasing the number of samples, are ongoing to confirm the results.

2. Kriton Grigorakis, “Compositional and organoleptic quality of farmed and wild gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors affecting it: a review”, Aquaculture, 2007, 272: 55-75.
P9

COMPREHENSIVE ASSESSMENT OF DNA QUALITY FOR ACCURATE QUANTITATIVE MEAT FOOD SPECIATION

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Keywords: Meat food; DNA purification; DNA integrity; Real Time PCR quantification.

Following the horse meat scandal, occurred in Europe in 2013, food research focused on meat species identification and quantification methods. Adding not declared species in a particular preparation allows a reduction of cost production for food industries. However, this can be defined as a food fraud with serious health, ethical and religious consequences. The European Union established the 2013/99/EU regulation, which specifies that meat products containing >1% of undeclared meat ingredients should be considered to be adulterated. For this reason the lack of an accurate method of species quantification is the main problem to properly distinguish between fraud and unintended processing contamination in mixed meat food.

Real Time qPCR is considered one of the most accurate methods for DNA quantification. Even though often underestimated, the DNA isolation step is crucial due to the presence of chemical inhibitors, proteins and/or damaged DNA in meat food. Therefore, many companies developed specific food DNA purification methods. Here we compare four commercial DNA extraction kits on minced meat samples: Gentra Puregene Kit (Qiagen, salting-out based method), Mericon DNeasy Food Kit (Qiagen, modified CTAB based method), NucleoSpin Food DNA Purification Kit (Machery-Nagel, column based method) and Wizard Magnetic DNA purification Kit (Promega, paramagnetic particles based method).

In cooperation with Coop Italia company, five reference matrices of pork and beef mixed meat were prepared in these percentages: pork/beef% 0.1; 1; 5; 10; 30. Pork meat was considered as contaminant species. The DNA extractions were performed according to the manufacturers’ specifications adapting the protocol to 1 g of starting material. For each sample we performed three extractions with the same kit. DNA yield of each extract was determined using a fluorometer (Qubit, Invitrogen) according to the manufacturer’s specifications; DNA was purity evaluated by nanospectrophotometer measurement (VivaSpec LS, Sartorius) considering the 260/280 and 260/230 absorbance ratios. DNA integrity was assessed using a 2100 BioAnalyzer (Agilent), considering the average fragment size. A preliminary quantitative evaluation of the percentages (pork/beef) on each sample was performed by Real Time qPCR (Stratagene MX3005P) based on a nuclear single-copy-gene species-specific target associated with an animal DNA gene detection system (Ren et al., 2017). Variance analysis on the extraction data was performed with the ANOVA test with Bonferroni correction using the Stata statistical software.

DNA yield: Gentra purification kit resulted more efficient than the other kits (p<0.05); on the contrary the Wizard average yield appears significantly worst than the others (Fig. 1a):
DNA purity: no significant difference was detected in 260/280 absorbance values between the purification kits (p>0.05) even if the Mericon and NucleoSpin coefficients were the ones closest to the optimum value (1.8); for 260/230 absorbance, Mericon and Nucleospin performed significantly better (optimum values: 2.0-2.2, p<0.05) than Gentra and Wizard.

DNA integrity: BioAnalyzer data indicated that Mericon was the best purification kit (p<0.05), followed by Nucleospin and Gentra; Wizard was confirmed to be the worst (p<0.05, Fig. 1b)

Quantification by qPCR performed on DNA from Nucleospin, Mericon and Gentra kits were in agreement with pork input values (Table 1).

![Graphs showing DNA yield and integrity comparisons](image.png)

**FIG. 1:** a) DNA yield comparison: on X axis there are the percentages (Pork/Beef) samples, on Y axis the DNA concentrations (ng/μl). b) DNA integrity comparison: on X axis there are the percentages (Pork/Beef) samples, on Y axis the DNA fragment sizes.

<table>
<thead>
<tr>
<th>MEAT REFERENCE MATRICES (Pork/Beef %)</th>
<th>NucleoSpin Food DNA Purification Kit%</th>
<th>Mericon DNaseasy Food Kit%</th>
<th>Gentra Puregene Kit%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% Pork</td>
<td>0.16%</td>
<td>0.21%</td>
<td>0.78%</td>
</tr>
<tr>
<td>1% Pork</td>
<td>1.05%</td>
<td>1.79%</td>
<td>1.09%</td>
</tr>
<tr>
<td>5% Pork</td>
<td>3.54%</td>
<td>7.75%</td>
<td>7.54%</td>
</tr>
<tr>
<td>10% Pork</td>
<td>14.16%</td>
<td>11.99%</td>
<td>17.43%</td>
</tr>
<tr>
<td>30% Pork</td>
<td>27.93%</td>
<td>52.85%</td>
<td>34.87%</td>
</tr>
</tbody>
</table>

**Tab 1: comparison between expected percentage values and true percentage values for pork meat quantification.**

Based on the statistical comparison, Nucleospin Food kit best performed for purity, and also for yield had good values. Gentra had much higher yield, despite lower purity. Mericon was the best kit for integrity, followed by Nucleospin and Gentra with similar values. Wizard resulted as the worst kit for all parameters.

The preliminary data on quantification of genomic DNA by qPCR provided a quite accurate estimation of the contaminant species abundance. Further analyses are needed to obtain more robust data about qPCR estimation. A successful DNA purification for quality and yield is crucial, and should be considered when analyzing more complex matrices, such as meat stuff or ragout, where other ingredients or cooking process may compromise purity and integrity. The selection of the best DNA extraction method, allows the purification of representative DNA sample for downstream quantification analysis of meat food species.

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CORRELATING STRUCTURE OF BIOACTIVE PEPTIDES IN FOOD OF ANIMAL ORIGIN WITH THEIR EFFECT AND STABILITY

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Keywords: functional food, medicinal effect, transport, stability.

During fermentation and/or processing of food, peptides showing beneficial medicinal effect could be released from “parent” protein(s). Such bioactive peptides (usually 2-20 amino acids in length) can exert different activity on organism (antioxidative, immunomodulatory, antihypertensive, antimicrobial, hypolipidemic, opioid, osteoprotective and antithrombotic). Due to difference in chemical composition and processing conditions, different food sources show different distribution of bioactive peptide with particular effect. However, due to interlinking of metabolic pathways and fact that most bioactive peptides employ several mechanisms to exhibit their effect, majority of bioactive peptides are multi-active, e.g. showing more than one effect. Furthermore, often activity of whole hydrolysate rather than individual peptides is tested. This further complicates linking activity of peptide with its amino acid sequence. However, effect(s) exhibited by particular peptide will depend on type and position of amino acid. For example, high frequency of aromatic amino acids in opioid peptides could be explained by their involvement in formation of stable complexes with opioid receptors. When considering position of amino acids, first amino acid and amino acid next to opposite terminus will have the biggest effect on peptide activity. Several factors, such as number and type of amino acid residues, size and overall charge of peptide, concentration of bioactive peptides, pH, metabolic state and age of studied organism and nutrition, can have synergistic effect on determining stability of peptide. Two major problems occur when connecting amino acid sequence of peptide with its stability: correlating effect of simulated gastric digestion (either as static or dynamic model) with in vivo digestion and the fact that some effects (like antioxidative and antimicrobial) are rarely tested in vivo. However, Pro was present in 67.22 % sequences showing activity in vivo, suggesting that specific shape of this amino acid hinders formation of peptide-protease complex. But, higher stability, thus slower degradation of bioactive peptides can lead to toxicity and development of allergic response. Activity and stability of bioactive peptides can also be affected by processing. Major factors influencing stability during processing are temperature, pressure, changes in pH, extent of hydrolysis and/or duration and type of microorganism used for fermentation.

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THE DEVELOPMENT OF METHODS TO DETECT THE ADDITION OF FLAVOURINGS IN COUNTERFEIT WHISKY

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**Keywords:** Scotch Whisky, Counterfeit, Oak Lactones, Chiral Chromatography.

In the European Union, Regulation 110/2008 precludes the addition of flavouring compounds in whisky. This requirement is also seen in other whisky process definitions around the world. The detection of added flavourings is therefore particularly useful for providing conclusive evidence of the adulteration or counterfeiting of whisky produced in such countries.

As part of its whisky authenticity analysis, the Scotch Whisky Research Institute (SWRI) screens each suspect whisky sample using a sensory panel, to identify aromas that are not typically associated with a genuine product. Samples with uncharacteristic aromas then undergo analysis by SPME GC-MS to establish whether added flavourings are present. Although the SPME GC-MS analysis provides a means to identify certain compounds present in the authenticity samples, in other instances, the chemical properties of the flavour active compound may prevent its detection by this method. This may be due to low volatility, poor affinity to the SPME fibre used, or that the compound is below the limit of detection for the instrument.

This poster describes the work undertaken to identify added flavourings that had not previously been detected by the SWRI’s standard GC-MS analysis, as well as other compounds that may also be used as markers for the addition of flavouring.

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DEVELOPING RAPID ANALYSIS METHODS TO IDENTIFY COUNTERFEIT SPIRITS

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Keywords: Rapid Analysis, Counterfeit Spirits, Photometric Analyser, Miniaturised ESI-MS.

The European spirit drink industry is extremely valuable to the European economy; hence it is important to protect the reputation of EU spirit drinks from the impact of illegally produced spirits. To achieve this aim, The Scotch Whisky Research Institute (SWRI) are leading the spirit drinks work package of the EU funded collaborative “FoodIntegrity” Project under FP7 topic “Assuring Quality and Authenticity in the Food Chain”. The spirit drinks work package has targeted the development of rapid analysis methods as one of its primary aims. The spirit drinks sector is particularly interested in agile and portable solutions that can be readily employed in the field and easily moved through customs at airports. These in-field screening solutions must be cost effective and easy to use, providing quick answers that can be backed up by laboratory techniques. Current laboratory methods to identify counterfeit spirit products are often complex and require sophisticated analytical instrumentation. Hence, a requirement for rapid analysis also extends back to the laboratory, where quicker and more authoritative tests are desired.

Rapid analysis methods have been evaluated and several offer measurable improvements over current techniques. This work has also highlighted where new authentication techniques have shown potential and areas for future development.

Acknowledgements: The project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688. R-Biopharm AG, Microsaic Systems PLC.
P13

NMR AND CHEMOMETRICS TO COMPARE EUROPEAN AND NON-EUROPEAN OLIVE OILS

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Keywords: NMR, chemometrics, olive oils.

NMR-based metabolomics represents a valuable tool to study complex mixtures such as foodstuffs and obtain their metabolite profile. Combined approach of NMR and multivariate statistical analysis allowed olive oils to be characterized in terms of geographical origin and cultivar [1-2]. Since metabolomic studies provide complex data sets it is necessary to take advantage of specific chemometric methods [3]. Within “FoodIntegrity” project, 1H-NMR spectroscopy was used to analyze 50 virgin olive oils mostly monovarietal, 35 of them coming from European countries (Italy, Spain and Portugal) and 15 from non-European ones (Australia, Argentina, Uruguay, Turkey and Tunisia). NMR results submitted to different multivariate analyses such as PCA, LDA PLS and PLS-DA allowed olive oils to be characterized in terms of provenance and cultivar.

References
**P14**

**RECENT DEVELOPMENTS IN FOOD SCREENING BY NMR: THE FOODSCREENER CONCEPT**

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**Keywords:** NMR, authenticity, FoodScreener.

Nuclear Magnetic Resonance (NMR) has been used for structure analysis of pure compounds over decades. The extraordinary potential of NMR has started to be recognized and exploited in the analysis of mixtures in the context of biofluids, food and beverages. This was possible due to the availability of high throughput automation technology, the increase of sensitivity and modern pulse sequences, with simultaneous suppression of big signals, such that simple proton NMR has become more and more applicable, providing highly information rich data, even in very complex mixtures.

Due to minimal sample preparation (typically only addition of buffer and, in some cases, pH adjustment), the high-throughput automation, and the very high reproducibility, also due to the standardization of all steps from sample collection to the final processing of data, the proton-NMR technique offers a low-cost-per-sample screening. Two different analysis tasks can be applied to one single measurement: targeted and non-targeted analysis. The targeted analysis deals with the quantification of several compounds, possible due to the large and linear dynamic range and truly quantitative NMR spectrum. The non-targeted approach applies statistical methods to the same data to qualitatively validate or classify a sample.

On these bases, Bruker, with the help of experts in different fields, has developed an innovative solution for the automatic analysis of wine, fruit juices and honey, using Nuclear Magnetic Resonance: the Bruker FoodScreener.

The principle of this method relies on the acquisition of the spectroscopic fingerprint specific to each individual sample. These profiles are then compared to large databases of authentic samples using multivariate statistical approach. This is used to extract different types of information, e.g. determination of variety in wine analysis, addition of mandarin juice in orange juice, addition of sugars in honey, evaluation of the geographical origin of a sample or non-targeted detection of atypical compounds. Furthermore, the fully automated quantification procedure is currently able to identify and quantify a multitude of compounds for every food matrix: standard compounds, sugars, amino acids, degradation parameters, fermentation products, just to give few examples.

Combining these two different approaches, Food-Profiling by NMR allows quality control and testing of safety issues and authenticity in a unique way. Some examples of applications will be shown.
SAMPLING ISSUES ASSOCIATED WITH FRAUD DETECTION IN THE ALCOHOLIC SPIRIT DISTRIBUTION CHAIN

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Keywords: fraud detection, sampling, Spirit industry, supply chain.

Food fraud and economically motivated adulteration is of serious concern to the food and drinks industries. Many analytical tools have been developed for the detection of fraud or adulterated food products and ingredients in the food supply chain. Approaches can be 'targetted' whereby one or more specific adulterants are tested for, or alternatively an 'untargeted' approach can be adopted whereby changes to certain characteristics of the material may be monitored for example using spectral imaging. Regardless of the approach taken, some element of a sampling plan needs to be put in place. The effectiveness of a sampling plan is dependent on the sample size and the frequency of sampling. Generally the effectiveness of a sampling plan can be assessed using an operating characteristic curve which indicates the probability of acceptance depending on the sampling plan. These operating characteristic curves generally assume that the defective product is distributed randomly in the batch of test material. However, fraudulent or adulterated product will often be heterogeneously distributed in the supply chain. This potentially affects the likelihood of detection. The objective of this work was to assess the ability of a number of different sampling strategies to detect fraud depending on the heterogeneous distribution of fraudulent material. An alcoholic spirit supply chain was modelled as an example of a supply chain where fraud can occur and 'fake' product can be locally distributed in the market place. A statistical modelling approach was developed in R to mimic various supply chain scenarios. Modelling indicated that in certain situations, a systematic sampling sampling can in certain circumstances increases the probability of detection compared to random sampling when there is localised or heterogeneous distribution of defective product. The modelling approach was very flexible and can be adapted to different product supply chain scenarios.
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ASSESSMENT OF PORK AUTHENTICITY BY MEANS OF MULTI-ELEMENT ANALYSIS

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Keywords: Authenticity, pork meat, ICP-TOF-MS, LDA.

In the last years, the increased level of consumer awareness in food choices and the strengthening of the prevention and suppression of food frauds has spotlighted the concept of “authenticity” of a food product. Therefore, genuinity and origin of raw materials has became an increasingly important issue for the food chains, consumers and industries.

In this scenario, in 2011 the European Community started to improve consumer information about the origin of foods, introducing, for some products (eg. beef), the mandatory indication on the label (Regulation 1169/2011). More recently, France and Italy, in order to increase consumer transparency, have extended the requirement of origin declaration to meat and dairy in prepared foods (2016/1137 French Decree) and to milk and dairy products (9th December 2016, Italian Decree), respectively.

In order to trace “scientifically” the geographical provenance of food, specific analytical approaches were developed, including those based on multi-element analysis, by the use of spectroscopy and mass spectrometry techniques.

The study of the origin of a meat-based food through the multi-element analysis is built on the principle that, as the elemental composition of feed reflects that of the soil where the vegetal components were grown, also the elemental composition of animal tissues could reflect a specific elemental profile.

In this work, the possibility to discriminate meat of pigs bred in three different geographical areas was explored through a multi-element analysis performed with both the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Varian Vista MPX) and Inductively Coupled Plasma-orthogonal-acceleration Time-of-Flight Mass Spectrometer (ICP-oa-TOF-MS, GBC Optimass 9500). In particular, 12 samples of domestic Heavy Pig, bred in Northern Italy and used to be processed into Italian PDO dry-cured hams (A), 7 samples of Nero di Parma, a local breed of black pigs reared in the province of Parma (B) and 8 samples of foreign pork, bred in Germany (C) and used in Italy to be processed into unbranded meat products, were analyzed. Muscle samples, were taken from the outer section of Biceps femoris muscle in fresh hams, wrapped in aluminum foil, vacuum packaged and frozen until analysis.

About 1 g of minced muscle and relative blanks were mineralized in triplicate with 4 ml of HNO3 by a pressure digestion with microwave heating (UltraWAVE, Milestone, Italy) and then, after filtration on 0.45 μm filters (Millex®-HA, Millipore) and appropriate dilutions, subjected to ICP-OES and ICP-TOF-MS analysis for determining 28 elements. Ca, Mg, Na, K, P, Fe, Cr, Ni, Mn and Zn were quantified by ICP-OES, while Li, Al, V, Co, Cu, Ga, As, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Ce, Ti, Pb and U by ICP-TOF-MS. Element concentration data were normalized to dry matter content, in order to compensate for the differences in the moisture degree of meat samples. As, Ag, Ce, Cd, Ti and U were excluded from the statistical analysis, as they showed concentration values under LOD in all or almost all samples.

In a first step, the univariate analysis of variance (One-Way ANOVA procedure, SPSS Statistic V22.0) was applied to compare elements between the groups of investigated pork samples (A, B and C).
Al, presenting an extremely high variability in A group, was excluded from analysis. In total, 19 elements (Ca, Mg, Na, K, P, Fe, Mn, Zn, Li, V, Co, Cu, Ga, Rb, Sr, Mo, Cs, Ba and Pb) were considered. Table 1 shows the mean values of element concentration (expressed as mg/Kg of dry material or μg/Kg of dry material) and the results of ANOVA test (Tukey HSD post-doc test, P < 0.05).

The test pointed out that Na, K, P, Fe, Mn, Zn, Co and Cu allowed to discriminate (P < 0.05) the Nero di Parma samples (B) from samples A and C. On an average, Nero di Parma showed the highest content of Na, Fe, Mn, Zn, Co and Cu and the lowest values of K and P. Moreover, Fe and Zn could discriminate samples belonging to the three groups, because the foreign pork samples (C) exhibited the lowest values of both elements. At a later stage, Principal Component Analysis (PCA) was performed, based on the elements which proved to be discriminant in one-way ANOVA (Tab. 1). Three components were extracted accounting for 82.7% of total variance (% variance explained by PC1 = 47.8, PC2 = 23.0, PC3 = 11.9). The scatter plot of samples onto the PC1-PC2 plane, showed that some samples were sub-grouped according to breeding origin: the Nero di Parma samples (B) were separated from the A and C ones, which, conversely, overlapped (figure not shown). In order to foresee if the domestic Heavy Pig samples could be discriminated from the foreign ones, a Linear Discriminant Analysis (LDA) was attempted for explorative purposes, despite the low number of samples. Unlike previous PCA analysis, the discriminant functions were obtained under the constraint to differentiate the three groups using a variable number lower than the number of samples belonging to the smallest group. In this respect, variables less related to each other among those listed in Table 1 (Pearson r < 0.65), were retained. Thus, Na, Mg, Co, Cu, Zn and Pb were submitted to Stepwise LDA analysis. Figure 1 shows the results of LDA analysis, which highlights a good separation among the three groups. Mg, Zn and Pb resulted as the most important variables for the discrimination of the breeding area of investigated pigs. A higher number of samples will be analysed in the future to build a reliable model to discriminate pork samples according to geographic origin.

**Tab. 1: Mean values of element concentration in pork samples, one-way ANOVA and Tukey HSD post-doc tests.**

<table>
<thead>
<tr>
<th>Element*</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>1090 a</td>
<td>969 b</td>
<td>994 b</td>
<td>0.003</td>
</tr>
<tr>
<td>Na</td>
<td>1938 a</td>
<td>2376 b</td>
<td>1965 a</td>
<td>0.000</td>
</tr>
<tr>
<td>K</td>
<td>15253 a</td>
<td>13404 b</td>
<td>14205 a</td>
<td>0.003</td>
</tr>
<tr>
<td>P</td>
<td>8574 a</td>
<td>7610 b</td>
<td>8001 a</td>
<td>0.017</td>
</tr>
<tr>
<td>Fe</td>
<td>20.8 a</td>
<td>30.4 b</td>
<td>16.6 c</td>
<td>0.000</td>
</tr>
<tr>
<td>Mn</td>
<td>224 a</td>
<td>283 b</td>
<td>232 a</td>
<td>0.007</td>
</tr>
<tr>
<td>Zn</td>
<td>80 a</td>
<td>120 b</td>
<td>62 c</td>
<td>0.000</td>
</tr>
<tr>
<td>Co</td>
<td>2.48 a</td>
<td>3.50 b</td>
<td>2.92 a</td>
<td>0.000</td>
</tr>
<tr>
<td>Cu</td>
<td>1778 a</td>
<td>2070 b</td>
<td>1743 a</td>
<td>0.007</td>
</tr>
<tr>
<td>Pb</td>
<td>8.75 a</td>
<td>18.9 ac</td>
<td>19.2 bc</td>
<td>0.012</td>
</tr>
</tbody>
</table>

* Mg, Na, K, P, Fe and Zn in mg/Kg of dry material; Mn, Co, Cu and Pb in μg/Kg of dry material; different letters along rows mean significant difference (Tukey HSD post-doc test, P < 0.05).

**Fig. 1: LDA plot of pork samples bred in different areas.**
P17
A NEW APPROACH TO DETECT THE ILLEGAL USE OF MECHANICALLY SEPARATED MEAT IN MORTADELLA DI BOLOGNA IGP: A PRELIMINARY INVESTIGATION

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Keywords: mechanically separated meat (MSM), histological method, calcium, Mortadella di Bologna IGP.

Introduction
Mechanically separated meat (MSM) is the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means and resulting in the loss or modification of the muscle fibre structure. Based on the current EU Regulation, low and high pressure MSM are defined according to the alteration of bone structure and calcium content. According to the hygiene requirements after production, MSM can be used in meat preparations which are clearly not intended to be consumed without first undergoing heat treatment, meat products or only for heat-treated meat products. However, there are some Italian meat products protected by geographical indications (PGI label) such as Mortadella di Bologna IGP for which the use of MSM is forbidden according to their production specification. The distinction of MSM from fresh meat, minced meat and meat preparations or the use of MSM in meat products only on the basis of microscopic detection of muscle tissue, bone and cartilage particles is not possible, although it is considered a promising method. Calcium content alone does not allow differentiation between low pressure MSM and other meat products. Studies based on combinations of different parameters could be useful and are recommended.

Materials and methods
Two raw minces of Mortadella di Bologna IGP (from two different producers) were mixed with different amounts of low pressure (75%, 50%, 25%) and high pressure MSM (50%, 25%, 15%). Proximate composition, hydroxyproline, phosphorus and calcium content determination and histological examination were performed on the prepared raw mixtures without cooking, raw minces and corresponding Mortadella di Bologna IGP at the end of the production process. Histological samples (three replicates for each sample) were prepared according the AFNOR procedure (1999) and Calleja staining. Bone and cartilage fragments were observed by a microscope DM2500 (Leica, Wetzlar, Germany) by 100x magnification. The number of bone and cartilage fragments was counted and histological section area was measured by a stainless hardened digital caliper.
Results
The number of bone fragments (FO), cartilage fragments (FC) and the sum of both (FT) per cm² as a function of the percentage of MSM in the investigated samples evolved according to a linear relationship both in low pressure (Figure 1) and high pressure (Figure 2). MSM. The highest correlation coefficients \( r^2 \) of the relationship were observed for FT/cm². The limit of detection calculated for this parameter was 1.6 that can be considered as a minimum threshold level to confirm the presence of MSM in Mortadella di Bologna IGP. A linear relationship \( (r^2 = 0.9225) \) was observed between the number of total fragments FT/cm² and the calcium content (Figure 3). 28 mg/100g is the calcium content corresponding to the threshold level 1.6 FT/cm².

Conclusions
A new approach based on a combination of histological and chemical parameters was tested to detect the illegal use of mechanically separated meat in Mortadella di Bologna IGP. The sum of bone and cartilage fragments per cm² (FT/cm²) might be proposed as a parameter for qualitative analysis of MSM in Mortadella Bologna; 1.6 is the minimum threshold value to confirm the presence of MSM in Mortadella di Bologna IGP. Moreover, a good correlation between the parameter FT/cm² and calcium content was observed in this investigation. MSM could be present in Mortadella di Bologna IGP samples showing a calcium content higher than 28 mg/100 g; therefore, it is advisable to submit those samples to histological examination.

References
AUTHENTICATION AND TRACEABILITY OF ITALIAN RICE CULTIVAR CARNAROLI BY WHOLE GENOME SEQUENCING

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Keywords: Food authentication, traceability, Next-generation sequencing (NGS), DNA polymorphism.

Food authentication and traceability is one of the major concern to the food industry, strictly correlated to food fraud and food adulteration. Rice (Oryza sativa L.) is one of the most important crops, supplying food for over half of the world's population. Authenticity of rice products has become a key issue in the food industry addressed to protect the interests of quality conscious consumers, stakeholders, and importing countries. DNA markers offer a powerful tool to address the validation of food authenticity and traceability of primary products. Progress in NGS technology has provided opportunities to detect large number of DNA polymorphisms, even in the closely related cultivars. In this study, a whole-genome sequencing of Italian rice cultivar Carnaroli has been carried out from genetically pure certified seed. The sequencing yielded about 22.5 million reads. After quality trimming 21.5 million reads were mapped onto the reference sequence of Oryza sativa ssp. japonica cv. Nipponbare (IRGSP-1.0), providing about 90% coverage of the rice genome and an average coverage of 15.12x. Preliminary results, found 450,414 candidate DNA polymorphisms between cultivar Nipponbare and Carnaroli. These were classified into 383,080 SNPs (85%) and 67,334 InDels (15%) by polymorphism types, 150,688 homozygous (85%) and 299,726 (15%) heterozygous by zygosity type, 371,801 intergenic (82.5%) and 78613 (17.6%) by genomic location. The distribution of DNA polymorphisms was found to be uneven across and within the rice chromosomes. In particular, chromosome 8 and 10 showed the highest density of DNA polymorphisms (14.7% and 14.5%, respectively). This study represents the first report of whole genome sequencing of Italian rice cultivar Carnaroli and will contribute to develop targeted and un-targeted method for rice authentication and traceability.

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OPTIMIZATION OF DNA EXTRACTION IN HIGH PROCESSED MEAT FOR NGS EXPERIMENTS

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Keywords: Food; food frauds; DNA extraction; high processed matrix.

Food authenticity becomes a necessity for global food policies, since food placed in the market without fail as to be authentic [1]. Nucleotide- and protein- based methods for food authentication are mostly used for species detection and identification. Genomics and proteomics are usually applied to identify false description and mislabeling of foods. Interesting examples are: detection of GMOs, seafood authentication, authentication of kosher and halal meat, detection of horse meat and pork in food labeled as beef, game meat authentication, botanical origin of foods, species origin authentication [2]. In particular, DNA-based methods, molecular methods, are highly specific, reproducible, sensitive and characterized by high discriminatory power, rapid processing time and low costs, but they are strongly limited by the presence of inhibitors in food. Moreover, complex food matrices have very low DNA contents, poor quality and degraded DNA; food processing, like cooking, may degrade DNA molecules and introduce substances that could give ambiguous results during the following test [3]. Good results in molecular assay in general, are highly dependent to the preliminary steps: DNA extraction, purification and concentration. For all of these limitations and for our will to use Next Generation Sequencing technology on the detection of food frauds, the aim of this work is trying two different approach to DNA extraction. In fact, we demonstrated if we are able to obtain a good DNA from the beginning steps, we could create good libraries and during the sequence reaction we not find damaged sequences and the reads results will be more clear. The extraction methods, we would optimize, use silica approach as affinity matrix for obtaining DNA, but in various ways. i) mobile solid phase: the DNA will blind, in defined conditions of temperature, pH and ionic strength, to the silica coated on the surface of magnetic beads. ii) column-based system: the DNA will blind to silica membrane helped by ethanol and binding buffer. Sample we will use are high processed meat created from/for the Advanced Research Laboratory with different know percentage of meat and cooked as the original recipes. The purpose of this work is to find the best DNA extraction kits for high processed matrix, also if started DNA is damaged/ fragmented, for monitoring the supply chain from food frauds and guarantee the food safety of high processed foods. With this optimization we would obtain a right quantity and good quality of DNA, without inhibitors of the amplification reaction (PCR) to improve the following step of molecular detection in NGS technology.

DETERMINATION OF CROCINS IN SAFFRON AS MARKER OF TRACEABILITY AND AUTHENTICITY

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Keywords: saffron, crocins, UHPLC-MS/MS, traceability.

Saffron is a precious spice known as “red gold” obtained from the dried stigmas of the Crocus Sativus L., a plant of the Iridaceae family. Its importance is related to the peculiar quality and sensory properties, in particular aroma, colour and taste that are mainly due to the presence of safranal, crocins and picrocrocin, respectively. Recent studies have highlighted the different beneficial health values beyond its colouring and flavouring proprieties (Melnyk et al., 2010). Because of the high value and cost, frauds and adulterations of saffron are increasing and this arises main issues of traceability and authenticity. Adulteration has been achieved mainly by inclusion of other colouring and flavouring substances, cheaper and of lower quality. Saffron quality is usually evaluated by spectrophotometric analysis based on the ISO 3632 standards that allow the classification of the spice in three quality categories. This analysis however, does not reflect the main compositional complexity of the spice that, in turn, depends on the interplay of factors including origin, process (post-picking-up, drying) and storage, key aspects of its authenticity and traceability.

Saffron is characterised by a number of volatile and aroma-yielding compounds that recent literature has indicated to be about 150. Main qualitative properties depend also on presence and concentration of many non-volatile active components, including carotenoids, like crocins, zeaxanthin, lycopene, α-carotenes, as well as polysaccharides (Lech et al., 2009). Crocins are known to exert various pharmacological effects on various illnesses and anti-tumor effects by inhibition of cell growth (Aung et al, 2007).

Crocins, are the red-coloured, water soluble-carotenoids of saffron and present in the range between 6 and 16% (on dry matter basis) of saffron depending on variety, growing conditions and processing methods (Gregory et al., 2005). They are glycosyl esters of crocetin with different sugar moieties, such as glucose, gentiobiose, neapolitanose or triglucose; they can be present in saffron in cis- and trans- isomeric forms. When saffron is exposed to white fluorescent light, trans isomers change into cis ones (Sarfarazi et al., 2015).

Figure 1. Structure of crocetin and crocins.
The aim of this study is to determine presence and content of the crocins isomers of saffron of different origin and obtained by different process conditions and to use the cis-trans content ratio as index of quality, authenticity and traceability.

Analysis was carried out by UHPLC-MS/MS. A preliminary step of the study implied the optimization of the extraction efficiency for both crocins and different extraction solvents were tested (Rocchi et al., submitted) Analyses were then carried out on saffron samples by using MeOH/H2O mix as extracting solvent coupled with Ultrasound Assisted Extraction (USAE).

Results have been processed by Principal Component Analysis (PCA) that allowed to discriminate with good accuracy the origin of saffron stigmas. This study confirms the feasibility to study the crocins isomers as marker of traceability of saffron samples.

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SESQUITERPENE UNTARGETED FINGERPRINTING APPROACH AS A TOOL FOR OLIVE OIL AUTHENTICATION

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Keywords: olive oil, sesquiterpenes, authentication, fingerprinting.

Olive oil is one of the most valued food products included in the Mediterranean diet because of its sensorial quality and beneficial effects on human health, features that can make it an objective of frauds such as blending with other vegetable oils. The authentication of olive oils involves another important field: the verification of the geographical and varietal origin, which is necessary due to the increasing of the number of certified quality brands throughout Europe in recent years.

A suitable tool to achieve this purpose could be the analysis of sesquiterpenes hydrocarbons in olive oil, semi-volatile compounds originating from secondary metabolites of olive fruit. The fact that the sesquiterpenes composition is not significantly modified during the oil extraction process and whose presence is under genetic and environmental control, suggest that these compounds would be able to become in an authenticity marker of olive oil.

Traditionally, the analysis of volatile or semi-volatile fraction was based on a target approach, which determines a few marker compounds. However, the state-of-the-art strategy in food analytical authentication consists in finding specific patterns, known as fingerprints, in highly dimensional analytical data for the varietal and/or the geographical origin to be authenticated. Thus, the aim of the present work is to apply an untargeted fingerprinting approach to the analysis of raw analytical data obtained from olive oil samples from different countries and olive tree cultivar (Bianchera from Trieste (Italy) and Arbequina from Siurana (Catalonia, Spain)) through Solid Phase Micro Extraction and Gas Chromatography/Mass Spectrometry analysis (SPME-GC/MS). The results obtained show how helpful this approach is for the improvement of analytical data evaluation, due to we are able to differentiate between samples belonging to each group.
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FINGERPRINTING APPROACH WITH TRIACYLGLYCEROL PROFILES FROM LOW RESOLUTION ANALYSIS AS A TOOL TO AUTHENTICATE OLIVE OIL

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Keywords: olive oil, triacylglycerols, authentication, fingerprinting.

The high nutritional and economic value of olive oil (OO) makes this product an objective of fraudulent blending with oils of different origin, or mislabeling with respect with its variety or geographical origin. Lipid fingerprints, for instance those based on acylglycerol profiles, are promising candidates to detect olive oil adulteration, and to verify its botanical (variety) origin. Several analytical methods exist to determine triacylglycerol profiles, differing in their resolution, analysis time, cost... HPLC-RID has been traditionally used although it has a low resolution which makes it difficult the proper identification and quantitation of the triacylglycerol species. Alternatively, several high resolution methods have emerged with this purpose, most of them being more expensive and less available for some routine labs.

The state-of-the-art strategy in food analytical authentication consists in finding, in highly dimensional analytical data, patterns (known as fingerprints) specific for the category to be authenticated, different from those in adulterated samples. The advantages are that by fingerprinting of the chromatographic analytical signals, there is no need of peak identification and quantitation. Thus this approach can be helpful in low resolution signals such as those from HPLC-RID.

Here, we have analyzed the triacylglycerol profile by HPLC-RID of 70 virgin and extra virgin olive oil samples from 3 varieties and various geographical origins, as well as of olive oils adulterated with seed oils. Subsequently, chemometrics has been applied to raw analytical RID data to develop models to verify the botanical origin and to detect adulteration. Data pretreatment has involved peak alignment to reduce noise in data from variability in the retention times, as well as several preprocessing strategies such as 1st and 2nd derivative. Then, PLS-DA or PLS regression have been applied to develop authentication models that have been internally validated by leave 10%-out cross-validation.

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P23

STANDARDIZATION OF BY-PRODUCTS FROM EDIBLE OIL REFINING INDUSTRY TO ASSURE THEIR RELIABLE, COMPETITIVE AND TRACEABLE USE AS FEED INGREDIENTS

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Keywords: by-products, feed, acid oils.

Foodintegrity comprises the whole feed and food chain. Therefore, the traceability and quality of feed ingredients is a key aspect to control and standardize. The use of by-products as feed ingredients is usual because of their low price compared with native products. The acid oils (AO) and fatty acid distillates (FAD) are by-products coming from refining of edible oils and fats. AO come from the soapstocks obtained during the alkali neutralization step in chemical refining and FAD from the distillation step in physical refining. In both cases the major components are free fatty acids. These fat materials have been used in feed for years due to their high energetic value. In addition, a higher use of these by-products in feed formulation would contribute to the sustainability of the food chain.

However, their use in animal feeding finds some limitations, mainly coming from the high variability of composition shown by AO and FAD. As a consequence of this unpredictable variability, they lead to unexpected animal production results in some cases and this decreases the quality perception of these fat materials among feed producers and farmers. This variability, in many cases, is partially originated from the blending of these by-products from different origins in the refineries. In many refineries, soapstocks obtained from chemical refining of various fats and oils are mixed in the same tank and the same can happen with FAD. Thus, in these cases the final composition of the AO and PFAD will depend on the fats and oils that have been refined during the tank filling period. Thus, this indicates the need for more exhaustive information of the composition and stability of AO and FAD in the market to be used as reliable feed ingredients.

According to these facts, the objective of our study is to characterize the AO and FAD coming from industrial refining to study their sources of variability and suggest standardization strategies.

A total of 92 samples of these fat by-products have been taken and their fatty acid composition has been determined. Results have shown a high variability in the composition, even for similar oils and blends. PCA has revealed grouping of samples mainly according to their botanical origin. This grouping has been compared with the composition declared by the producers, and some disconformities have emerged. This reflects the need of standardization for these types of by-products, and that fatty acid profiles are among the parameters that can provide information on sample identity and composition.

This study has been funded by a research grant (AGL2015-64431-C2-2-R) from MINECO/FEDER (EU).
TRACING THE GEOGRAPHICAL ORIGIN OF LENTILS (LENS CULINARIS MEDIK.) BY INFRARED SPECTROSCOPY IN COMBINATION WITH CHEMOMETRICS

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Keywords: geographical origin, lentil, infrared spectroscopy, chemometrics.

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, the potential of infrared spectroscopic fingerprinting technique for the geographical origin traceability of lentils was investigated. In particular, lentil samples from two different countries, i.e. Italy and Canada, were collected from retail markets and analysed by Fourier transform near- and mid-infrared spectroscopy (FT-NIR, FT-MIR). After a suitable pretreatment of the raw spectral data, Linear Discriminant Analysis (LDA) was used examining the FT-NIR and FT-MIR fingerprints separately and in combination in order to evaluate the spectral range mostly influenced by geographical origin. The LDA classification results were expressed in terms of recognition and prediction abilities (cross validation and external validation). Good classification results were obtained for both FT-NIR and FT-MIR ranges with FT-MIR one giving better prediction abilities, i.e. 95% and 92% for cross and external validation, respectively. The combination of the FT-MIR and F-NIR did not improve the model performances. These findings demonstrated the suitability of the methods developed to discriminate geographical origin of lentils and confirmed the applicability of the infrared spectroscopy, in combination with chemometrics, to solve geographic origin issues of foodstuffs.

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MEASURES AND ACTIONS THREE YEARS AFTER THE HORSEMEAT INCIDENT OF 2013

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Keywords: horsemeat, beef, supply chains, Elliott Review.

The horse meat incident of 2013 (the so called ‘horsegate’ crisis) highlighted complexities in the food supply systems. Horsegate saw beef meat being fraudulently adulterated with horse meat resulting in widespread recalls and subsequent investigations across both the food service and retail channels throughout the European Union (EU). Managing complex supply chains can be difficult and the crisis subsequently revealed complicated global supply and sourcing arrangements involved in securing supply in beef chains. The investigations exposed vulnerabilities in the beef supply chain, particularly at EU level where consignments move freely between union countries with minimal checks.

Six months after the crisis, an independent review into the integrity and assurance of food supply networks was commissioned by the UK government. This review was undertaken by Professor Chris Elliott of Queen’s University, Belfast. The final report recommended eight pillars of Foodintegrity to industry and government; Consumers First, Zero Tolerance, Intelligence Gathering, Laboratory Services, Audit, Government Support, Leadership and Crisis Management. This poster examines the extent to which these recommendations have been implemented (as of September 2016) using personal communications with Professor Chris Elliott and relevant industry bodies including the International Meat Traders Association (IMTA) and British Retail Consortium (BRC) Global Standards.

Following the review, industry attitudes have changed substantially with industry recognising that even ‘a little bit of cheating’ or shortcuts are unacceptable. Transparency among the supply chain is now the key initiative undertaken by industry and several campaigns have helped rebuild consumer trust. Testing and surveillance systems within the meat industry have been integrated into normal industry practice. Despite strides made forward in intelligence sharing among industry, intelligence is still not shared with Government/regulator. Regulators within the EU share information among their contact points but not with industry and thus information sharing is still ‘siloh-like’ among regulators and industry. Testing of meat products has improved substantially but there are concerns that wider surveillance and testing on other products and commodities is lacking. It is believed that significant steps have been taken to address the recommendations regarding auditing with standards being developed and adapted to help address food fraud and Foodintegrity. However, the number of audits have increased rather than decreased as recommended, but food retailers and food service operators have moved towards unannounced platforms.
In addition, the Government are more prepared for future incidents through the establishment of the National Food Crime Unit (NFCU) and the Cross- Government Group on Foodintegrity and Food Crime. However, the poor financial resource allocated to the NFCU is regarded as a bottleneck to enabling meaningful actions and activities to be carried out by the organisation.

Horsegate raised the profile of food fraud and crime in supply chains and despite improvements to date, further collaboration between industry and Government is required to fully align with the recommendations.

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EFFECTIVENESS OF GERMINATION TO REDUCE WHEAT GLUTEN EPITOPES

**Keywords:** wheat, gluten epitopes, germination, mass spectroscopy.

Gluten is a key factor of wheat technological quality conferring to the dough its viscoelastic properties. However, it is currently associated with several disorders including non-celiac gluten sensitivity, gluten ataxia, dermatitis herpetiformis and wheat allergy. Thus, several attempts have been made to reduce wheat gluten allergenicity, such as germination. Indeed, during wheat kernels germination, proteases break down proteins into smaller units to provide free amino acids to the embryo; consequently, epitopes can be broken down, reducing the allergenicity of gluten. In this work, wheat kernels germination was performed for eight days at 25 °C. Then, the protein profile was studied using Osborn method followed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Germinated wheat kernels were subjected to in vitro gastrointestinal digestion together with ungerminated kernels. To investigate germination effect, gluten epitopes elucidating celiac disease were identified and quantified using mass spectroscopy technique. Furthermore, free amino acid amounts were determined on the mixture obtained after digestion.

Protein profiles revealed an important difference between wheat protein fractions before and after germination. Gliadin and gluten were severely degraded due the activity of protease of germinating seeds. Regarding gluten epitopes related to CD, the multivariate analysis showed a high significant effect of genotype, germination and their interaction. Germination induced a drastic reduction in total immunogenic peptides (35.72- 57.75 %), total toxic peptides (30.19-78.86 %) and total immunogenic toxic peptides (34.56- 61.51 %). Furthermore, it induced an important increase in free amino acids amounts. Indeed, correlation coefficients and principal components analysis established that the hydrolysis of gluten was highly correlated to the increase of free amino acids contents. This study suggests that germination might be considered a biotechnological alternative to provide a low “allergenic” raw ingredient for special wheat based foodstuffs.
INNOVATION IN HACCP SELF-MONITORING PLANS AND PRECISION LIVESTOCK FARMING IN MILK CHAIN: A BIOELECTRONIC PLATFORM FOR THE IDENTIFICATION, DAILY CONTROL AND MANAGEMENT OF BIOMARKERS

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Keywords: Dairy supply chain; Good Dairy Farm Practices; Traceability; Biosensoristic devices.

The Hazard Analysis and Critical Control Points (HACCP) approach is the widely used on-enterprise strategy to control and manage the safety of food production process as well as to support traceability and liability. The ALERT technological strategy, based on the bioelectronic multiprobe platform BEST patented by the ISS, implies the transfer of updated know how to innovate HACCP and self-monitoring plans with new biomarkers and chemical/toxicological aspects. The milk chain is particularly challenging and challenged by practices (e.g. water dilution of milk or mixing with milk from different species) possibly hindering safety issues, or unintended/unexpected contaminations. Indeed, the milk chain would benefit of early identification of anomalies to shield from commercial frauds and guarantee both authenticity and traceability of the food product. Innovative technologies for monitoring such factors as farm animal health, productivity, food wholesomeness and traceability can make a substantial difference. BEST is a patented, metabolome-based bioelectronic platform for precision livestock farming, integrating and daily monitoring anomalies in parameters relevant to undesirable substances, animal health/welfare and milk quality/composition.

The ALERT Consortium (www.alert2015.it) integrates public scientific bodies (Istituto Superiore di Sanità, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Consiglio Nazionale delle Ricerche) and qualified Italian SMEs encompassing the fields of dairy production, sensor technologies and marketing (Centrale del Latte di Roma, Lattepìù, Amel, Biosensor, Nutriservice, Leonardo Business Consulting). The Consortium is funded by the Italian Ministry of Economic Development under the Call “New technology for Made in Italy”. ALERT is organized in a Technology Cluster implementing the BEST hardware/software, a Risk Management Cluster implementing the use of BEST as a system for safety management of the dairy production chain, and a Market Cluster organizing the industrialization aspects.

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VALIDATION OF NMR FINGERPRINTING METHODS FOR THE ANALYSIS OF AQUEOUS EXTRACTS OF WHEAT AND FLOUR

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Keywords: NMR spectroscopy, wheat, flour, validation.

The goal of this work was to set up validation procedures and new quality control parameters 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 ILa,b 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 ILCc 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-tetradeuteropropionic 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.

References


Acknowledgments: All the researchers listed in references a and b as participants to ILCs are gratefully acknowledged.
A PROTEOMIC APPROACH FOR THE SAFEGUARD OF A TYPICAL AGRI-FOOD PRODUCT: FIORE SARDO PDO

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Keywords: UREA-PAGE, pecorino cheese, food fraud, heat-treated milk.

Fiore Sardo PDO is one of the oldest Mediterranean hard cheeses, dating back to Bronze Age, exclusively produced in Sardinia (Italy) from raw whole ewe's milk curdled using lamb rennet. The rind varies from deep yellow to dark brown in colour and encases a paste that varies from white to straw-yellow. The sharpness of the flavour depends on the length of maturation. The young Fiore Sardo is about a couple of months old, the ripened type is more than six months old.

It was awarded Protected Designation of Origin (PDO) status from the European Commission in 1996 (EC Regulation no.1263/96). The “Consortium for the Protection of Fiore Sardo Cheese” safeguards the original cheesemaking protocol, which contemplates the use of raw milk as one of its most essential feature, that gives the product its unique characteristics.

Some manufacturers, improperly, submit raw milk to therminization (68 °C for 15 s), even for the manufacture of cheeses that should be produced from raw milk. Heat treatment of milk may have several advantages, such as safeguarding the hygiene of industrial production pipelines, increasing product safety and cheese yield. The use of raw milk is one of the most relevant characteristics of Fiore Sardo PDO, while other Sardinian sheep milk cheeses, generally referred to as pecorino, can be produced from heat-treated milk, hence the need for a method that can discriminate between Fiore Sardo PDO, produced according to the specifications, from that does not comply.

Aim of the present study was to evaluate the proteomic profile of Fiore Sardo PDO and to investigate possible differences between cheese made from raw milk (R) and from heat-treated milk (HT). Starting from the same ovine bulk milk, 8 cheese wheels of Fiore Sardo PDO were produced, four with raw milk and four with heat-treated milk, and all the further productive steps were conducted in the same conditions for both types (R/HT) of cheese wheels.
Samplings at different ripening times (141, 183, 197, 294 and 394 days) were made for both types (R/HT) of cheese wheels. Finely grated cheese samples (0.2g) were dispersed in a reducing buffer solution. The mixture was held at 40 °C for 45 min, mixed (1:1) with a marker dye solution and subjected to denaturing monodimensional electrophoresis (1D-UREA-PAGE). After electrophoretic separation, the gels were stained with Blue Silver (2).

1D-UREA-PAGE analysis has highlighted the presence of a protein with a molecular weight of ̴40 kDa, only in cheese wheels produced from raw milk (R), regardless of cheese ripening time. The band of interest was excised from the gel and protein identification analysis are currently ongoing by liquid chromatography mass spectrometry (LC-MS/MS). Protein identification will provide further information on the protein of interest.

In conclusion, 1D-UREA-PAGE represents a rapid method to evidence proteic differences related to heat-treatment of milk, providing a potential useful tool for discriminating Sardinian sheep milk cheese made from HT milk (pecorino cheese) and that made from raw milk (Fiore Sardo PDO cheese), for the safeguard of consumers and producers interest.

References


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ANNOTATED LITERATURE DATABASE: A REVIEW ON ANALYTICAL DNA METHODS APPLIED TO COMPLEX FOOD MATRICES

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Keywords: DNA-based methods, complex food, database, food frauds.

Introduction
Counterfeiting of food products has a major detrimental effect on the EU food industry as consumers start to doubt the authenticity of European brands. Whereas food safety within Europe is well coordinated and has a high profile, this is not the case for detection of food frauds or the enforcement of associated legislations.
In recent years, consumer preference for food with declared provenance has led to an increase in the marketing of foods from designated origins/productions and a strengthening of European legislation regarding the labelling of food.
In the later centuries, many food frauds have been discovered, such as methanol in alcoholic spirits, or horse meat in beef burgers. So, in the last decades many detection methods were developed.

Objectives
To assess critically the scientific literature and patents reporting application of analytical DNA methods to authenticity testing of complex food products.

Methods
The bibliographic research was performed using PubMed and EBSCO libraries by keywords. The articles were analysed with “Lucene 4.0.0” and carried out on “lukeall-4.0.0-ALPHA” to have a user-friendly interface.

Results
Since the list of articles to analyse was very long (more then 100), we adopted an automatic approach to document classification. In order to create a textual index of all considered documents we developed a java code exploiting the “Lucene 4.0.0” library. In order to browse the above-mentioned index, we adopted the “lukeall-4.0.0-ALPHA” java library. This library allows us to search within index by using a simple keyword query or a complex query with Boolean operator and multiple keywords.
The literature database has been developed for a critical evaluation of analytical DNA methods and tools applied to complex food matrices, in a format for easy referencing. This searchable database contains all essential bibliographic information for the retrieval of the relevant documentation. We have analysed 117 publications in the period of about 20 years: from 1996 up to 2017.
Conclusions
An annotated literature database has been developed for a critical evaluation of literature on analytical DNA methods and tools applied to complex food matrices, in a user-friendly format for easy referencing. This survey on the current state-of-the-art of know-how, methodologies, and reference materials have been mainly applied to ingredients of two complex foods: ready sauce and stuffed pasta dish. According to this evaluation the most appropriate DNA fingerprint technologies will be chosen for further testing based on: feasibility, demonstrated usefulness in authenticity issues, and consultation with industrial partners.

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METROFOOD-RI “INFRASTRUCTURE FOR PROMOTING METROLOGY IN FOOD AND NUTRITION”

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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 Foodintegrity, leading to important relapses on different application fields (agrofood; sustainable development; food quality, safety, traceability and authenticity; environmental safety; human health).

Currently, thirty-six partners from 17 Countries, together with an international partner (FAO) are involved in the METROFOOD-RI. Each partner brings its wide and consolidated network of international collaboration, which will ensure a very broad range of action, open also to Developing Countries and new markets and able to meet the needs of the scientific community and all stakeholders at a global level. METROFOOD-RI will be built on a Hub&Node model constituted by a Central Hub in Italy, and a network of 17 National Nodes, one for each Country. Some National Nodes – typically those of the Countries with more Partners – will be in turn organized in a network of Centres. In that Countries, dedicated Joint Research Units have been established.

Acknowledgements: METROFOOD-RI is currently under its “Early Phase”, founded by EC trough the H2020 INFRADEV-02 project PRO-METROFOOD “Progressing towards the construction of METROFOOD-RI” (GA n.739568).
A NEW QUANTITATIVE REAL-TIME PCR APPROACH FOR THE DETECTION OF LUPINE ALLERGENS IN FOOD PRODUCTS

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Keywords: Lupinus albus, food allergens, real-time PCR, food analysis.

Lupine (Lupinus albus) has been increasingly used as a functional food and as an ingredient in all kind of food products (bakery, confectionary, snacks) due to its nutritional value and technological properties. However, allergy to lupine has been reported with an increasing prevalence in last years [1]. Currently, there are 11 allergens identified in Lupinus spp., of which two are included in official database, Lup a 5 (L. albus) and Lup an 1 (L. angustifolius) [2]. For that reasons, lupine was recently included in European Union regulations as a food whose presence must be declared in the list of labelled ingredients of pre-packaged foods, regardless of their amount [3]. Analytical methods able to detect and quantify lupine allergens with high specificity and sensitivity are of great importance to help food industry in allergen management and to guarantee life quality of sensitised individuals. In this work, a real-time PCR approach with a TaqMan™ probe was developed to detect and quantify a lupine allergen in food products. Reference mixtures of known quantities of L. albus in rice (10% to 0.0001%, n=11) were prepared and 27 commercial samples were purchased from local markets in order to verify the compliance with label. DNA extraction was performed using the NucleoSpinFood kit. The sequence encoding the allergenic protein PR-10 of L. albus was choose for the design of specific primers and probe. The results showed that qualitative PCR confirmed the specificity of primers to Lupinus spp., with a sensitivity of 0.0005% (w/w) of lupine in rice. The real-time PCR assay with a TaqMan™ probe was specific for L. albus and L. luteus, showing absolute and relative sensitivities of 1 pg of lupine DNA and 0.0005% (w/w) of lupine in rice, respectively. The parallel amplification of both, the target sequence and an endogenous control gene, enabled the development of a normalised quantitative calibration model based on the ΔCt method in the range of 10-0.0005%. The method exhibited adequate real-time PCR performance parameters (PCR efficiency=96.4%, R2=0.9865 and slope=-3.41), being successfully validated. From the 27 tested samples, 7 were positive by the lupine-specific PCR assay, but only 5 were confirmed by real-time PCR, with the estimated concentrations between 3.01% and 17.43%. From the 14 samples that mentioned “may contain traces of lupine”, none demonstrated its trace presence, suggesting the common practice of precautionary labelling. It is possible to conclude that useful and effective tools were proposed for lupine detection and quantification in food products, to verify labelling compliance and to guarantee consumer's protection.

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ISOTOPIC AND METABOLOMICS CHARACTERIZATION OF SLOVENIAN APPLE JUICE AND THEIR USE IN DETERMINATION OF GEOGRAPHICAL ORIGIN

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Keywords: Apple juice, Stable isotopes, Geographical origin, Slovenia.

Apples and apple products have an important role in human food consumption. The popularity and demand for apple juice consumption has been steadily increasing, which consequently is leading to an increased level of fraud. Therefore the determination of authenticity of apple juice is on one side of great economic importance, while on the other it is also an important issue in quality control and food safety.

The presented research is based on studies, in which a combination of different isotopic ratios of bioelements (2H/1H, 13C/12C, 15N/14N, 18O/16O), multi-element analysis and major primary and secondary metabolite profiles were exploited to differentiate the geographical origin of Slovenian apples. These parameters were used to establish the first database of authentic Slovenian apple juice, which can be further used to verify the authenticity of commercially available apple juice in Slovenia. In first study six apple (Malus domestica Borkh) cultivars (Topaz, Idared, Golden Delicious, Goldrush, Gala, Gloster) were collected in 2009 growing season, covering different geographical regions (Alpine, Dinaric, Pannonian and Mediterranean) in Slovenia. The results revealed that the stable isotope parameters in sugar, pulp and water were the most significant variables for differentiating between the regions. Good separation was achieved between the geographical regions in Slovenia based on the δ18O and δ2H values in water and Rb and S levels in apple fruit juice. The second study, which took place during the 2011
and 2012 growing seasons included three apple cultivars (Idared, Golden Delicious and Topaz) from five different geographical regions. In the first part, major primary and secondary metabolites were tested to obtain regional discrimination, while in the second part elemental and isotope composition were also used. Regional discrimination of juice samples was most successful when taking into account the following phenolic compounds: flavanols (catechin, epicatechin, procyanidin B1 and procyanidin B2+B4); flavonols (quercetin-3-rhamnoside and quercetin-3-glucoside+quercetin-3-galactoside) and the levels of trans-piceid. The overall prediction ability was 60.9%. A much better separation (prediction ability of 83.9%) was obtained using the δ2H and δ18O content of juice water, the δ15N and δ13C content of the pulp; (D/H)I and (D/H)II in ethanol and the concentration of S, Cl, Fe, Cu, Zn and Sr. The factors that best distinguished the different types of cultivar were the average fruit mass, the amino acids (aspartic acid and threonine), the sugars (fructose and sucrose), the phenol components (phloridzin, cryptochlorogenic and neochlorogenic acid and all of the determined flavanols (catechin, epicatechin, procyanidin B1, procyanidin B2+B4)), isotopic parameters (δ2H and δ18O content of fruit juice water and the δ13C and (D/H)I content of ethanol), and the concentration of the following elements: S, Mg, K, Cu, and Ti. The third study involved the validation of the authentic Slovenian apple juice database obtained within the 2011-2012 study using thirty-six commercially available apple juices from Slovenia. The results clearly showed how the stable isotopic composition of the most important bioelements and sugar content together with LDA analysis could be used to distinguish apple juices produced in Slovenia from those originating from other EU countries, independent of their botanical origin. The total prediction ability was 90.0%. Three samples (10%) had been adulterated with beet sugar. Although this is a relatively low percentage of adulterated samples, correct labelling should be enforced through legislation to protect the rights of both the consumer and the producer.

The first systematic characterization of Slovenian apple juice was made and represents innovative and interdisciplinary approaches and methods in the field of authenticity and traceability. Information available through our study will be of great help to the food industry and for protecting producers, consumer and product quality.

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ION MOBILITY SPECTROMETRY: A RAPID TOOL TO ASSESS EGGS FRESHNESS

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Keywords: egg products, freshness, GC-IMS

In the last decade, the attention on the authenticity and quality of food commodities increased for commercial and safety reasons and that is why, from both industries and research institutes, the demand for the development of rapid methods able to detect frauds increased as well. Eggs, mostly in the egg products form, are largely used for the creation of different industrial products and their freshness is a crucial step for the production of safe and high quality commodities; however, there are not several rapid ways able to assess if egg products are as fresh as declared. In this sense, our research group developed in the past an artificial olfactory system solution [1].

In this study, GC-IMS (Flavourspec® instrument – Gas Dortmund Company - Germany, constituted by a Gas Chromatograph coupled with an Ion Mobility Spectrometer) technique is proposed in view of its ability to record the volatile profile of liquid or solid samples without any relevant pretreatment. In particular, GC-IMS is presented as a rapid, low cost and low sample demanding way able to assess eggs freshness, thanks to the identification of specific marker spots in the final 2D graph that change their intensity or that can be detected or not, according to the time and storage conditions of the egg products. An attempt to identify these spots have been performed analyzing the same samples with a GC-MS equipped with a 50/30 mm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber. After the identification of some markers with the mass spectrometry approach, these compounds have been searched in the GC-IMS libraries and, when available, their specific reference standards have been injected in the Flavourspec® system, with the aim to confirm the retention time and the drift time of the spot.

After a complete method validation, this GC-IMS technique could be implemented in production sites: the total time of the analysis (from sample preparation to report/outcomes generation) is lower than 50 minutes, allowing the acceptance or the rejection of an egg product batch before its introduction in the plant and consequent exploitation in the production process.

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ENSURING THE AUTHENTICITY & SAFETY OF FOOD WITH NEW ADVANCES IN LC-MS/MS WORKFLOWS - BRINGING ROUTINE CLOSER THAN EVER

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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 or allergen free?

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. We will also highlight new developments from SCIEX in routine methods for the authenticity of meat and allergen detection in foodstuffs using LC-MS/MS.
APPLICATION OF FOOD FRAUD PREVENTION GUIDE IN POULTRY SECTOR THAT USE QUALITY LABEL CLAIMS

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Keywords: food fraud, poultry sector, quality brand, agro food industry.

Although the biggest part of Food fraud incidents may in a direct or indirect way affect human health as well as consumers rights about food product information, there is still neither a clear definition of “food fraud”, or no specific tools or mechanisms that may be used in the agro food sector to prevent and evaluate them. In order to help Food Business Operators to assess Food Fraud vulnerabilities Premiumlab in 2015 developed a special procedure (Food Fraud Preventive Guide). The objective of this exercise was to apply the above mentioned procedure in a practical way using the example of poultry meat production that use quality label claims. The analysis of prices of poultry meat on the marked showed that the use of specific quality claims on the label increases price almost three times and the use of the ecolabel may increase it almost six times. So there is a clear economic motivation in this case. Poultry slaughterhouses that intended to be responsible to put this information on the label definitely need a special tool to verify whether this information is reliable. In order to perform vulnerability assessment as well as develop effective measures to decrease a probability of Food fraud our study was based on the following normative documents:

3. FOOD FRAUD PREVENTIVE GUIDE.
4. Specific check list for Food Fraud inspection.
In order to evaluate all the possible weak points in poultry production food chain, we carried out audits of different poultry slaughterhouses. These facilities have different production volumes, and they also use different label-claims. In the first case we performed an audit of the medium size slaughterhouse which was responsible for labeling: four points of control have been found during inspection, such as: Suppliers, Reception, Classification, and Labeling. Analysis of mentioned points of control showed that slaughterhouse has appropriate methods of Fraud prevention. Independent certification body for suppliers validation; methods of verification of traceability, reliable and trustful sub-contracting and the special labeling system in place to prevent any adulteration. In the second case the audit of the small size slaughterhouse revealed the following situation: one part of final production was not labeled by the slaughterhouse but was delivered back to the poultry farms that were responsible to put all the relevant information on the label. The information provided from this slaughterhouse to farms was only "the day of the week" and the average weight of each carcass. There was no further control regarding any quality claims that may be put on the label by the farmer. In the described experiment we found that in poultry meat sector the risk of Food fraud is really high. It may be significantly decreased if the slaughterhouse is responsible for labeling uses the verification of suppliers by an independent certification body. It will minimize the probability of the Food fraud and increase quality of raw material as well as the company's image. At the same time the scenario where the farmer is responsible for final product labeling represent really high vulnerability of Fraud with the almost total absence of control measures and as a consequence the countless number of Fraud opportunities. The procedure of Food Fraud Guide was successfully applied in all cases in order to analyze vulnerabilities, establish points of control and elaborate preventive measures. Still, it is necessary to highlight that that there is a need to elaborate a special audits check list for each specific agro food sector.
"CONSUMEHEALTH" USING CONSUMER SCIENCE TO IMPROVE HEALTHY EATING HABITS

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Keywords: food choices, Marie Skłodowska-Curie Action, behavioral economics.

Introduction
Although today’s consumer can make informed decisions about which foods, and in what quantities, are best for a healthy life style, in recent years in the European Union there has been an increase of diet-related health problems caused by unhealthy and over-consumption of food (e.g. overweight, obesity, and other chronic diet-related diseases).
This Marie Skłodowska-Curie Action (MSCA) will deal with healthy eating habits, a key issue for the EU in contributing to safe, healthy and nutritious food for preserving life and making positive impact on health and society.

Research questions
The objective of this MSCA is twofold: (1) understand what drives consumers to make healthier food choices and (2) provide evidence-based recommendations for stakeholders and policy makers to develop and communicate innovative win-win solutions to improve eating habits and contribute to the well-being and healthy ageing of citizens.

The following research questions will be answered:
• Which food information (e.g. type, sources, format, 2 sided-messages) is most successfully conveyed to the population and how?
• Which micro-environment changes (e.g. food presentation, attractive names, suggestive selling) are most effective in changing behavior and how?
• Which individual preferences, levels of involvement, expectations, interest in a particular subject (e.g. nutrition) and demographic parameters influence the food choice for healthy eating patterns and how?
• How do the findings from different techniques in consumer behavior research (e.g. hypothetical vs non-hypothetical) proposed in this action differ and how can they be integrated?

Overview of the action
This MSCA will consist of spending 24 months at Cornell University Food and Brand Lab. A detailed study of healthy eating patterns and behavioral economics will be carried out through lab and field experiments. Workshops and courses on food consumer research methods will provide excellent training. Visits to conferences, stakeholder meetings and other research centers (Michigan and Oklahoma State Universities), already scheduled, will be made. Finally, acquired expertise will be disseminated during the 12-month mandatory return period at the Department of Food Science, University of Parma, through seminars and courses, and through a workshop in collaboration with the European Food Safety Authority –EFSA.
Expected results
Most existing research on eating behavior has limited impact on health or public policy. This is partly due to the no translational nature of many studies, i.e. the difficulty in applying findings in practice. It is also partly due to the absence of a useful framework that organizes the conclusions. This research however will investigate healthy dietary habits through behavioural economics, modelling choice in different settings (online, in lab and in field settings such as cafeteria and shop market) to discover which changes (micro-environments and types of messages) and interventions are most effective. This new approach is expected to increase the validity of the results and produce information useful for the development of new techniques, contributing to discussion among food scientists.

References

Acknowledgments: “This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 749514”
THE PRESENCE OF SURROGATES IN ROASTED COFFEE

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Keywords: roasted coffee, surrogate, test, starch.

Coffee is a beverage that is prepared by cooking roasted and grinded seeds of the coffee plant, usually in water or milk. Coffee is usually served warm. This drink is very popular in many countries. There are several ways to prepare coffee, the most popular are filter coffee, espresso, instant coffee, Irish coffee.... and in our country - black coffee.

Roasted coffee is obtained by a certain production processs, roasting of the green coffee to a level of frying product which provides a characteristic color, odor, taste and flavor. Roasted coffee in the further manufacturing process may be subjected to grinding. If the product is declared as roasted ground coffee, flavorings, spices, edible parts of plants or other cannot be added.

On the market can be found the products that are a mixture of roasted ground coffee with roasted ground surrogate under the name “mixture of coffee and coffee surrogate”. Declaration of these products must contain the information on the amount of a surrogate in the mixture, expressed as % (m/m) compared to a surrogate in the final product, where this information has to be located in the same field of view as well as the name of the product.

Barley, rye, wheat, roots of chicory, carob, chickpea, soya edible fruits rich in starch or sugar can be used as coffee surrogate.

The problem arises when surrogates are added to the roasted ground coffee but they are not indicated on the label. This misleading consumers and can potentially be dangerous (e.g., people suffering from celiac disease can intake potentially present gluten rye or barley).

As most surrogates contain starch, surrogate presence in coffee, may be proved by detecting the presence of starch. In the SP laboratory the presence of starch, is determined in three ways: a test-starch (the visual method - qualitative method), enzymatic method Megazyme MEG-K-TSTA (quantitative method), and a polarimetric method - Ewers (quantitative method).

The aim of this study is to determine which amount of added surrogate (i.e. % of starch) can be determined with certainty by conducting a test of the starch. The principle of the test method of starch is based on the characteristic chemical reaction of the starch with an iodine solution with and leads to a blue coloration.

Series of samples that contained various participants’ surrogates has been prepared. Chickpea was used as a surrogate. Prepared samples contained 0.5%, 1%, 2%, 5% and 10% of starch. The samples were prepared so that chickpea amount was added to the samples of coffee which will provide a corresponding quantity of the starch. In these samples starch was analyzed.
These results showed that with certainty the presence of starch can be determined with qualitative method for concentration of 1%. Percentage present surrogate is possible to calculate if it is known that it is a surrogate in question, and an accurate concentration of starch it contains. The results were confirmed by analyzing the samples by standard methods.

*Table 1: Results of the test for starch*

<table>
<thead>
<tr>
<th>Percentage of starch (%)</th>
<th>Test (qualitative method)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0,5</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
</tr>
</tbody>
</table>

Analysis of starch is required when it comes to quality control of roasted ground coffee and, as such, is always done in SP laboratory. This is one of the ways which protect consumers from misleading information.
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WHEAT FOOD WITH A POTENTIAL FOR LOW-IMPACT ON SUBJECTS PREDISPOSED TO CELIAC DISEASE. A COMBINED PROTEOMIC AND PEPTIDOMIC APPROACH

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Keywords: Durum wheat, in vitro digestion, gluten peptides, protein analysis.

Wheat is one of the most important food crops, with an annual world production of about 715 million tons; with a relatively low protein content (8-15%), wheat is the most important protein source in the human diet. Gluten, the water insoluble fraction of wheat flour protein, is responsible for technological quality and is mainly composed of two prolamin fractions: gliadins (α, β and ω) and glutenins. The ingestion of these proteins is responsible for: 1) celiac disease (CD), an autoimmune enteropathy with a prevalence of about 0.7-2% in the human population, in genetically predisposed individuals and 2) gluten sensitivity, a newly recognized pathology.

Flours from one hundred Triticum durum genotypes were subjected to in vitro digestion. Peptides containing sequences known to trigger CD were identified and quantified with liquid chromatography coupled to mass spectrometry. A subset of T. durum genotypes grown in four Italian regions were analysed, in order to evaluate environmental influence on the production of CD related peptides. The different seed storage protein fractions were extracted from wheat flour with the sequential procedure of Singh and collaborators (1991) and quantified by Bradford assay. A relative quantification of single gliadin subunits by SDS-PAGE coupled with their identification by ORBITRAP LC-MS was utilized to correlate single protein amounts with peptides responsible of CD disease. Multivariate analysis of variance (MANOVA) and principal component analysis (PCA) was performed at this purpose.

Eleven peptides were identified as containing sequences known to elicit CD response. The amount of immunogenic peptides was found to be highly variable among the different wheat varieties. Among the 100 genotypes analysed, 10 showed an immunogenic peptide amount lower than 500 ppm, while 4 genotypes produced more than 1550 ppm of immunogenic peptides after in vitro digestion. Environmental effects were evaluated: the amount of immunogenic peptides was not statistically different among three of the four Italian regions analysed, while it was slightly higher for Sicily. The relative quantification of gliadins and glutenins HMW and LMW fractions, showed significant differences both between the genotypes tested,
and between environments. SDS-PAGE analysis of the gliadin fraction undeline differences in the number of subunits and in the amount of single subunits in the same genotype grown in different environments, and also between different genotypes. ORBITRAP LC-MS analysis allowed for the identification of different classes of gliadins and a positive correlation was evaluated between the amount of a specific \( \alpha \)-gliadin and the amount of toxic or immunogenic peptides in each cultivar in relation to the growing environment. Wheat varieties having a potential low impact on CD predisposed subjects were identified on the base of immunogenic peptides produced after in vitro digestion. Given the variety, which is the most important determinant, some areas of cultivation exert an influence on the amount of seed storage proteins, in particular on the gliadin fraction. The combined proteomic and peptidomic approach was useful to quantitize the different cultivars and to identify of functional markers for breeding/selection of wheat varieties with low stimulatory capacity of T cells.

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**Acknowledgments:** This research was financially supported by the Smart Wheat project (POR FESR 2014-2020).
Food represents not just a source of calories, energy and the building blocks for cellular processes, but also a source of compounds important for promoting health and sustaining a range of physiological processes. Much of the interest in plant-derived bioactive peptides present in foodstuffs lies in their potential pharmaceutical and/or nutraceutical benefit. Well known examples are soymetide (from soybean) and oryzatensin (from rice). The advantage of bioactive peptides over synthetic pharmaceuticals is particularly strong in less developed parts of the world, both from a cost and an availability point of view. Among the benefits to human health claimed for plant peptides are antibiosis, a reduction in blood pressure, a reduction in blood cholesterol level, antithrombosis and antioxidation, the enhanced absorption of trace minerals, cytoimmunomodulation and opioid activity. Some of the bioactive peptides present in plant-based foodstuffs are hydrolysis products of proteins which in their native form are pharmaceutically inactive. They can be generated by microorganisms (especially as a product of fermentation), by gastrointestinal digestive enzymes (in particular pepsin, trypsin and chymotrypsin) or by in vitro procedures involving treatment with either a proteolytic enzyme or following exposure to high temperature and/or extreme pH conditions. A number of peptides accumulate during the aging or storage of foodstuffs, notably in cheese and yoghurt.

A literature survey covering the presence of bioactive peptides in plant-derived foodstuffs is presented. Examples are given of plant peptides associated with a beneficial effect on human health. The key structural features of these peptides are defined and their mechanism of action described. Current understanding of the way in which these molecules are adsorbed, distributed, metabolized and finally excreted is discussed. A particular focus is given to potentially immunomodulatory peptides. Inspection of crop proteomic data revealed that at least 6,000 proteins may harbour bioactive peptides. The most frequently represented crop species were Brassica spp., soybean, barley, rice, bean, peach, pearl millet, tomato, potato, wheat, grapevine and maize. The analysis of these proteins using a Gene Ontology approach has provided a number of insights regarding their occurrence and relevance.

The production of bioactive plant-based peptides from residues, waste and other by-products of food processing is an attractive proposition, since this would represent a low-cost and otherwise valueless feedstock.

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THE TROUBLESOME HONEY: REVIEWING ADVANCES AND PITFALLS OF BOTANICAL AND GEOGRAPHICAL ID OF A COMPOSITE PRODUCT

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Keywords: honey botanical geographical origin.

Honey is a worldwide known product, hand-collected by men since very ancient times. Bee/hives domestication started very early: back to Maya in the Americas, with stingless bees of the genus Melipona and Scaptotrigona. From Egypt to Europe, domestication was based on highly adaptable species of the genus Apis, A. mellifera becoming the most commonly managed bee and deliberately introduced around the world. Estimating total domesticated bee population in any given area is a difficult task, due to complexity of controls on bee-keeping activities, often a subsistence and movable farming. Recently, the task became even more difficult due to bee mortality, heavily increased in the last decades. The management of bee hives strongly influences the quality and price of the main product, the honey, combined with the complexity of its natural origin.

In fact, honey is made mainly by nectar. Nectar is a natural sugar solution that angiosperm plants produce into flowers, as a reward to pay pollinator’s services, or extra-florally, to attract ants that defend the resource from other phytophagous insects. Nectar is produced by the vast majority of plants, yet the amount available may be different, as its relative content of water, carbohydrates, minerals, volatile and phenolic compounds, etc. Bees tend to optimise their foraging efforts: botanical sources especially abundant in time and space may drive bee foraging to repeatedly collect the nectar from a single or few sources. The honey produced from nectar may come predominantly from a single (most abundant) defined botanical origin that characterise its colour, flavour and taste, increasing its quality and price. But it is also, to an unknown extent, somehow modified by bees.

Differences in honey taste are perceived and appreciated by consumers, therefore efforts are concentrated on tools and protocols that have been proposed to establish the botanical origin of honeys. Since some honeys are especially valued, also their geographic origin has been acquiring more and more importance. Notwithstanding new and promising techniques continuously emerge and are reported, difficulties lies in the composite origin of this product.

Here a point will be made on the importance of considering the many influences that should be taken into account when analysing honey: those added by plants (density, distribution, timing of nectar production), by honey bees (flower handling abilities, colony requirements, flying performances), and by humans (bee keepers selection of bees, hives location, honey production processes). A review on the advances and pitfalls of different methods to assess honey origin will be discussed. Hopefully, this will raise a new perspective of this composite product that may help to disentangle which analytical tools and protocols may satisfied market expectations of proper labelling.
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A NOVEL APPROACH TO AUTHENTICATE WHOLE AND REFINED GRAIN DURUM WHEAT (TRITICUM DURUM DESF.) BASED ON UNTARGETED LIPIDOMICS

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Keywords: Authenticity; Common wheat; Durum wheat; Untargeted Lipidomics.

Pasta constitutes a dominant portion of a standard Mediterranean diet, supplying a large fraction of the needs for energy-rich materials, such as considerable amounts of carbohydrates, proteins, fiber or minerals. As a result, 14.3 million tons of pasta are produced worldwide according to the survey carried out by the Associations of Pasta Manufacturers of the European Union (UN.A.F.P.A, 2015).

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. Nowadays, wheat is mainly authenticated by genomics and proteomics approaches. This fraud has a huge impact both 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 the present work the possibility of using an untargeted metabolomics strategy was explored in order to discriminate between common and durum wheat lipidome. A first study was conducted by analyzing 52 samples from two durum and common wheat varieties. Afterwards, an extended and independent sample set (172 samples and five varieties) was used for as a confirmatory study to verify the stability and consistency of the models obtained. Among the metabolites resulted statistically significant in both preliminary and confirmatory study, alkylresorcinols, and in particular heptadecyl-resorcinol (AR 17:0), could be further used for the discrimination of common and durum whole grain flour, being present in the outer layer of the kernel and thus lost during refine process. By contrast, digalactosyl diglyceride (DGDG 36:4), an abundant membrane-forming lipid mainly concentrated in the common wheat inner layer, may be distinguish durum wheat adulteration also in refined flours. Putatively identified markers were evaluated applying The receiver operator characteristic (ROC) curves analysis resulting in individual
marker AUC >90% both in preliminary and confirmatory study. In addition, the untargeted analysis was shown to be an effective approach differentiating between authentic durum wheat and its adulterated admixture down to 3% adulteration level, which is the maximum contamination level allowed by Italian legislation. The results demonstrate that untargeted lipidomics, in conjunction with chemometric tools has potential as a screening tool for the detection of wheat fraud.

<table>
<thead>
<tr>
<th>Marker</th>
<th>DGDG 36:4</th>
<th>AR 17:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary set</td>
<td>95.7 (90.3-100)</td>
<td>100 (100-100)</td>
</tr>
<tr>
<td>Confirmatory set</td>
<td>96.7 (93.8-99.5)</td>
<td>98.0 (94.5-100)</td>
</tr>
</tbody>
</table>

*Figure 1. Receiver operating characteristic (ROC) curves of heptadecyl-resorcinol (AR 17:0) and digalactosyl diglyceride (DGDG 36:4) in the training and validation sets.*
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PCR BASED OFF-TARGET METHODS FOR THE DETECTION OF ALLERGENIC INGREDIENTS IN FOOD

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Keywords: Food allergen, species-specific marker, qPCR.

Food allergies are a major health concern in industrialized countries and may affect up to 3% of the adult population and 6-8% of the children in Europe. The level of exposure necessary to provoke a reaction varies from food to food and from person to person. Most often, reactions are elicited after exposure to 1-100ppm of an allergen, but sometimes, only minute amounts are required. Effective treatments for food allergies are not available and sensitive consumers must rely on avoiding the allergens, with an accurate food labelling which inform the consumer on the ingredients in a processed food. Within the European Union, both Regulation (EU) No 1169/2011 (2011) and Commission Directive 2007/68/EC (2007) include a list of 13 species as a food allergen for which labelling is mandatory. This research focuses on the development of reliable methods for the detection of food allergens. The approach is an off-target analysis of the DNA of the allergenic species as a marker of the allergenic ingredient. This off-target method can be considered complementary to those based on the analysis of proteins (target methods), but may be also used whenever a target method for the detection of the allergenic protein fails. The development of species-specific markers is simplified by the availability of genomic databases for most of the species under study, and through the bioinformatics analysis, it has been possible to obtain species-specific and allergen specific markers. PCR, qPCR and Multiplex qPCR with SYBR®Green™ have been developed to specifically detect different allergenic species, and they have been tested both on incurred and on commercial food products. The method based on real-time PCR could allow also the quantification of allergenic species if proper standard materials such as IPRS (Internal Plasmid Reference Standards) are utilised. The qPCR methods have been compared with ELISA.

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FOOD AUTHENTICITY TESTING BY THE USE OF STABLE ISOTOPES: MODERNIZING A TRADITIONAL METHOD FOR COMMERCIAL PURPOSES ON TESTING GEOGRAPHIC ORIGIN OF FOOD AND NATURALNESS OF FOOD INGREDIENTS

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Keywords: stable isotopes, food authenticity, geographic origin, natural ingredients.

The constant race between food fraud and food testing was always a triggering cause of technological and methodological developments in the sector. The more and more prominent demand for analytical proof of fraud and manipulation has expanded the spectrum of techniques, both targeted and non-targeted, while the need for speed, versatility and quality from the industry has made the analytics a real commercial service, in all aspects. Imprint Analytics, having exactly this orientation, moves from traditional applications to a modern structure of industrial analytical service. Using the knowledge from the fundamental research, new isotopic tools are presented as ready products in the testing fields of geographic origin of food and naturalness of food ingredients. The innovative Reference Sample System (RSS) is utilized as an all-in-one package which corresponds to diverse needs and delivers high quality and precision. Its purpose is not only to deliver evidence of falsified food products in respect to their declaration of geographic origin but further to provide support to the user in terms of logistics, monitoring, quality control and marketing actions. Similarly, recent methodological and technical developments in compound specific isotope analysis has brought us to a sophisticated though simple and quick test of food ingredients’ source, to identify natural versus synthetic materials. Simple mass spectrometric and single-isotope indices have been proven insufficient to catch up with the food fraudsters, resulting in lack of trust and confidence in the ingredients’ supply chain globally. Using 2D-isotope analysis as a user-friendly tool helps in quick and reliable testing of many compounds in practically any sample matrix. Last but not least, a focused summary of the experience from the market is presented, shedding light on emerging aspects of food fraud related to the above two topics. Correlative examples are given as part of the analytical, doc-free proof explaining how these can be used for quality management, quality assurance and marketing, turning science into solutions.

Acknowledgments: The research was funded by the Austrian Research Promotion Agency (FFG) (Project-# 848264, e-Call-# 5077301).
IDENTIFICATION OF POTENTIAL FOOD BIOMARKERS TO DISCRIMINATE BETWEEN ORGANICALLY AND CONVENTIONALLY GROWN TOMATOES

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Keywords: Authentication; Organic food; HRAMS; IRMS.

Consumer’s interest in organic foods is increasing and so is the need for robust analytical tools for their authentication. The EU organic food market is the second largest in the world behind the US. Organic farming in the EU is supported by EU law, Regulations (EC) No 834/2007 and 889/2008/EC.[1,2] However, the lack of reliable biomarkers to discriminate between organic and conventional products makes this market susceptible to foods labelled as “organic” that have, in fact, been produced conventionally. Scientific contributions can be found in several organic farming research projects which have been funded under the EU framework programmes since the mid-1990s. Nevertheless, the initial results shown in the recently completed Organic-data-network project (2012-2014) conclude that there is a need for further methodology harmonisation in data collection. From an analytical point of view, the authentication of organic food products is a challenging problem. To date, analysis of stable isotopes has proven them to be valuable indicators of agricultural practices, although a complete discrimination has not been found. However, the MS profile of a food sample can be regarded as an analytical signature of the food product and thus can help in discriminating between different practices, reflecting the impact of both endogenous and exogenous factors as well as the food’s properties. But, the potential of these tools to assemble the data sets of crop/fertilizer correlation has not been extensively explored for application to organic vegetables.

Thus, the principal aim of this study was to investigate the potential of advanced technologies based on high resolution accurate mass spectrometry (HRAMS) for the identification of biomarkers capable of distinguishing between organically and conventionally grown tomatoes in greenhouse under controlled agronomic conditions, and in a climatic region of leading EU production such as the Mediterranean. Tomato samples were extracted using methanol and analysed by liquid chromatography Q-Orbitrap mass spectrometry (LC-Q-Orbitrap-MS). In addition, nitrogen stable isotope ratios (IRMS) were used as a valuable supporting tool for the authentication of organic production. Mass profiling data were used to establish (dis)similarities between agricultural management practices. Figure 1 shows the mean area found for the case of the two glycoalkaloids compounds detected in the organic and conventional tomatoes samples.
Principal component analysis (PCA) showed promising results according to farming systems and significant differences in peak areas were observed for 8 bioactive components (3 flavonoids, 2 glycoalkaloids, 1 xanthophyll carotenoid, 1 sucrose ester and 1 vitamin derivative) in organic samples (see Figure 2). In light of these preliminary results, it is possible to conclude that metabolomic fingerprinting/profiling of low molecular weight food components is a promising approach for the identification of potential food biomarkers to discriminate between organically and conventionally grown tomatoes. The combination of IRMS and HRMS techniques are a useful analytical tools to supporting the authentication of organic crops (see Figure 3).

**Figure 1.** Mean area of the two glycoalkaloids detected in the organic and conventional tomatoes samples analysed.

**Figure 2.** PCA of MS-profiling data for 8 bioactive components detected.

**Figure 3.** Trend of IRMS and HRMS data for the case of the two glycoalkaloids detected in the organic and conventional tomatoes samples.

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**References**


BIOTECHNOLOGY AND CONSUMER’S NEEDS: RESULTS OF A CITIZENS’ JURY

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Keywords: Genetically modified (GM) animals, public consultations, citizens’ jury, GM policies.

Introduction
In recent decades, increasing food demand, technological innovation, global exchange and asymmetric information have changed the relationship between food consumers and producers. In addition, new legislation on food and agriculture issues has failed to adequately embrace public opinion during the policy making process. In the last years, many policy makers have agreed that involving the wider community in setting priorities for decision making is desirable (Rowe et al., 2008). This paper examines results of a Citizens’ Jury event organized in Parma (Italy) in 2012, as the final phase of the EU project PEGASUS (Public Perception of Genetically modified Animals – Science, Utility and Society, 7th FP). The objective of the exercise was to understand the elements which generate the development of policy recommendations for drugs derived from polyclonal antibodies from GM rabbits, and policy recommendations associated with the development and commercialization of GM salmon (Mora et al., 2011; Mora et al., 2012; Menozzi et al., 2012).

Methodology
Two public engagement activities were conducted in Newcastle, UK and Parma, Italy (Brennan et al., 2012a). This paper describes the second event which was held in Parma, the 21st/22nd April 2012. This paper reports only on the event held in Parma.

Results
The Parma CJ developed and refined (through their feedback) 32 policy recommendations. Both case specific, i.e. policy recommendations for drugs derived from polyclonal antibodies from GM rabbits, and associated with the development and commercialisation of GM salmon, and general. The CJ ruled that every business development and marketing of products (drugs or food) derived from GM animals must follow certain ‘guidelines’ or general recommendations.

At the end of Parma CJ events, the jurors were asked to vote on whether they supported the development and sale of GM salmon in the European Union and the development and use of GM rabbits to produce polyclonal antibodies in the European Union. Results show that the development of GM animal applications aiming to produce new drugs are more acceptable compared to food applications, such as GM salmon.
Conclusions
The Parma CJ aimed to assess both possible policy recommendations and the effectiveness of a CJ process in producing credible outputs capable of improving the policy process. Results suggest that CJ can be a method for involving citizens with a deeply and highly technical and scientific topic and evidence (Brennan et al, 2012a). The methodology, although widely used across the US, Europe, Australia and New Zealand, is still quite new in Italy (Carson, 2006).

The CJ was able to explore, through several group activities and cross examination of experts, personal opinions of the participants and capture their position. Conclusions from the juror's discussion suggested that the consumers want to be aware when they are actually buying GM products and clear labelling must be provided. Moreover, the price of GM products must not be a barrier to purchase. In addition, according to the participants, if these products were found to be necessary, the Public Authority should ensure that the price would be at least 30-40% lower than the price of conventional products.

Acknowledgments: The authors would like to thank the European Commission for its financial support of the PEGASUS project under the 7th Framework programme (theme 2 – Food, Agriculture and Fisheries, and Biotechnology, Grant agreement no.: 226465).

References
AUTHENTICITY AND GEOGRAPHIC ORIGIN OF TOMATO PRODUCTS WITH NON TARGETED SCREENING MASS SPECTROMETRY AND CHEMOMETRICS

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Keywords: tomatoes, authenticity, non-targeted screening mass spectrometry, chemometrics.

In an increasingly global and competitive market, transparency, evidence and authenticity in terms of geographic origin represent an added value of the product. Mérieux NutriSciences and Mutti have worked together on a project with the aim of analytically discriminate tomato products in terms of geographic origin. In particular, through experimental design, regarding:

a) purpose: geographical origin clear distinction capacity of tomatoes used for the manufacture of tomato products (Italian vs. Chinese);

b) sampling: with excellent traceability information and managed in its variables such as seasonality, production process, storage etc;

c) Non-Targeted Screening Model based on;
   a. LC/HRMS;
   b. Chemometrics (SIMCA PCA and PLS-DA) checked and verified through pre-defined acceptance criteria;

we have developed a method and a reference system to clearly distinguish the geographical origin of tomatoes used for the tomato products within the project boundaries.

Figure 1: LC/HRMS Non Targeted Screening Chromatogram
Figure 1: LC/ RMS Not Targeted Screening approach allows the acquisition of a large amount of information with high level of selectivity. The use of internal standards minimizes output variability of non-targeted molecules allowing a later effective and meaningful chemometric analysis. Non-Targeted Screening Mass Spectrometry Approaches properly interfaced with well defined chemometric analysis, allow a clear geographic origin discrimination of the products within the limits defined by the experimental design of the project.

Figure 2: Coomans' plot shows a clear Geographic Origin distinction and classification of tomato products from Italy (blue areas) and from China (green areas). The quality control used for the verification intra / inter batches (mixtures Italy / China 50/50) shows a correct classification outside of each of the two classes. All authors thank Mutti SpA and Mérieux NutriSciences companies for the financing of this research, certainly important for the dissemination of analytical services designed to verify the full transparency of final products in terms of Authenticity and Foodintegrity.
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AUTHENTICITY AND GEOGRAPHIC ORIGIN OF TOMATO PRODUCTS WITH NON TARGETED SCREENING MASS SPECTROMETRY AND CHEMOMETRICS

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Keywords: Wine vinegar, Protected Designation of Origin, NIRs, PLS-DA.

Some high quality wine vinegars, traditionally linked to a specific geographical area, are protected by European regulations under the indication of “Protected Designation of Origin” (PDO). As the high quality demand has significant increased over the last years, and food quality is directly related to commercial value, adulteration and unfair competition in the vinegar industry has to be considered to develop appropriate protection tools. For this reason, wineries and regulatory councils are demanding effective analytical methods to allow a rapid and inexpensive analysis to verify the origin of the vinegars in order to protect their brands and to prevent from adulteration. In this regard, Near Infrared spectroscopy (NIRs) in combination with advanced multivariate data (a.k.a. Chemometrics) has demonstrated its usefulness in the authentication of foodstuff without the use of chemical references and in a non-destructive manner. In this sense, the aim of the study was to investigate the potential of NIR as a rapid, inexpensive and non-destructive methodology for vinegar characterization and classification considering three of the five wine vinegar PDos produced in Europe ("Vinagre de Jerez", "Vinagre de Condado de Huelva" and "Vinagre de Montilla-Moriles").

Spectra from 77 wine vinegars of different categories within the 3 PDos and 16 wine vinegars without a PDO and purchased from the market have been analyzed and compared in the infrared region of 12000-4000 cm⁻¹. After the adequate data pre-processing, and prior to classification approaches, Principal component analysis (PCA) was performed as an exploratory analysis to detect groupings, outliers and relationships between variables and classes. PCA pointed out a trend of grouping according to aging category (Figure 1-A) as well as some similarities between the spectra of the categories of the three Spanish PDos: the absorption bands most involved in aging changes, and also related to sweet category, were those from 5200 to 6500 cm⁻¹ (Figure 1-B). Then, Partial Least Squares-Discriminant Analysis (PLS-DA) was applied to obtain classification models for a wine vinegar category classification and a PDO classification.
The unique characteristics of the Spanish PDO wine vinegars, which directly affect to the NIR spectra, allowed a good classification according to the category (aged and sweet categories within each PDO) and origin (PDO wine vinegars and vinegars without this quality indication) by the development of PLS-DA models. The results demonstrated that NIRs together with chemometrics could be a powerful combination to differentiate and authenticate PDO wine vinegars and their commercialized categories, due to the high ability of classification and prediction obtained (90-99% good classified).

Acknowledgements: The authors want to thank “Consejería de Economía, Innovación y Ciencia” of the “Junta de Andalucía” for the financial support provided through the project P12-AGR-1601, and the “Ministerio de Educación Cultura y Deporte” (MECD, Spanish Government) for the FPU pre-doctoral fellowship. In addition, authors want to thank the Spanish Regulatory Councils of the wine vinegars PDOS for their invaluable help with the acquisition of the samples for the study. Finally, authors want also to thanks University of Copenhagen, specifically to Franciscus Winfried J van der Berg and the department of food science for providing the equipment and their knowledge in the methodology.

References

Figure 1. PCA models of NIR spectral data of the three wine vinegars PDOS. The scores plot (A) and loadings plots (B) of the first principal components (PC1, PC2, and PC3) obtained are shown.
DIFFERENTIATION OF CHIA, FLAX AND SESAME: POLYPHENOL FINGERPRINTING BY HPLC-ESI-QTOF (MS)

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Keywords: Biomarkers; Polyphenols; Seeds; HPLC-ESI-qTOF (MS).

Nowadays, there is a widespread preference for the consumption of foods that, in addition to its nutritional properties, provide components that could reduce the risk of certain diseases, leading to the concept of “functional food”. Chia, flax and sesame seeds are a great source of polyphenols and have greatly increased their popularity among consumers due to their beneficial properties like reducing cancer risk, diabetes and heart disease, among others. The antioxidant activity shown by these compounds is responsible of the mentioned properties. Due to their great nutritional capacity, these seeds can be added to processed foods to increase their nutritional value.

Foodintegrity is the state of being whole, complete, undiminished or in perfect state in terms of quantity and quality, and this quality means to reach specific standards in sensory, microbiological and origin aspects of foodstuffs. There is a gap in the available scientific information to evaluate food attributes for complex foods. Many times it is difficult to assess the presence of a particular ingredient of nutritional importance within a complex food. So far, when a food product can be sold for a premium price because of its characteristics, it is of paramount importance that label claims and declarations on origin and identity can be certified.

The aim of this work was to find polyphenols within each seed, which could be used as authenticity biomarkers in complex foods containing these seeds.

For this study we used defatted chia, flax and sesame flour obtained after oil extraction. Polyphenols were extracted by sonication of defatted flour with ethanol 50% and identified and quantified by

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liquid chromatography coupled with time of flight mass spectrometry detection (HPLC-ESI-qTOF). The identification was done by MSn spectra, UV-Vis spectra and exact mass comparison and quantification was performed by external calibration curves using structurally related compounds. Nineteen polyphenols were found in the sesame flour, of which eleven were lignans (derivatives of Enterolactone and Enterodiol), five phenolic acids (being these the majority ones); and only three flavonoids which were (-)-Epigallocatechin-3-O-gallate, Apigenin, Apigenin-6-C-glucoside.

On the other hand, fourteen compounds were found in chia flour and all of them were phenolic acids being the majority derived from Caffeic acid (e.g. Salviaflaside and Rosmarinic acid). Finally, fifteen compounds were found in flax flour, six were phenolic acids (mainly Caffeic acid derivatives such as O-caffeoyl-fructosyl-glucoside and Rosmarinic acid hexoside), one was Quinic acid and eight were flavonoids derived from Quercetin mostly.

There were ubiquitous compounds in the analyzed seeds such as Rosmarinic acid and Quinic acid. On the other hand, each seed showed distinctive compounds. In the case of sesame flour the lignans (e.g. Sesamolin, 7-Hydroxymatairesinol, Matairesinol-rhamnoside); in the case of the flax flour the flavonoids (Cyanidin-3-O-glucoside, Eriodictyol-7-O-glucoside, Gallocatechin, among others) and in the case of chia some phenolic acids like Caftaric acid, Fertaric acid, Salvianolic acids, Sagerinic acid and Danshensu.

Therefore, these key polyphenols could be used to differentiate each seed as well as markers of authenticity in complex foods. Further research is needed to evaluate the stability of these proposed markers after food processing.

**Acknowledgments:** FP7, CONICET and National University Córdoba

*Equally contributing authors.*
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**PRODUCT QUALITY – TRANSLATION OF INTERDISCIPLINARY SCIENCE TO REAL WORLD SOLUTIONS – INTELLIGENT QUALITY ASSURANCE**

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**Keywords:** Discolouration, Product Quality, Non-targeted analysis, Quality Assurance.

Product quality, “The group of features and characteristics of a saleable good which determine its desirability and which can be controlled by a manufacturer to meet certain basic requirements”¹, is vital for any manufacturer. In terms of food manufacturing this translates into consistency, being able to provide consumers with the desired flavours, textures and appearance.

Fera were asked by ABP Food Group, UK to investigate a product quality issue relating to appearance, specifically the discolouration of their packaged meat. Their product was developing discolouration during production. However, only a small number of samples were showing these signs of discolouration and there was no pattern of occurrence between production runs. The manufacturer’s internal investigations were not able to identify the root cause.

Taking the principle of measuring ‘normality’ we profiled representative non-discoloured samples, and analytically compared them to profiles acquired from samples showing signs of discolouration. Statistical analysis of these data gave us tentative biomarkers, chemical knowledge of these gave us the reason why they were different between samples and biology translated this into the root cause of the discolouration.

The presentation will give an overview of the methodology used, the analytical findings, show why the meat was discoloured and ultimately the steps available to rectify the quality issue.

**Acknowledgments:** This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613688.

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RISK ASSESSMENT OF PESTICIDES IN AQUACULTURE FISHES

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**Keywords:** Seabass, Seabream, PCBs, OCPs.

The amount of world fish production is 195,776,207 tons (capture 94,637,130 tons and farmed 101,139,077 tons) in 2014. Aquaculture of sea bass and sea bream are of major importance for the economy of the Mediterranean region. Turkey is the second largest producer of both sea bream and sea bass after Greece. Environmental contamination by polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) is a global concern. There is little information on PCB/OCP concentrations and their potential health risks in cultured fish from Turkish farms. The main objective of this study was to determine PCBs - OCPs levels of cultured sea bass and sea bream obtained from four different Turkish farms. Samples were obtained from four different large exporter fish farms. Sixty-four cultured sea bass and sixty-four cultured sea bream samples were collected in two different times. These samples were randomly purchased from farms located at the west coast of Turkey (Aegean Sea, Turkey). In four farms, specimens were reared in net cages in the sea, fed with commercial diets. The PCB and OCP residues of the fish fillets were GC/MS determined according to the methods described by Lehotay [2007] and TS/EN [2004] respectively. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are representative compounds of persistent organic pollutants. In recent years, an important number of studies has been focused on contaminants in several edible marine species from several areas in Europe. A very recent study, documented the bioaccumulation of some PCBs in farmed gilt-head sea bream reared in different aquaculture farms of the Mediterranean area, revealed important differences between inland and in-shore sea farms, suggesting that commercial feed is an important source of PCBs [Pinto et al., 2008]. In this study, PCBs and OCPs in sea bass and sea bream samples were measured under the limit of quantification. The European Union (EU) set a maximum residue limit (MRL) of 75 ng/g (wet weight) for ΣPCBs (PCB 28, 52, 101, 138, 153 and 180) in fish flesh and aquacultured products. In this study, polychlorinated biphenyls (PCBs) and organochlorine pesticide (OCP) residues of sea bass and sea bream cultured in Turkey were examined. The PCB and OCP concentrations of all sea bass and sea bream samples were found lower than the maximum limit levels set by EC Regulations, being totally safe for human consumption.

**Acknowledgments:** This work was supported by the Research Fund of Istanbul University, Project Number BVP-21044.

**References**


THE AUTHENTICATION OF PREMIUM ORANGE ORGANIC JUICES USING HS-SPME-GC-MS

Keywords: Organic farming, authentication, volatile compounds.

Orange juice is the most consumed fruit juice in the world. Consumer demand and the popularity of organic products have grown tremendously in the last decade. This trend on organic juice consumption is driven by the increasing awareness of the benefits of healthy eating and the demand for more sustainable agri-food systems. This scenario has led to the emergence of significant interest in the authenticity of products declared as organic. Nineteen premium orange juices (ten conventional and nine organic juices) were collected from French and Spanish markets.

Figure 1. Compounds responsible of the discrimination in the organic and conventional commercial orange juices. Footnote: Unit = Ion peak area divided by 106.
A total of twenty eight volatile compounds were reported in orange juices. All the compounds are well-known odorants contributing to the flavour of orange juices. An independent student’s t test was carried out to provide the impact of the production system in the samples (table 1). Clearly different amounts of three aldehydes and one ester compound were found in the organic and the conventional samples. The contents of hexanal, Z 3 hexenal, 6 methyl 5 octen 3 one and ethyl butanoate were higher in the conventional juices (50-100%) (figure 1). In this sense, it is the first time that a high concentration of the above compounds has been found in a heterogeneous group of juices without supervising the genetics, geographical origin and processing technologies. Thus, the role of aldehydes and ester compounds in organic juices should be investigated with further studies.

Table 1. Statistical comparison for each compound and variable importance in projection in the HS-SPME-GC-MS data. Footnote: Unit = Ion peak area divided by 106. Mean comparison values (student t-test) at different significance level. Significance level: ns= non significant, * = P <0.05, ** = P <0.01, *** = P <0.001.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conventional</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Σ Aldehydes and ketones</strong></td>
<td>174.44 ± 19.22</td>
<td>141.9 ± 44.27</td>
</tr>
<tr>
<td>Pentanal</td>
<td>11.01</td>
<td>14.63</td>
</tr>
<tr>
<td>Z-3-hexenal</td>
<td>19.37 ± 1.63</td>
<td>11.20 ± 1.87</td>
</tr>
<tr>
<td>Hexanal</td>
<td>3.95 ± 0.80</td>
<td>2.05 ± 0.55</td>
</tr>
<tr>
<td>E-2-hexenal</td>
<td>0.89 ± 0.16</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>6-methyl-5-hepten-2-one</td>
<td>146.15 ± 18.2</td>
<td>126.38 ± 43.66</td>
</tr>
<tr>
<td>Octanal</td>
<td>8.64 ± 1.08</td>
<td>9.65 ± 2.37</td>
</tr>
<tr>
<td><strong>Σ Alcohols</strong></td>
<td>119.6 ± 4.28</td>
<td>95.8 ± 7.82</td>
</tr>
<tr>
<td>Z-3-hexanol</td>
<td>4.21 ± 0.54</td>
<td>6.19 ± 1.78</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>0.59 ± 0.04</td>
<td>0.66 ± 0.12</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>31.05 ± 4.21</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td><strong>Σ Terpenoids</strong></td>
<td>1828.38 ± 633.6</td>
<td>958.4 ± 797.78</td>
</tr>
<tr>
<td>α-pine ne</td>
<td>21.51</td>
<td>236.32</td>
</tr>
<tr>
<td>β-myrcene</td>
<td>23.61</td>
<td>282.67</td>
</tr>
<tr>
<td>Limonene</td>
<td>26.30</td>
<td>274.62</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>27.58</td>
<td>224.62</td>
</tr>
<tr>
<td>Linalool</td>
<td>29.33</td>
<td>284.62</td>
</tr>
<tr>
<td>E-limonene oxide</td>
<td>31.79</td>
<td>294.62</td>
</tr>
<tr>
<td>β-citronnellol</td>
<td>35.52</td>
<td>314.62</td>
</tr>
<tr>
<td>β-cyclocitril</td>
<td>36.11</td>
<td>324.62</td>
</tr>
<tr>
<td>Z-carveol</td>
<td>36.46</td>
<td>334.62</td>
</tr>
<tr>
<td>Nerol</td>
<td>37.70</td>
<td>304.62</td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>46.14</td>
<td>354.62</td>
</tr>
<tr>
<td>Valencene</td>
<td>49.18</td>
<td>384.62</td>
</tr>
<tr>
<td>Nootkatone</td>
<td>59.69</td>
<td>374.62</td>
</tr>
<tr>
<td><strong>Σ Esters</strong></td>
<td>141.05 ± 15.88</td>
<td>115.52 ± 20.14</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>8.56</td>
<td>5.24</td>
</tr>
<tr>
<td>Methyl butanoate</td>
<td>11.71</td>
<td>6.23</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>14.63</td>
<td>7.79</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>23.73</td>
<td>21.4</td>
</tr>
<tr>
<td>Neryl acetate + geranyl acetate</td>
<td>41.7+41.9</td>
<td>82.65 ± 14.44</td>
</tr>
<tr>
<td><strong>Σ Total</strong></td>
<td>10152.5 ± 635.73</td>
<td>9850.5 ± 989.94</td>
</tr>
</tbody>
</table>
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CYCLIC FATTY ACIDS AS QUALITY MARKERS IN MEAT

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Keywords: cyclic fatty acids, ensiled feed, GC-MS, meat.

In the last few years, counterfeit of high quality meat with cheaper counterparts became 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 and to protect both consumers and producers from illegal substitutions. Current methods to identify the origin of species present in commercial meat are based on DNA and ELISA, but also UPLC, Raman spectroscopy, low-field NMR, mass spectrometry have been considered (1).

Cyclopropane and ω-cyclohexyl fatty acids are unusual alicyclic fatty acids of microbial origin, isolated respectively in lactic acid bacteria and in rumen. Recently, their presence was demonstrated in milk and dairy products combining GC-MS and 1H-NMR techniques (2). Cyclopropane fatty acids (CPFA) were present only in dairy products from cows fed with silages and their determination has been demonstrated to be molecular markers of quality for PDO cheeses, as Parmigiano Reggiano, where the use of silage is forbidden (3). In this context, Consorzio del Formaggio Parmigiano has proposed a modification of the Production Specification Rules, including the determination of CPFA among the official controls, and a national UNI method has just been validated (UNI11650).

Ω-cyclohexyl fatty acids (CHFAs), mainly cyclohexyl-undecanoic and tridecanoic acids occur in several acid-thermophilic bacteria such as Alicyclobacillus acidocaldarius. It is almost certainly that CHFAs are produced by bacteria in the rumen and 13-cyclohexyltridecanoic acid has been considered as a potential marker of ruminal acidosis in cow (3).

Because no data are reported in literature on the presence of cyclic fatty acids in meat, more than fifty samples of meat of different species (both ruminant and not ruminant) were analysed combining GC-MS and 1HNMR techniques. Twelve beef meat samples were obtained from a farm certified for the absence of ensiled feeds in the animal diet. Lipid extraction from meat samples following the Folch et al. (5) method was performed.

CPFA were detected in the GC–MS profiles of most of the commercial bovine meat samples, on the contrary they were absent in all the twelve samples of certified meat from cows not fed with fermented forages. The amount of the cyclopropane fatty acids detected in the commercial bovine meat samples varied from 100-400 mg/kg of the total fatty acid methyl esters. GC-MS analysis of the other meat samples (pork, horse, chicken, turkey and lamb) generally showed the presence of a signal at the retention time of CPFA and with the corresponding mass spectrum, with amounts between 60 and 100 mg/kg of the total fat. In some cases, the signal at the same retention time showed a little different mass spectrum, indicating the co-elution of another unknown substance. This interfering peak was also resistant to oxidation as a saturated fatty acid, but it has not been identified yet. Therefore GC-MS analysis was not able to confirm the presence or absence of CPFA in meat samples, but it required in some cases
The presence of the cyclopropane unit was confirmed by 1H NMR analysis of meat fat that evidenced characteristic signals in the region from -0.30 to -0.35 ppm, due to the methylene of the propane ring (6).

The presence of CPFA was confirmed only in bovine meat samples resulted positive by the GC-MS analysis, on the contrary CPFA resulted absent in pork, chicken, horse and rabbit meats as shown in Table 1.

These results suggested cyclopropane fatty acids could be used as markers of silage feedings and as a powerful tool for the authentication of high quality costly meat from cows not fed with fermented forages. Furthermore, CPFA (mainly lactobacillic acid) were recently found in farmed fish; therefore, we are exploring the possibility of using cyclopropane fatty acids as molecular markers able to correlate the presence of these fatty acids in lipid profile and fish feeding, and/or to distinguish farmed fish from wild fish.

Ω-cyclohexyl fatty acids, 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acid, were detected only in bovine meat and not in pork and horse meat. Preliminary data of GC-MS analysis showed that the semi-quantitation of ω-cyclohexyl fatty acids, combined with other fatty acids as branched chain fatty acids, permitted to discriminate beef from pork meat and to identify the presence and the ratio of beef/pork meat in minced meat, both raw and cooked (ragout), therefore even in complex matrix and after thermal treatment. Ω-cyclohexyl fatty acids can be proposed as markers of ruminant meat, especially of beef meat.

<table>
<thead>
<tr>
<th>Meat Samples</th>
<th>N° Samples</th>
<th>CPFA Range (mg/kg total fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Beef</td>
<td>14</td>
<td>100-400</td>
</tr>
<tr>
<td>Beef of Certified Origin (not fed with silages)</td>
<td>12</td>
<td>Negative</td>
</tr>
<tr>
<td>Other Meats (pork, horse, rabbit, chicken, lamb, turkey, goose)</td>
<td>26</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 1. Presence of CPFA in meat samples.

References
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ESTIMATION OF HEALTH RISK ASSOCIATED WITH FISH CONSUMPTION AT DIFFERENT AGE’S INDIVIDUALS

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Common sole (Solea solea), black scorpionfish (Scorpaena porcus) is demersal fish species and horse mackerel (Trachurus trachurus) is a pelagic fish species. The three fish is an economic important species in Turkey. Generally, this fishes consumed as grilling and frying. Scorpionfish soup is very tasty, and thus consumption is common. These fish species are mainly located in the Marmara Sea. Pollution is one of the main problems of the Marmara Sea. Mercury, lead, cadmium, arsenic and tin compounds play an important role in the contamination of these fish. In this study is to determine the levels of mercury, lead, cadmium, arsenic and tin during the year in fish samples examined and compare the results with international levels. The main purpose of this study is to estimate the health risk that can be caused by the consumption of these fish in different age groups using this data. The common sole, black scorpionfish and horse mackerel samples were and purchased from the Istanbul local fish market during the catching season. Determination of arsenic (As), cadmium (Cd), total mercury (Hg), lead (Pb) and tin (Sn) in each fish species were analysed according to US EPA (2007) method. Nine different age-categories (USEPA, 2008) were used for the estimation of health risks. These age categories are as follows: namely children 1-3 years, children 4-6 years, children 7-10 years, adolescents 11-14 years, adolescents 15-19 years, adults 20-24 years, adults 25-54 years, adults 55–64 years and seniors >65 years.

The limits of the European Communities are for Hg 0.5 mg/kg in fishery products, for Pb 0.3 mg/kg in fish, for Cd 0.05 mg/kg in lean fish, 0.1 mg/kg in wedge sole, eel, horse mackerel, sardine, and anchovy, for Sn 200 mg/kg in canned fish products (European Communities, 2006). According to the average of the values measured for each season, the legal limits were exceeded for mercury in sole. This value not exceeded in three species of fish for lead, tin and cadmium. Concentrations of contaminants in the food may pose a risk to the consumer even if the concentration does not exceed the limit values. The body weight of the consumer group and the amount of food consumed are influential in the formation of health risk. With this understanding, health risk is estimated by making THQ account in recent years. The THQ value is greater than 1, indicating the presence of risk. In this study, it was observed that arsenic-derived health risks could occur in the first and fourth group of individuals in the calculations made according to nine different age groups. Total THQ value were found above 1 in first (for children 1-3 years), second (for children 4-6 years) and fourth groups (for adolescents 11-14 years) for sole, in the first group for horse mackerel, and consumption of these fish species for this group of individuals brings a health risk.

Acknowledgements: This work was supported by the Research Fund of Istanbul University, Project Number BYP-21044.
THE SIGNIFICANCE AND HEALTH IMPLICATIONS OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM LOCALLY MADE SNACKS IN NIGERIA

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Keywords: water, sewage, lagoon, energy.

Introduction and Objectives
Generally, snacks are foods which are neither nuts, fruits nor vegetables but are made and eaten as they are sold. In Nigeria, there is a vast diversity of snacks foods, from bakery products to take-away foods. They are eaten by all age groups, particularly young ones and school children. Arguably, the proliferation of snack consumption has been the result of modern lifestyles, economic recessions and increased industrialization among other factors. Although world governments have tried to improve food/snacks supply safety, the incidences of borne disease has made snack consumption a significant health issue, especially in developing nations. The World Health Organization reported that in 2005 alone, 1.8 million people died from diarrheal disease. These deaths were mostly related to the consumption of contaminated food and water. The high consumption rate of locally-made snacks in Nigeria has thus warranted the need for the determination of the microbial quality and antimicrobial properties of these foods.

Methodology and Approaches
The study collected 7 diverse samples of traditional snacks bought from different parts of Nigeria. These samples were subjected to microbiological analysis to ascertain the presence of antimicrobial susceptibility patterns. The samples were aseptically blended and serial dilution of up to ten fold were made for the samples. The isolated were identified by conventional methods.

Analysis and Results
Certain bacteria were identified from the samples: Enterrobacterspp, Pseudomonas aeruginosa,Klebsiella pneumonia, Escherichacoli, Micrococcus spp, Baccillus spp, Streptococcus faecalis and Staphylococcus aureus. Further, the bacterial count on the snacks samples revealed that kulikuli and aadun contained the greatest bacterial load (6.6 x 10.7 cfu/ml and 5.6 x 10.7 respectively). The antimicrobial susceptibility pattern revealed that Pseudomonas aeruginosahad the greatest resistance to virtually all the antimicrobial agents tested.

Conclusion and Recommendations
This paper shows the presence of antimicrobial susceptibility patterns in locally-made snacks in Nigeria. Many studies have reported that snacks and foods prepared under unhygienic conditions are vulnerable to microbial contamination. There is an urgent need to study the pathogenicity and strain distribution of presumptive food pathogens and how they relate to the hygienic practices of snack preparations in Nigeria. Doing so could disclose the potential of food poisoning epidemics related to snacks consumption in Nigeria.
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USE OF MOLECULAR ANALYSIS TECHNIQUES FOR MISLABELLING DETECTION IN SEAFOOD PRODUCTS, COMMERCIALIZED WITH THE TERM “GALEOS”

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Keywords: mislabelling, galeos, COI, 16S rRNA.

Detection of fraudulent commercialisation of seafood products has been extensively reported. Although traditional methods of morphological identification offer a straightforward solution for recognizing fish species, it is not possible to use them on certain fish products such as fish fillets. Fortunately, DNA-based methods offer an alternative solution. A DNA methodology based on the PCR amplification and sequencing analysis of two mitochondrial genes (16S rRNA and COI) was developed, for mislabelling detection in seafood products commercialized with the term “galeos”. A total of thirty fish fillets were purchased from seven open markets (twenty four samples) and three fish markets (six samples), in the city of Thessaloniki (Greece). Total DNA was extracted from frozen fish tissue muscles, according to the CTAB methodology. The amplification of the mitochondrial 16S rDNA gene was done with universal 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 thirty specimens studied. The eight haplotypes were entered to the BLAST engine and revealed an almost 99% maximum identity with eight different species (Table 1). The amplification of the mitochondrial COI gene was done with the Fish-F2 and Fish-R2 universal primers, and the amplified fragment was almost 700 bp. The COI gene could not be amplified for samples with numbers 11, 12, 17, 18, 23 and 24, but these samples were classified to the species Hexanchus griseus with the 16S rDNA analysis (Table 1). In total, 680 base pairs at the 5΄ end of the COI gene were sequenced and seven different haplotypes were revealed. The generated haplotypes were analyzed using the Identification System on BOLD (Species Level Barcode Records) and using the BLASTn on GenBank, and corresponded to seven different species (Table 1). According to the Greek Food & Safety legislation requirements only the Mustelus spp. products should be sold as “galeos”, but the common name “galeos” is also used for Galeorhinus galeos. Finally, only twelve specimens out of the thirty specimens studied were classified as Mustelus spp., according
to the three methodologies. The rest of the samples were classified to six different Elasmobranchii species. Marine products labelled as “galeos” have higher commercial value (15-18 euros/kg), in contrast to other Elasmobranchii species with the common name “sapounas” (mainly Hexanchnus griseus and rarely Cetorhinus maximus), with much lower value (5 euros/kg). It is worth mentioned that the fish markets sell the authentic “galeos” species, whereas all the other Elasmobranchii species can be found in open markets.

<table>
<thead>
<tr>
<th>Sample location</th>
<th>16S - BLAST NCBI</th>
<th>COI - BLAST NCBI</th>
<th>BOLD ID</th>
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<tbody>
<tr>
<td>1 Open market 1</td>
<td>Squalus acanthias 99%</td>
<td>Squalus acanthias 98%</td>
<td>Squalus acanthias</td>
</tr>
<tr>
<td>2 Open market 1</td>
<td>Squalus acanthias 99%</td>
<td>Squalus acanthias 98%</td>
<td>Squalus acanthias</td>
</tr>
<tr>
<td>3 Open market 5</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca</td>
</tr>
<tr>
<td>4 Open market 5</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca</td>
</tr>
<tr>
<td>5 Open market 6</td>
<td>Mustelus manazo 97%</td>
<td>Mustelus punctulatus 100%</td>
<td>Mustelus sp. or Mustelus punctulatus</td>
</tr>
<tr>
<td>6 Open market 6</td>
<td>Mustelus manazo 97%</td>
<td>Mustelus punctulatus 100%</td>
<td>Mustelus sp. or Mustelus punctulatus</td>
</tr>
<tr>
<td>7 Open market 4</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca</td>
</tr>
<tr>
<td>8 Open market 4</td>
<td>Prionace glauca 98%</td>
<td>Prionace glauca 99%</td>
<td>Prionace glauca</td>
</tr>
<tr>
<td>9 Open market 4</td>
<td>Mustelus griseus 97%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>10 Open market 4</td>
<td>Mustelus griseus 97%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>11 Open market 5</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12 Open market 5</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13 Open market 3</td>
<td>Scyliorhinus canicula 99%</td>
<td>Scyliorhinus canicula 99%</td>
<td>Scyliorhinus canicula</td>
</tr>
<tr>
<td>14 Open market 3</td>
<td>Scyliorhinus canicula 99%</td>
<td>Scyliorhinus canicula 99%</td>
<td>Scyliorhinus canicula</td>
</tr>
<tr>
<td>15 Open market 7</td>
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<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>16 Open market 7</td>
<td>Mustelus griseus 99%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>17 Open market 5</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18 Open market 5</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19 Open market 5</td>
<td>Alopias vulpinus 99%</td>
<td>Alopias vulpinus 99%</td>
<td>Alopias vulpinus</td>
</tr>
<tr>
<td>20 Open market 5</td>
<td>Alopias vulpinus 99%</td>
<td>Alopias vulpinus 99%</td>
<td>Alopias vulpinus</td>
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<tr>
<td>21 Open market 2</td>
<td>Squatina squatina 99%</td>
<td>Squatina squatina 100%</td>
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</tr>
<tr>
<td>22 Open market 2</td>
<td>Squatina squatina 99%</td>
<td>Squatina squatina 100%</td>
<td>Squatina squatina</td>
</tr>
<tr>
<td>23 Open market 2</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24 Open market 2</td>
<td>Hexanchus griseus 99%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25 Fish market 2</td>
<td>Mustelus griseus 98%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>26 Fish market 2</td>
<td>Mustelus griseus 99%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>27 Fish market 3</td>
<td>Mustelus griseus 99%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>28 Fish market 3</td>
<td>Mustelus griseus 98%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
<tr>
<td>29 Fish market 1</td>
<td>Mustelus manazo 98%</td>
<td>Mustelus punctulatus 99%</td>
<td>Mustelus sp. or Mustelus punctulatus</td>
</tr>
<tr>
<td>30 Fish market 1</td>
<td>Mustelus griseus 98%</td>
<td>Mustelus mustelus 99%</td>
<td>Mustelus mustelus or Mustelus sp.</td>
</tr>
</tbody>
</table>

Table 1. Sampling location and identification results based on three different methodologies, for the samples of the present study. Samples classified to Mustelus spp. are denoted with bold letters.
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GREEN TEA: AUTHENTICATION OF GEOGRAPHICAL ORIGIN BASED ON UHPLC-HRMS FINGERPRINTS

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Keywords: green tea, authentication, fingerprints, UHPLC-HRMS.

Tea, product prepared from Camellia sinensis leaves, is one of the most widely consumed non-alcoholic beverages. It is popular not only due to its relaxing and stimulating effects but also due to a positive effect on a human health. Besides of other factors, the quality of green tea is associated with its production area. Therefore, it is necessary to have available appropriate analytical tools enabling verification the authenticity of tea, particularly its mislabelling. Since tea represents a very complex matrix, characterization of its composition is a rather difficult task. Currently, many analytical strategies dealing with the evaluation of food authenticity exist. Metabolomic fingerprinting, represents a novel approach that has demonstrated its potential to be a useful tool for the authentication purposes.

Figure 1: UHPLC-HRMS fingerprints of green tea methanolic extract in ESI+ (A) and in ESI- (B) mode of ionization.
The presented study was focused on the classification of 37 samples of green tea originated from China (n = 17), Japan (n = 13) and Korea (n = 7) by means of the above-mentioned non-target screening employing ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS). The main objective of this work was an assessment of the application potential of this approach for construction a statistical model allowing the samples classification according to the geographical origin. Within the development of an analytical procedure, sample preparation step was optimised considering a range of factors including extraction solvent and those influencing chromatographic separation. Also MS detector setting was adjusted to achieve as many as possible ‘features’ (ions, m/z) characterizing respective sample. An example of chromatographic records obtained by analysis of methanolic extract of green tea is shown in Figure 1 for both ionization modes.

The generated data were processed by multivariate chemometric analysis; principal component analysis (PCA) was used in the first step, followed by partial least square - discriminant analysis (PLS-DA). At first, data obtained using individual ionization modes were investigated separately. However, combining both data sets (both ionisation modes) enabled, as it is documented in Figure 2, even more efficient sample classification. The statistical model for classification of teas from China, Japan and Korea was created for this purpose.

Finally, the validation of developed PLS-DA model was performed. The recognition ability of the final model was excellent, 100%, and the prediction ability was also good, 94.5%. The tentative identification of the most important compounds (‘markers’) contributing to the distinguishing the samples based on the estimation / calculation of elemental formula of respective ions, m/z (MS1), using accurate mass, isotopic pattern and mass error was preliminary performed.

Acknowledgments. This work was supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015). This work was also supported by the Czech Republic National Agency for Agricultural Research (Project no.QJ1530272).
TOWARDS HONEY AUTHENTICATION: ASSESSING EUROPEAN HONEY ENTOMOLOGICAL ORIGIN BY A MOLECULAR IDENTIFICATION APPROACH

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Keywords: honey, authenticity, western honey bee, Apis mellifera subspecies.

Following the European Union (EU) legislation, honey should be produced by the western honey bee, Apis mellifera. Across Europe, 10 different A. mellifera subspecies can be found, comprising 3 different lineages (A, M and C) based on mtDNA [1]. In general, honey bees occupy allopatric geographical ranges according to their evolutionary lineages, allowing to establish an entomological origin for honey produced in different EU countries. Additionally, several honeys with protected designation of origin (PDO) detail the subspecies traditionally used in their production [2]. While numerous works focused on the botanical and/or geographical authenticity of honey, only a few have attempted its entomological authentication. For that purpose, DNA-based methods have been considered as the most suitable tools since they allow the unequivocal species identification. So far, only few works described the use of DNA-based methods to establish the entomological origin of honey [3,4] and those were focused on different species of honey bees, including Meliponini and/or Trigonini stingless bees. To our knowledge, this is the first attempt to distinguish among different European honey bee subspecies commonly used in honey production, with further application to honey authentication.

In this work, DNA markers were developed for the differentiation of A. mellifera subspecies DNA in honey. For this purpose, individuals of A. m. iberiensis lineage A (n=22) from Portugal and Spain (n=5), A. m. iberiensis lineage M from Spain (n=7), A. m. mellifera lineage M from France, Netherlands, Scotland and Norway (n=7), A. m. ligustica lineage C from Italy (n=4), A. m. carnica lineage C from Croatia and Serbia (n=4) and commercial Buckfast lineage C bees (n=10) were tested. Different sets of primers were designed targeting the cytochrome oxidase I gene. The specificity and sensitivity of the designed primers were assayed by qualitative polymerase chain reaction (PCR). Species-specific primers successfully allowed the identification of A. m. iberiensis lineage A by end-point PCR. The use of real-time PCR coupled with High Resolution Melting analysis allowed the separation of A. mellifera honey bee subspecies in
different clusters according to their lineages. The developed methodologies were applied to the analysis of authentic honey samples from Portugal (produced by A. m. iberiensis lineage A), Spain (produced by A. m. iberiensis lineage M), and Italy (produced by A. m. ligustica lineage C), allowing its successful entomological origin identification.

Acknowledgements: This work has been supported by FCT (Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013 – POCI/01/0145/FEDER/007265 with financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 and by the project NORTE-01-0145-FEDER-000011. S. Soares and J. Costa are grateful to FCT grants (SFRH/BPD/102404/2014 and SFRH/BD/75091/2010) financed by POPH-QREN (subsidized by FSE and MCTES).

References
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SMALL GRAIN CEREAL CHAINS: TRACKING DNA TO DEFEND AUTHENTICITY AND SAFETY

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Keywords: pasta, bread wheat, Kamut, mycotoxigenic fungi.

The growing awareness of food value and quality is reflected in the increasing request of consumers regarding the safety and authenticity of components, as well as the traceability of food production chain. The culture of quality and the certification systems are gaining more and more strength in the agricultural sector, not only to provide the necessary guarantees about the raw materials to the industry and consumers, but also to safeguard the qualitative and economic value of food products. Over the years, the use of DNA-based methods in the food fields has attracted growing interest and has been proposed as good alternative due to their accuracy, reliability and cost effective characteristics. DNA barcoding systems, molecular markers, next generation sequencing can be adapted and combined to counteract food frauds and to meet new challenges in food safety and quality.

In this work we have explored the applicability of the genetic approach to improve quality and safety aspects of wheat chains and their final products. Different methods are included in this study, ranging from the identification and quantification of cereal species and varieties in grains and food to the use of barcode approach for the detection of fungal contamination in cereal grain samples. More details are provided and will be discussed below:

a. Pasta made from Triticum durum is considered superior for several reasons, because of its sensorial and technological peculiarities. Italian law establishes that the pasta must be produced with only durum wheat, with a maximum accidental bread wheat's contamination of 3% (D.P.R. 187, 9 February 2001 and D.P.R. 41 5 March 2013). The Italian official method, based on the separation and quantification of albumins by isoelectric focusing (Resmini, 1968), is now negatively affected by high-temperature drying of pasta. After a full validation study (Ring test) in collaboration with public and private laboratories, we have identified a qPCR assay with performance characteristics that fit for the purpose of regulatory compliance on pasta chain (Figure 1);

b. Kamut® is a trademark registered by Kamut International, Ltd. (http://www.kamut.com/en/trademark.html) and indicates a specific tetraploid wheat genotype - i.e. Triticum turanicum, variety QK-77- cultivated as a certified organic grain, containing protein in the range of 12-18%
and selenium between 400 and 1,000 ppb. A set of SSR markers has been developed to track this genotype in grains, pasta and bakery products (Figure 2);

c. The identification and quantification of specific fungal species and the production of particular toxic metabolites (mycotoxins) in small grain cereals is fundamental in terms of safety. A BARCODE approach, based on the sequencing of ITS and LSU genomic regions, has been used to obtain the description of the fungal species present on a set of durum wheat samples, growing in different Mediterranean areas. A correlation between the diagnostic data obtained with classical morphological and molecular barcoding approaches has been done.

Acknowledgements. Work partially funded by Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese.

Figure 1. Real-time PCR plots of 15 durum wheat pasta samples. The results are obtained by using Terzi primers (used to verify the presence of amplifiable DNA) and Alary bread primers (specific for bread wheat DNA). (A) shows the plot of the ΔRn versus the number of cycles obtained from Terzi primer; (B) represents the corresponding dissociation curves. (C) shows the amplification curves of Alary bread primers and the equivalent dissociation curves (D); The analysis are acquired by using DNA extracted from each pasta samples.

Figure 2. The results with GeneMapper® Software 4.0 Microsatellites Analysis shows the different curves of Kamut, Core and Claudio varieties by using a set of SSR markers (marker 11 in the graphic) developed to track Kamut genotype in grains, pasta and bakery products;
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SESAME SEED LABELLING AND HEALTH PROTECTION OF ALLERGIC CONSUMERS: A LABORATORY SURVEY IN NORTHERN ITALY

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Keywords: Food allergy, sesame seed, consumer health protection.

In the general populations food allergy is reported to affect nearly 5% of adults worldwide. Despite the discrepancy in prevalence data, there is general agreement on a higher prevalence among young people in developed countries, where it is estimated that up to 8% of children suffer from food allergy. Despite the continuing rise in the prevalence of food allergies worldwide, accurate prevalence data are limited and statistics vary by country and food. At present, food allergy is primarily managed by strict avoidance of food containing allergens, which is why food allergen labelling is critical for ensuring that consumers make safe choices in the food they purchase. To protect allergic consumers, European legislation requires the provision of allergen information on food labels so that sensitized consumers can avoid the foods that trigger their allergic reactions. Under European Regulation No 1169/2011, the presence of allergens in a food product must be declared in the ingredients list on food labels. Here we report on the presence of sesame seed as a food allergen. Labelling of sesame is mandatory in Europe, and sesame seed is included in the list of allergenic ingredients to be declared on the food label (Annex II of Regulation EC 1169/2011). The aim of the study was to determine the presence of sesame seeds in samples of packaged food products in which sesame seed was either not declared in the list of ingredients or the voluntary labelling statement.

Packaged bakery and non-bakery food items were collected according to two sampling plans with different aims: i) to detect sesame seed as a hidden allergen in food products carrying no mention of sesame on the ingredient label; ii) to detect sesame seed as an allergen in food products carrying the voluntary labelling statement “may contain traces of sesame”. The parameters for the sampling plans were: estimated prevalence of approximately 2%, precision of 5%, and sample size calculated accordingly. A total of 32 samples were collected for each food category, as calculated (EPI INFOTM). Packaged food items were obtained from retail outlets between January and September 2016. In a preparatory step, the analytical performance of the detection method was determined. The RIDASCREEN®FAST Sesame test (R-Biopharm AG, Darmstadt, Germany) was used for the detection of sesame seed as a food allergen.
The test's limit of detection (LOD) is expressed as 0.2 ppm of sesame. To prepare the sesame-spiked samples, 0.0015 g (± 0.0001g) of sesame flour were added to wheat flour to a dry weight of 50 g and a theoretical initial concentration of 30 ppm. This spiked level was then used to prepare six sesame-spiked levels: 20, 15, 5, 2.5, 0.5 and 0.25 ppm sesame.

Each of the six spiked-level samples was analyzed in triplicate with the allergen detection kit. Sesame was correctly found in triplicate trials down to the spiked level of 0.5 ppm. Since the 0.25 ppm spiked level was detected in only one of the three trials, the test's LOD was fixed in the laboratory at 0.5 ppm of sesame protein in the food matrices, although the LOD according to the manufacturer is 0.2 ppm. Of the 32 food samples that did not mention sesame seed on the ingredient label, one breadsticks sample (3.1%) tested positive at a concentration about 326 ppm. Of the 32 food samples that carried the precautionary label statement “may contain traces of sesame”, one breadcrumbs sample (3.1%) tested positive at a concentration of 305 ppm.

The results of the present study were similar to those of previous studies by our research group, in which we reported the percentages of hidden egg (3.6%) and milk (2%) proteins out of a total of 1,566 food samples analyzed. Other Italian surveys reported that 3.3% of irregular food samples contained undeclared molluscs and 3.2% of food samples contained undeclared crustaceans. Labelling of sesame seed is mandatory in the European Union, Canada, and Australia but it is not part of mandatory food-allergen labelling requirements in the United States, Mexico, China, Hong Kong, Japan, Korea, and Mexico. Sesame seeds are difficult to control in food production plants and equipment due to their particulate nature and electrostatic properties, and unintentional cross-contact with non-sesame products made on the same production line is a major concern for the food industry, legislators, and allergic consumers. As calculated in seeds of sesame, the threshold dose of the most sensitive patients in their study ranged between 1.0 and 2.4 mg of sesame proteins, corresponding to 2-4 sesame seeds. By comparison, the contamination we found could be considered very consistent, indeed.

In conclusion, the percentage of sesame-positive samples in this survey was about 3%, which has implications for the health protection of allergic consumers. Monitoring of compliance with allergen labelling requirements could support individuals with sesame allergy in avoiding their allergen and reduce the risk of unexpected exposure to this increasingly included and undeclared ingredient in many foods.

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WHAT'S COOKING? FIRST APPROACHES OF SPECIES IDENTIFICATION IN COMPLEX FOOD MATRICES

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Keywords: mtDNA metabarcoding; NGS; species identification; complex food matrices.

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. Currently, the most common methods for species identifications are based on amplification by PCR and Sanger sequencing of marker genes, generally from mitochondrial DNA (mtDNA). The obtained sequence is then compared to reference databases, like GenBank or the more curated Barcode of Life Data System. The limit of this approach is that the Sanger sequencing is able to provide only the most represented DNA sequence in the mixture of amplicons. Thus, if a sample is composed by one major species with very low abundance ones, these are not identified. As well, if the minor species are high enough for detection, they may lead to an unusable sequencing data. Next Generation Sequencing (NGS) can overcome this limit, since it merges DNA-based species identification and high-throughput DNA sequencing. The advent of NGS methods led the metabarcoding to be largely used in many different fields of research. Starting from this premise, we applied the mtDNA metabarcoding to food in order to characterize the “species composition” of a food matrix.

As a first attempt for food metabarcoding, we focused on meat and fish species identification, applying two different sets of primers from literature: primers L15601 and H15748 (Lopez-Oceja et al., Forensic Sci Int Genet, 2016), targeting a fragment of 148 bp of the cytochrome b for meat; primers MiFish-U-F and MiFish-U-R (Miya et al., R Soc Open Sci, 2015), targeting a hypervariable region of the 12S rRNA gene (163–185 bp) for fish. To test the metabarcoding approach, we prepared three mixtures of meat:

1) 50% goat (Capra hircus), 50% water buffalo (Bubalus bubalis)
2) 25% roe deer (Capreolus capreolus), 25% red deer (Cervus elaphus), 25% ostrich (Struthio camelus), 25% fallow deer (Dama dama)
3) 70% beef (Bos taurus), 30% pork (Sus scrofa)

We then analyzed four aliquots of fish sticks, received in our laboratory for routine analyses. The producer of the sticks declared manifold fish species in the ingredients list (Theragra chalcogramma, Merluccius gayi, Merluccius productus, Merluccius hubbsi, Gadus morhua, Pollachius virens, flour, lard, whey powder, etc.), and for some of the aliquots it was not possible to retrieve a clean DNA sequence, probably due to a mixed preparation. DNA was processed by PCR amplification with primers+adapters and purification by magnetic beads. Amplicons were then processed for index PCR, purified, and normalized. Library pool was sequenced on the Illumina MiSeq platform with a 2x150 cycles paired-end workflow. The pipeline included a first step consisting in primer and quality trimming and mate pairing, then dereplication, chimera detection and removal, clustering at 97%. Finally, the reads were compared to the GenBank mitochondrial database by local blastn search. The top 95% read assignments were considered for the results.
The analysis on the meat samples provided a good results for mammalia (Fig. 1): in the goat-water buffalo mix, the two species were detected in a relative abundance similar to the input. In the more complex mix 2, the ostrich was not detected, while the others were correctly assigned. The beef-pork mix was correctly described, although the abundance did not correspond to the input amount.

Fig. 1: Pie charts of the results of metabarcoding on meat mixture samples.

The fish sticks showed coherent results among them and returned two major species (Fig. 2): Gadus chalcogrammus and Bos taurus. G. morhua was detected at a very low abundance, and was declared in the label; the significant presence of Bos taurus can be explained by the use of whey powder, as declared by the producer.

Fig. 2: Pie charts of the results of metabarcoding on fish sticks.

The outcome of the mtDNA metabarcoding on food matrices provided good results correctly identifying the major species. The occurrence of low abundance species may be explained with either PCR-induced errors or blastn misidentification or database inaccuracy. Traces of G. morhua may be due to such reasons or also to residual traces of DNA from the processing. Based on these results, such approach is very promising; however, further investigation is needed to have more robust results possibly by combining more than one marker gene.

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SELECTION OF DNA MARKERS FOR THE IDENTIFICATION OF ANACARDIACEAE FAMILY MEMBERS (ANACARDIUM OCCIDENTALE AND PISTACIA VERA)

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Keywords: Cashew nut, pistachio nut, molecular markers, PCR.

Cashew nut (Anacardium occidentale) and pistachio nut (Pistacia vera) are members of the Anacardiaceae family, both presenting great importance for human nutrition. They are widely appreciated for their pleasant taste/aroma, being commonly consumed as snacks or as ingredients in a variety of foodstuffs such as ice creams, chocolates, cakes, biscuits, among others. Besides their organoleptic characteristics, cashew and pistachio nuts are well-known for their potential health benefits (e.g. heart-protective properties), being usually associated with healthy food habits. However, as part of tree nuts, they are also classified as allergenic seeds, which are known to be responsible for triggering several moderate to severe IgE-mediated reactions in sensitised/allergic individuals. Cashew and pistachio nuts can be easily identified when consumed as snacks, but when integrated as ingredients in processed foods, their correct identification is not possible to accomplish. Therefore, it is of utmost importance the development of highly specific and sensitive analytical methodologies for allergen detection/identification [1]. In this work, we propose the use of polymerase chain reaction (PCR)-based methods as effective tools to trace cashew and pistachio nuts in foods. For this purpose, model mixtures of pasta containing known amounts of cashew or pistachio nut (50-0.0001%, n=13) were prepared. For PCR assays, all allergen encoding sequences from cashew and pistachio nuts were extensively in silico analysed, using the available nucleotide sequences at GenBank database, to allow the design of primers for the their specific detection. DNA extraction was performed using the commercial NucleoSpin Food kit. Yield, purity and integrity of the extracts were evaluated by UV/Vis spectrophotometry and agarose gel electrophoresis. In the case of cashew nut, PCR assays were developed targeting three different allergenic encoding genes (Ana o 1, Ana o 2 and Ana o 3), which presented absolute sensitivities of 1-10 pg of cashew nut DNA and relative sensitivities of 0.05-0.01% of cashew in pasta. Regarding the pistachio nut, three PCR assays were also developed targeting Pis v 1, Pis v 2 and Pis v 5 genes. Absolute limits of detection (LOD) ranged from 1-10 pg of pistachio nut DNA and relative sensitivities of 0.05-0.005% of pistachio nut in pasta were obtained. Pis v 2 revealed some minor reactivity with mango and cashew nut (all belonging to the Anacardiaceae family), thus PCR products were sequenced. Primers enabling the best absolute/relative sensitivities were used for the development of real-time PCR systems. In spite of presenting distinct sensitivities, it was demonstrated that different DNA markers can be used for the unequivocal identification of members of the Anacardiaceae family, namely cashew and pistachio nuts in processed foods.

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DETECTION OF MILK AND CHEESE FRAUD BY ELISA METHODS

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**Keywords:** milk fraud, cheese fraud, ELISA.

Cow’s milk is the most popular type of milk produced worldwide. In addition to cattle, many other kinds of livestock provide milk used by humans for dairy products. These animals include buffalo, goat, sheep, camel, donkey, horse, reindeer and yak. The consumer prices of milk from these different species differ greatly. Cow’s milk is generally cheaper than milk obtained from other species. This encourages fraudulent mixing for economic benefits which is also a risk for consumers allergic to cow’s milk. Similar illegal handlings can be expected with the more expensive grain milks (from barley, oats, rice and spelt), legume milks (from lupin, pea, peanut and soy), nut milks (from almond, cashew, hazelnut and walnut) and seed milks (from hemp, quinoa, sesame seed, sunflower seed and coconut). The Milk Fraud/Bovine ELISA was developed to detect the presence of cow’s and buffalo’s milk in the milk of other species and sources. This method is based on a specific mouse monoclonal antibody that recognizes a small 5 amino acids containing epitope on bovine k-casein which is similar in buffalo’s milk but not present in the milk of other species and sources. The assay was validated and found to be suitable for the detection of both raw and heat treated cow’s milk in milk of other species and sources at a level 0.1% and above.

Another ELISA method was developed for the detection of cheese fraud, that is when cow’s milk is used for the production of cheese, which is claimed to be produced only from goat’s milk, sheep’s milk or milk of other species. A monoclonal antibody applied in the Cheese Fraud ELISA recognizes a small 7 amino acids peptide fragment of the para-k-casein part of bovine k-casein (cow and buffalo). This specific epitope is not present on the proteins from milk of other species (i.e. goat, sheep, camel, etc.). Due to this small epitope being detected in competitive assay format, the Cheese Fraud ELISA can be also used to screen for k-casein after proteolysis (breakdown of proteins into smaller polypeptides) in old cheeses. The method was validated and the limit of detection of cow’s milk in cheese was found to be 1%.
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PURSUING AUTHENTICITY AND VALORIZATION OF MEDITERRANEAN TRADITIONAL PRODUCTS, REALMED PROJECT

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Worldwide, consumer demand for food quality and distinctiveness is growing, as well as concern over issues related to food authenticity, geographic origin, health and nutrition and sustainable production. The use of standards and certifications can act as a warranty of quality to gain the trust and confidence of consumers. Indeed, food products have to respond to current ethical, environmental and socially sustainable claims. This is particularly critical in the Mediterranean region, where several unique and traditional food products of an exceptional quality play a fundamental role in local socioeconomic activity and in the conservation of cultural and natural heritages.
The latter is under threat from the cumulative impacts of land use and climate change affecting the Mediterranean Basin.

REALMed project aims to add value to Mediterranean food products by providing means to assure authenticity and quality. We will focus on high premium products typical of the national identities involved in the project - Moroccan argan oil, Portuguese and Spanish meat products from Iberian black pigs, Italian and Slovenian truffles and Tunisian lamb. Through a comprehensive set of activities, including national and international benchmarking, we will achieve an assessment and analysis of national capacity needs, as well as create opportunities for value chain improvements. Analytical techniques that show great potential for determining geographic origin (stable isotope ratios and elemental profiles) and authenticity (molecular characterization) will be combined. The data generated will be used to create a database, spatial-temporal models and ‘authenticity’ maps available for control authorities as well as for stakeholders. These ‘user friendly’ information tools will be a scientific basis for a Mediterranean Identity Label of the selected products, transferable to other foods of the Mediterranean area and elsewhere. Coordinating individual countries efforts to recognize, certify and defend local products will ensure competitiveness, sustainability and help implement prevention measures against fraudulent food practices.

The REALMed project will also reduce the disparities in food traceability, quality and safety assessment methods that exist amongst the Mediterranean regions through knowledge and technological transfer between partners and regions. Establishing a sustainable cooperation platform of experts in food traceability and authenticity, and having a balanced partner structure and early stakeholder involvement at all stages will ensure the success of the project. The expert platform will remain operational after the project’s closure and will continue to collaborate with producers and policy and decision makers.

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DETECTION OF GELATIN ADULTERATION USING BIO-INFORMATICS, PROTEOMICS AND HIGH-RESOLUTION MASS SPECTROMETRY

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Keywords: adulteration, gelatin, high resolution MS, bioinformatics.

Introduction
Gelatine is composed of highly processed proteins, which is widely used as a gelling and thickening agent in a variety of food products, including meat, confectionery products and water-based desserts. It is also widely used in the pharmaceutical industry. Gelatine is obtained by hydrolysis of collagen, which is extracted from materials such as bone, hide and skin from animal slaughterhouses. Nearly 80% of gelatine are produced from pig byproducts. However, vegetarian, Halal and Kosher gelatine, are normally prepared from seaweed, fish or bovine skin or bones. The objective if this study was to demonstrate that detection of specific peptide biomarkers in the digested gelatin samples using HPLC-QqOrbitrap is an effective strategy to detect gelatine adulteration.

Methods
Processed meat samples containing gelatine (e.g. cold cuts from pork, chicken and beef) 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 followed by a trypsin digestion (100:1 ratio). The reactions were stopped and supernatants analyzed using Thermo ScientificTM Q-ExactiveTM OrbitrapTM MS. The chromatography was achieved using a 30 minutes linear gradient along with a Thermo ScientificTM BioBasic™ C8 100 × 1 mm column at a flow rate of 75 µL/min. The Q Exactive MS was operated in full scan MS, targeted MS2 and Data Independent Acquisition (DIA) modes.

Results
Gelatine contains mainly highly denatured collagen proteins, mostly type 1 collagen. The protein sequence is highly conserved between animal species. There are substantial residue substitutions and the level and locations of post-translational modification (proline) is variable. Additionally, collagen proteins have unusual amino acid sequences. Glycine (G) is found at almost every third residue, and collagen contains large amounts of proline (P) and can be found as hydroxyproline. Therefore, we have methodically analyzed in silico type 1 collagen sequences for various species and identify several species specific peptide biomarkers. Based on in silico investigation, we have generated lists of peptide biomarker candidates, precursor (MS1) and product (MS2) mass lists were used to survey and collect MS
data. We were able to detect 24 pork collagen type 1 tryptic peptides including 12 species specific peptide biomarkers previously identified using our in silico investigation. As expected, several of these peptides contained hydroxyproline. MS2 spectra were collected for all tryptic peptides and were coherent with the amino acid sequences. Following repeat analysis, 4 complementary peptide biomarkers ([831-846]/[847-879]; [949-974]/[975-996]) were used to develop a specific assay to detect gelatine adulteration with pork byproducts. The digestion protocol was optimized (16-24h at 40˚C) to obtain maximum abundance. Pork adulteration was tested from 0.1% to 20% (w/w) and good reproducibility and linearity was observed. Sequence, mass and MS/MS signature ions of species-specific peptide biomarkers will be presented with a detailed analytical strategy to perform systematic pork adulteration surveys using high-resolution MS.

**Novel aspect**
Systematic methodology based on a proteogenomic mapping and methodical selection of specific peptides to assess gelatin adulteration by high-resolution MS.
PRINCIPAL PHENOLIC ACIDS COMPOSITION OF TRITICUM SPP. WHOLE GRAIN AND CORRESPONDING MILLING CO-PRODUCTS

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Keywords: Phenolic acids, Triticum spp., milling co-products, bran.

Cereal grains belonging to Triticum spp., generally known as wheat cereals, are one of the most important crops because of their end-use, mainly for products such as pasta, bread, biscuits, cakes, etc. Wheat grain (Fig. 1) is mainly structured in three parts, the endosperm (81-84%), that plays a role as storage of energy (starch granules), the bran (14-16%), outer layers protecting the grain, and the germ (2-3%), in which the genetic material is enclosed. During cereal grain processing a large amount of co-products are generated, principally the parts that comprise the bran and outer layers. Co-product streams amount for about 23-27% of the milling output [1] and they are mostly directed to the feed industry.

Figure 1. Wheat seed anatomy.
Nonetheless, the fractions removed still preserve their high nutritional quality. Phenolic acids content, indeed, progressively decreases through the aleurone layer into the inner parts of the kernel, indicating their higher concentration in the outer and usually discarded layers. Phenolic compounds are secondary metabolites which are essential to plants because of their protecting role from UV radiation, inhibiting pathogens and providing structural integrity to the cell wall. Furthermore, phenolic compounds play a significant role in health benefits owing to their highly antioxidant capacity. In wheat grain phenolics are mainly represented by either cinnamic and benzoic acid derivatives. Two phenolic components could be distinguished in wheat seed, the insoluble or bound fraction, most abundant, [2] and the free component [3]. Phenolic acids in whole grain and corresponding milling fractions were determined through a liquid chromatography/tandem mass spectrometry (UHPLC–MS/MS) method. Phenolic acids are principally found in the outer bran layer of wheat grain, in according to several other studies [4-6]. The bran layer is extremely stratified not only in phenolic composition, but also in the degree of ester and ether bonds and the compounds to which the phenolics are cross-linked. Ferulic and sinapic acid were established as the dominant phenolic acids in a wheat grain and its co-products. The high amount of phenolic compounds found in wheat species suggest that these co-products may represent a valuable source for the recovery of bioactive compounds and they could be further used as ingredients in food products, in which they could exert technological or health functions.

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Reference
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TOWARDS STANDARDISATION OF NON-TARGETED METHODS – STATUS QUO

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Keywords: non-targeted methods, standardisation, validation, food authenticity.

Non-targeted methods become more and more important in food authentication. Doubtless the potential of these approaches is tremendous but the way towards standardisation and thereby their limitation for a broad application appears to be one bottleneck. The lacks of appropriate validation strategies and approaches for standardisation have been identified also in the FoodIntegrity project. Several activities have been conducted and launched in the recent past and some are presented herein:

1. The German Federal Institute for Risk Assessment (BfR) in cooperation with the EU funded project FoodIntegrity held an international symposium on 28–29 November 2016 in Berlin/Germany entitled “Standardisation of Non-targeted Methods for Food Authentication”. The main issues addressed at the symposium covered (1) general aspects of standardisation of non-targeted approaches in food authentication, (2) the most current status quo on validation and standardisation of analytical methods and statistical models, and (3) the exchangeability of data between laboratories, system challenges, and quality assurance measures. The conference was a forum for experts and interested stakeholders to exchange scientific knowledge and expertise and to extensively discuss open questions in the research field of food and feed authentication using non-targeted methods.

2. The U.S. Pharmacopeial Convention (USP) drafted a guidance document on developing and validating non-targeted methods for adulteration detection. This guideline sets the frame for a good practice using non-targeted methods for food and feed authentication.

Further cross linked activities (e.g. in the metabolomics community) are listed and discussed.
DEVELOPING A TARGETED PARALLEL REACTION MONITORING METHOD FOR THE DETECTION OF MILK-DERIVED INGREDIENTS IN COMPLEX MATRICES

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Keywords: milk proteins, high resolution MS, allergens.

Introduction
Food allergen testing is often associated with enzyme-linked immunosorbent assays (ELISAs), however modern advances to analytical instrumentation promise alternative analytical methods for confirmatory detection and quantification. Mass spectrometry (MS) is a widely recognized highly specific, sensitive and accurate analytical tool for the identification and characterization of proteins and peptides. MS approaches may be the future of food allergen detection, as a single analysis can simultaneously interpret multiple allergens in complex matrices. Within the food industry, different types of milk-derived ingredients, with different protein profiles, are valuable additions to the functional properties of food. The aim of this study was to create a high-resolution, accurate-mass (HR-AM) targeted parallel reaction monitoring (PRM) mass spectrometry method to detect milk and milk-derived ingredients in complex matrices.

Methods
Six milk-derived ingredients were solubilized in protein extraction buffer, trypsin digested, C18 spin cleaned and injected onto a Q ExactiveTM Plus OrbitrapTM LC-MS/MS system. Protein discovery was performed in data-dependent acquisition mode and peptide identification and relative quantification data were evaluated to select milk allergen peptide and precursor targets for a PRM inclusion list. Milk ingredients were further analyzed using an in-house targeted PRM method. PRM precursor/transition targets were evaluated and filtered by modifications [carbamidomethyl (C), oxidation (M), phosphorylation (S, T, Y)], uniqueness and minimum number of transitions. Several trials were completed to reduce the number of targets.

Preliminary data
Over 1300 total milk proteins (6500 peptides) were identified in discovery mode using a bovine (Bos taurus) sequence database. Approximately 100 proteins were identified as being “high confident” after applying selected filters (target FDR is 0.01, minimum two peptides per protein and each peptide is greater than 5 AA in length), and exporting for label-free quantification analysis. Forty-four “high confident” proteins were identified as milk allergens. After removing duplicate peptides and peptides with an idotp less than 0.80, 10 proteins, 64 peptides and 100 precursors remained. Identified peptides
belong to the casein (\(\alpha\)-S1-casein, \(\alpha\)-S2-casein, beta casein and kappa casein), whey (\(\beta\)-lactoglobulin, \(\alpha\)-lactoglobulin), serum albumin and lactoferrin groups. Casein peptides derived from nonfat dry milk, whey protein concentrate 80 and sodium caseinate were 100-fold greater in peak areas when compared to the other ingredients. Peak areas from whey peptide were comparable among all ingredients. Milk samples were re-tested using the inclusion list and targeted PRM method. After two experimental runs, the target list was reduced to 21 peptides and precursors with additional filtering possible following evaluation in food matrices. Future experiments will examine the performance of peptide targets when milk-derived ingredients are incurred into several matrices including a wheat-based cookie.

**Novel aspect**
This study is the first to develop a PRM method to detect milk-derived ingredients.
AUTHENTICATION OF ITALIAN AND SPANISH OLIVE OILS USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION TANDEM MASS SPECTROMETRY

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Keywords: olive oil; geographical origin authentication; quality grade; U-HPLC-HRMS/MS.

Olive oil represents a highly valuable commodity which might be subjected to fraudulent activities. Particularly, dilution with cheaper plant oil, substitution by a lower quality grade and/or geographical provenance mislabeling are typical issues of high concern. In the latter case, especially the highly valued extra virgin olive oils originating from the countries of the Mediterranean region are vulnerable to fraud. In addition to classic methods, various techniques based on mass spectrometry, nuclear magnetic resonance and vibrational spectroscopy have been increasingly used to authenticate olive oils. Regarding laboratory strategies, verification of geographical origin is a considerably challenging task. In the recent years, untargeted metabolomics, study on small-molecule metabolite fingerprints, has shown a good authentication potential demonstrated at numerous studies dealing with the geographical origin of various commodities such as tea, coffee, fermented beverages, honey, meat as well as olive oil.

Within this study, ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (U-HPLC-HRMS/MS) employing Q-Exactive Plus followed by multivariate data analysis was utilized for the purpose of geographical origin authentication of olive oil. A newly developed analytical method was tested on a wide set of Italian monovarietal extra virgin olive oils (EVOO; n = 154) and Spanish olive oils represented by monovarietal and coupage olive oils of different quality grade (n = 93). Unsupervised principal component analysis (PCA) followed by supervised orthogonal partial least square discriminant analysis (OPLS-DA) were used to create statistical models for classification of samples according to (i) EVOO geographical origin (Italy x Spain), (ii) quality grade of Spanish oils (EVOO x non-EVOO), and (iii) blending of Spanish olive oil (monovarietal x coupage), the results are presented in Figure 1.

The constructed statistical models showed good prediction ability for all the tested criteria (Q2 > 0.6). Their applicability was successfully verified using leave-one-out cross validation (LOOCV) method and external validation set of 48 samples. Numerous discriminative markers belonging mainly to the class of phenolics such as tyrosol, elenolic acid and ligstroside aglycone were found for the individual tested groups. To obtain even more robust model, additional data obtained on the olive oil samples representing e.g. different harvest years, would be needed.
Figure 1 Multivariate statistical analysis (PCA, OPLS-DA) on olive oil data. Authentication according to (i) geographical origin (Italian and Spanish olive oils), (ii) quality grade (Spanish olive oils) and (iii) blending (Spanish olive oils).

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NON TARGET SCREENING STRATEGY BASED ON GC-Q-TOF FOR SCOTCH WHISKY AUTHENTICATION

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Keywords: whisky, authentication, GC-Q-TOF, screening.

Whisky is one of the most popular spirit drinks in the world. Unfortunately, this high valued commodity is vulnerable to fraud. To detect fraudulent practices and document quality parameters, a number of laboratory tests based on various principles including chromatography and spectroscopy has been developed. In most cases, the analytical methods are based on targeted screening strategies. In our study, to isolate and pre-concentrate as much as possible volatile and semi-volatile compounds, ethyl-acetate extraction was used. In the next step, non-target approach, fingerprinting of whisky components based on gas chromatography coupled with tandem mass spectrometry (Q-TOF mass analyzer) was employed. The data obtained by analysis of a unique set of 180 authentic whisky samples (differing in region of origin, maturation in various cask and age) provided by the Scotch Whisky Research Institute were assessed by advanced chemometric methods. Principal component analysis (PCA), partial least squares discriminant analysis (PLS–DA) and orthogonal PLS–DA were applied for classification model construction. Very good separation according maturation casks (bourbon, sherry,

Figure 1: Distribution of whisky samples aged in cask used for a sherry (red), port (yellow), red (brown), white (purple) wine and bourbon (green) production – PCA.
red, white and port wine) was achieved (see Figure 1A), significant markers most contributing to the clustering were identified (e.g. diethyl tartrate or vanillin acetate). Selected markers enabled separation not only between samples aged in ‘wine’ and/or ‘bourbon’ casks and even the samples of Highland origin, as shown at Figure 1B. In the next phase, 20 fake samples and 24 ‘unknown’ samples provided again by Scotch Whisky Research Institute were analyzed and the data processed in the same way. As documented in Figure 2, some differences could be observed in volatiles profiles of authentic and fake samples. Employing the chemometric model developed for this purpose, marker compounds enabling distinguishing fake samples were found. Based on their mass spectra, several food additives (e.g. triacetine - E 1518) were identified. These ‘new’ markers might be added on the target analytes list for a routine control.

![Figure 2: Total ion chromatograms of fake and authentic samples.](image)

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VOLATILE FINGERPRINTING USING PTR-MS PAVES THE WAY FOR SOUTH AFRICAN LAMB TO ACQUIRE PGI STATUS

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Keywords: extensive grazing, geographical origin, lamb fat, lamb meat.

Globally, increased attention is being given to the labelling and branding of specific sheep meat such as the Northumbrian lamb (UK), Ronaldsay lamb (Scotland), Texelse Lambsham (Netherlands), Ternasco de Aragon (Spain), Patagonian lamb (Argentina) and Agnello della Maremma (Italy). In South Africa, lamb typically produced in the Northern parts (the Karoo region) of the country is known as Karoo lamb. The meat is appreciated and valued by consumers for its unique sensory quality (e.g. herbaceous aroma and flavour) due to the diet of the sheep. Indigenous, herbaceous Karoo bushes and shrubs are key components of the diet and believed to function as a natural herb/spice. Due to the quality and value associated with Karoo lamb, fraudulent activities may occur. For instance, lamb may be sold as Karoo lamb when in actual fact it has been produced in a feedlot or different region. Similar to Karoo lamb, other characteristic sheep production sites also exist in South Africa where the typical diet of the sheep, associated with the region and traditional farming practises, lends the lamb meat unique sensory qualities. However, scientific evidence is required to authenticate the meat and prevent fraudulence. This was achieved by measuring the volatile fingerprints of South African lamb meat and fat using proton transfer reaction-mass spectrometry (PTR-MS). Meat and fat of the Longissimus lumborum (LL) of lambs from six different regions were assessed. Analysis showed that the volatile fingerprints were affected by the origin of the meat. A distinct fingerprint mass spectrum for lamb fat of the six different regions was developed (Fig. 1). The Karoo samples contained the ions m/z 77, 97, 135, 137 and 138, and had highest concentration of the ions m/z 41, 43, 59, 71, 81, 83 and 89. The monoterpene mass ratios m/z 81, 135 and 137 were particularly useful for indicating dietary differences. The fat had a higher concentration of monoterpenes, validating the direct link with the herbaceous plant samples, which could serve as markers for future classification purposes. The classification of the origin of the lamb was achieved by examining the calculated and recorded fingerprints in combination with chemometrics. Four different partial least square discriminant analysis (PLS-DA) models were fitted to the data to classify lamb meat and fat samples into “region...
of origin" (six different regions) and “origin” (Karoo vs. Non-Karoo). Performance of the models was assessed by external validation. The estimation models classified samples 100% correctly. However, validation of the first two models gave only 42% (fat) and 58% (meat) correct classification of region. Whereas, the validation results of the second two models were better with 92% (fat) and 83% (meat) correct classification of origin. The separation between the Karoo and Non-Karoo regions was clearly distinguishable (Fig. 2). Overall the significant differences between the Karoo and Non-Karoo samples indicated the typicality of Karoo lamb with the result being promising in the view of development of an authentication test required to strengthen its PGI status.

Figure 1 Mean fingerprint mass spectrum for lamb fat of the different regions generated by PTR-MS.

Figure 2 PLS-DA scores plot on axes 1 and 2 of the mass spectral data of lamb meat (upper) and fat (lower) determined by PTR-MS for origin (Karoo vs. Non-Karoo) classification. (CK) Central Karoo; (HK) Hantam Karoo; (NK) Northern; (BL) Bushmanland; (RU) Rûens; (SE) Semi-extensive.

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FOOD FRAUD IDENTIFICATION IN ARGENTINA

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Keywords: food mislabeling, food adulteration, species identification.

Detection of food adulteration or contamination is an underdeveloped area in Argentina. At the moment, there are no controls from any state or private organisms to prevent this kind of frauds. In spite of that, in the last years there is an increase demand to solve different issues related to the identification of the species components present in a diversity of food and food products. In the present work we introduce the main cases resolved in our laboratory, mainly food crime, coming out to give response to judicial authority.

These cases were solved using different DNA extraction methods depending of the types of matrices such as organic extraction, DNAzol®, magnetic beads DNA extraction kit, silica columns, etc. Genotyping was done using PCR-RFLP, PCR using species specific primers, DNA sequencing, pirosequencing, among others.

A cheese sold as pure goat generates an allergy reaction to a person who knows was allergic to cow proteins. This fact leads him to demand to its producer. A DNA analysis revealed that actually the cheese was adulterated with cow milk.

A market survey of a Patagonian city carried out by the Town Hall Bromatology Department allowed to detect that in a butchery a sausage supposedly from beef was adulterated with horse meat.

Game meat is an alternative to domestic animals meat. However, protected deer species as the marsh deer (Blastocerus dichotomus) or the pampas deer (Ozotoceros bezoarticus) are hunted illegally in protected areas and sold as different kind of meat in informal markets. Species origin identification of this meat concern equally to food authorities as well as fauna wildlife authorities. A simple DNA analysis allows distinguishing if an illicit was perpetrated.

In all the cases these methods allowed to resolve successfully the issues raised, showing the food contamination or adulteration, providing evidences for justice cases resolution and assuring food quality and integrity.
DIFFERENTIATION OF ORGANIC AND CONVENTIONAL FARMING OF TOMATOES BY NON-TARGETED METABOLOMIC PROFILING VIA TRAVELLING-WAVE ION MOBILITY HIGH RESOLUTION MASS SPECTROMETRY

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Keywords: UPLC-IMS-HR-Q-ToF MS, metabolomic profiling, metabolomic fingerprinting.

Nowadays the appreciation of food quality and traceability are important issues to both costumers and manufacturers. The quality of food products is often linked to their origin and affects the consumer's choice looking for the quality of regional products or specialties from abroad. Both 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. by organic or conventional farming of plant-derived food. Thus, it will highly relevant to ensure the traceability of these items from farm to fork, not only for consumers but also for manufacturers and retailers. To detect food fraud in the manufacturing and supply chain specific and robust analytical methodologies are required.

In our FOODOMICS project a valid methodology for food authenticity testing by metabolomic profiling is established via liquid chromatography travelling wave ion mobility quadrupole time of flight mass spectrometry (UPLC-TW-IMS-HR-Q-ToF MS). This project is funded by the German Federal Ministry of Food and Agriculture (BMEL).

The method development was based on the commodity tomato. Here the focus was set on the differentiation of organic and conventional farming practices. Representative authentic material comprising specified stages of fruit maturity was derived from different countries of origin, comprising multiple plant varieties grown under organic and conventional farming conditions. After a QuPPe-like extraction step sample extracts were subjected to UPLC-TW-IMS-HR-Q-ToF MS in full scan HDMSE mode with ESI positive and negative polarity.

The classification of organic and conventional tomatoes was evaluated on the basis of statistical discriminative models in a metabolomic fingerprinting approach by multivariate data analysis (PCA and OPLS-DA) using Progenesis QI v2.3 (Nonlinear Dynamics, UK) and EZInfo3.0 software (Umetrics Inc., Sweden).

Subsequently chemical markers differing significantly in their abundance profiles in organically and conventionally grown fruit were selected, characterized using a metabolomic profiling workflow applying a three-point coordinate system based on the exact mass, the retention time and the collision cross section (CCS) in TW-IMS (EM_RT_CCS) and further identified due to proprietary and Chemspider chemical databases. Marker compounds were classified according to their identification status and included into respective condition-specific metabolome databases. Descriptive models for classification of tomatoes from organic and conventional farming were established and validated using a blind sample approach to verify the applicability of the models in routine analysis.
ON-SITE DNA TESTING FOR SPECIES AUTHENTICITY IN FOOD AND NATURAL HEALTH PRODUCTS

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Keywords: DNA, real-time PCR, food authenticity.

Authenticity of food and natural health products (NHP) remains a risk to businesses and consumers. Fraudulent products carry an economic and health risk, yet the complexities of the global supply chain, make it increasingly important, and difficult, to ensure the authenticity of products on the market. In many cases, authenticity of ingredients is a matter of species identification. In these cases, DNA testing can often be used to identify species contained in a product and provide a measure of authenticity. However, these tests can be labour-intensive and costly. In addition, samples must often be sent to an appropriately equipped facility and results can be difficult to interpret for non-experts. Real-time PCR provides a potential solution to these issues for authenticating species in food and natural health products. The method is reliable, rapid and relatively cost-effective and results are easy to interpret. New mobile technology also enables testing to be run on site. These benefits, coupled with simplified kits, extend the benefits of DNA testing to anyone with minimal training. By moving beyond traditional lab-based tests, the power to authenticate species ingredients can be extended throughout the supply chain. This presentation reviews the benefits and potential of this testing for food and NHP businesses and regulators using case studies from seafood and natural health products, two areas with high profile media coverage of species mislabeling leading to recent prosecutions.
The sugar composition of honey is mainly dependent on its floral source and differs in various honeys. It is also affected by climate, processing, and storage conditions. Fructose and glucose are the major components and account for 85–95% of the honeybee honey sugars. Their concentrations of fructose and glucose, as well as their ratios are useful parameters for the classification of monofloral honeys. The remaining carbohydrates are a mixture of at least 11 disaccharides, 11 trisaccharides, and several larger oligosaccharides. Minor honey sugars may be useful for the determination of floral origin and may act as a “fingerprint” for a sample’s floral source.

High levels of sucrose may indicate a variety of adulterations, such as adding cheap sweeteners, like cane sugar or refined beet sugar, during early harvest. Due to these factors, various regulations require a minimum amount of reducing sugars and a maximum amount of sucrose among other honey quality parameters.

An HPAE-PAD method was successfully developed and validated for the sugar analysis of 12 commercial honey samples using the Dionex CarboPac PA210-4μm column. This method enabled us to detect the addition of industrial sugar syrups (adulteration) to honey samples.
Keywords: Complex Systems, Foodintegrity, Food Safety, Risk-based auditing.

Introduction
Today’s food sector is a conglomerate of international, long, fast changing and highly connected and depending networks which follows new consumer trends, chemical-, physical- microbiological- and technical innovations and demographic, cultural, legal and economic developments. Food chains have changed dramatically due to technical and demographic changes over the last decades. Although huge efforts are made to ensure high quality and integrity, food-borne outbreaks and food scandals still happen to an undesired extent. Questions on how we can react appropriately to the rising complexity of the food chain, what risk-based approaches are and how they can help to cope with today’s challenges arise.

Objectives
This commentary paper aims to give insight in the current approach in the European food control and audit system, the current underestimation of complexity and the role of trust for food control and auditing. Furthermore, the emergence of risk-based approaches in Foodintegrity management is shown and a framework to structure complex system risk-based auditing is proposed (Figure 1).

Results, Discussion, and Conclusion
The current food control system seems to cause a reactive, vicious cycle of more, stronger regulations and standards on every level, which result in dissatisfaction of food chain stakeholders (Figure 2). Based on the current underestimation of complexity and the current audit practices, it was concluded that a new paradigm in risk-based auditing is needed that addresses the behaviour of complex systems to ensure trust along the food chain. Further research and testing of the proposed framework for complex system risk-based auditing is needed to specify and identify risk factors for specific food chain stakeholders as basis for more effective auditing. A suitable method to test this framework seems to be serious gaming as an analytical and educational method with incorporates knowledge and data of specific food chain stakeholders.

Acknowledgements: The Authors thank INTACT Consult GmbH in Lebring, Austria for enabling this research and their viable input.
Figure 1 – Framework for complex system risk-based auditing.

Figure 2 – The reactive food control vicious cycle.
In this presentation the application of stable isotope fingerprints in food and beverage fraud detection is explored. Data are shown that show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the technology used is also provided.

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect whether the labeled product is authentic or if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.

Detecting food and beverage fraud can be achieved using stable isotope measurements because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable technique in food and beverage fraud prevention and food safety.
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FROM THE OCEAN TO THE TABLE. AN INTEGRATED MASS SPECTROMETRY APPROACH TO IDENTIFY THE FISH THAT IS ON YOUR PLATE

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Keywords: fish authenticity, high resolution MS. food fraud, proteomics.

The fishery market has growth in sales for the last 15 years. As a result, fish demand is producing a worldwide overexploitation of resources and fraudulent practices in the industry that account for 30% of the sales. In most cases, high priced fish species are substituted for lower value species. The identification of commercial fish species is a relevant issue to ensure correct labeling, maintain consumer confidence and enhance the knowledge of the captured species, benefiting both, fisheries and manufacturers.

Here we propose a proteomic approach, based on top down proteomic analyses using ESI-MS/MS in a high resolution orbitrap mass spectrometer for the identification of fish species with commercial interest. ESI-Orbitrap protein mass fingerprint from thermo-stable proteins purified from fish tissue were used for the identification of a commercial hake filet with no label regarding the fish other than Product from South Africa. Further identification and characterization of this sample was performed using standard shotgun proteomics and PRM targeted analysis.

We believe that fisheries and manufacturers can take advantage of this methodology as a tool for a rapid and effective seafood product identification and authentication, providing and guaranteeing the quality and safety of the foodstuffs to consumers.
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SET-UP OF UNTARGETED PROTEIN FINGERPRINTING USING LAB-ON-CHIP MICRO-ELECTROPHORESIS APPLIED TO SALMON TRACEABILITY

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Keywords: microcapillary lab-on-a-chip electrophoresis, salmon, origin determination.

Over the last years food traceability has earned the attention of the international scientific community, as well as the general interest of the consumers, who are more and more concerned about the origin, the integrity and the safety of the goods they are buying. New food frauds are popping up on a daily basis, and they are harder and harder to detect. Instead of searching for a specific contaminant or anomaly, untargeted analytical methods aim at describing the complex “fingerprint” of a reference food, in order to identify any irregularity independently of its source. Salmon, a fish belonging to different genera (like Salmo salar, also known as “Atlantic salmon”, and Oncorhynchus spp., commonly defined “Pacific salmon”) is a widely consumed commodity sold at different prices, depending on the quality of the product. Quality (and price) is strongly affected by both the diet and the general life conditions of the fish. Salmons can be fished in cold waters all over the world, but the majority of the salmon-based food products are prepared using flesh of farmed salmons, which are characterized by a lower quality and a lower cost. Even though salmons are well known for their beneficial properties on human health, contamination with mercury, pesticides, and persistent organic pollutants has become a widespread problem in certain salmon habitat; therefore, correct information on the geographic origin of the product is relevant for the consumer. Previous papers were aimed at enabling the discrimination between farmed and wild salmon, and verifying their geographic origin, particularly using NMR technique[1].

In this work we tried for the first time to assess the potentiality of the microcapillary electrophoresis perfomed on chip for the correct assignment of salmon origin. The use of the chip allows the compartmentalization of both samples and reagents, thus avoiding the contact of the liquids with the instrument. The optimization of the extraction of the total protein fraction was achieved. Some samples from wild caught and farmed salmons coming from different areas of the globe were analyzed using the 2100 Bioanalyzer, generating untargeted electrophoretic profiles. Raw data were extracted and analyzed using statistical tools (post analytical data mining). Preliminary results confirm that this approach could be used to generate clusters, providing new options to the identification based both on the geographic origin, and on the life conditions (farmed vs wild caught salmons).

Keywords: combustion, food quality, labeling, protein.

The nutritional composition of food plays a very important role in food industries for research and quality control purposes. Food market globalization requires accurate control of product characteristics to protect commercial value, to safeguard consumer health, and to maintain manufacturer reputation. The selection of food products available in the market is very wide, and consumers’ decisions depend on a set of information that includes quality valuation. New regulations regarding all processed food and most raw foods include a series of tests aimed at determining food contents and their contribution to a healthy diet. One of the tests used in the production process is the determination of protein content of food and animal feed. The exact determination of the amount of protein through the determination of the nitrogen content is fundamental for the nutritional quality of animal feed and for the safety of final food products intended for human consumption. Official regulations establish the protein content and labeling requirements, which enable consumers to define price and quality comparisons based on % protein declarations. For this reason, the use of a simple and automated technique allowing fast analysis with excellent reproducibility, and that can avoid the risk of handling toxic chemicals is required. An alternative to the classical Kjeldahl method, based on the Dumas (combustion) method, has been developed. The Dumas assay precedes Kjeldahl analysis by more than 50 years. The former technique was developed by Jean Baptiste Dumas. In recent decades, the advent of easy-to-use and highly accurate combustion nitrogen analyzers rekindled interest in the Dumas method. The Dumas combustion method is comparatively quicker, cheaper, easier to perform, safer and more environment friendly. The Dumas method is approved by different associations (AOAC, AACC, AOCS, ASBC, IDF, ISO and IFFO). The technique must be robust and capable of analyzing fresh and processed products in various physical states (powders, slurries, dilute liquids, emulsions, gels, pastes and other) and deal effectively with products from either animal or plant sources. The Thermo Scientific FlashSmart Elemental Analyzer (Figure 1), based on the dynamic combustion method (modified Dumas method), provides rapid and automated nitrogen determination without use of hazardous chemicals and offers advantages in precision over traditional methods. The FlashSmart Elemental Analyzer allows runs at both high and low nitrogen levels with no need to change configurations and without matrix effects. Sample protein content is calculated automatically using a conversion factor in the Thermo Scientific EagerSmart Data Handling Software. This paper presents nitrogen and protein data of several food and animal food in a large range of nature and concentration to show the repeatability, accuracy and precision obtained.
Keywords: Oryza sativa, Waxy gene, ALK gene, DNA markers.

Rice (Oryza sativa L.) is one of the most important cereals in the world, being Italy its main producer in Europe with nearly 200 different varieties present in the germplasm [1]. Italian rice varieties have different characteristics, from which the starch composition is a highly relevant parameter. Starch is composed of two polysaccharides, amylose and amylopectin, whose ratio is determinant for the rice cooking properties. After cooking, high amylose content varieties have dry, firm and separate grains, while low amylose ones usually have tender, cohesive and glossy texture [2]. Gelatinisation temperature and gel consistency are also important properties that are related to the amylopectin content [3]. Amylose synthesis is catalysed by the granule bound starch synthase (GBSS) that is encoded by the Waxy gene (Wx) [2], while the amylopectin synthesis is driven by the starch synthase IIa (SSIIa) encoded by the ALK gene [3], both located on the chromosome 6. Various nucleotide polymorphisms have been associated with the Wx [2] and ALK [3] genes, namely (CT)n repeats and several single nucleotide polymorphisms (SNP). This work intends to exploit nucleotide polymorphisms in both genes aiming at identifying molecular markers of authenticity of Italian varieties, focusing on Carnaroli rice. The Carnaroli rice is a high quality and priced variety belonging to the group of Japonica, produced mainly in Piedmont. It is considered one of the finest Italian rice varieties due to its excellent cooking resistance, given by a low tendency to lose starch and a good ability to absorb liquid while creaming, being, therefore, ideal for the preparation of traditional risotto.

In the present work, the Italian rice varieties of Carnaroli, Sant'Andrea, Carnise, Karnak, Gladio, Volano, Barone, Ronaldo, Gloria and Sole Cl were obtained from producers. DNA from rice grains was extracted with NucleoSpin food kit. In silico analysis was performed in the Wx gene to design primers targeting the (CT)n microsatellite, the G/T first intron and the A/C SNP in exon 6. In the ALK gene, primers targeting SNP in exon 8 (A/G at 4041 bp and GC/TT at 4172 bp) were used [4]. In the Wx gene, the preliminary sequencing results suggest that Gladio has a different number of CT repeats
from all the other varieties; Carnaroli, Carnise, Karnak and Gladio show a G in the first intron SNP, while other varieties have T; Carnaroli, Carnise and Karnak present a C in the exon 6 and the others A. In the ALK gene, Carnise and Sole CL display an A in the exon 8 SNP, while the others show G; Carnise, Gladio, Ronaldo and Sole CL have a GC polymorphism in the exon 8, while the others present TT. These findings are being exploited for the development of a method based on high resolution melting (HRM) analysis as a promising and high-throughput tool to differentiate closely related species or even varieties.

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References
Keywords: food manufacturers; halal control point (CP); hygiene.

Muslims follow the Islamic dietary code by consuming halal (lawful or permitted) food that are free from haram (unlawful) components. Halal food products are a growing demand across the world and demonstrates a strong potential for growth. Halal integrity is defined as the assurance of safe (pure), quality (good) and free from mal-practice (lawful) food from farm to fork. To ensure the safety and integrity of food products and that they fulfil the religious dietary requirement, hygiene monitoring is a valuable method to determine presence of filth and organic contamination. The purpose of this study was to determine the Adenosine Triphosphate (ATP) hygiene and microbiological quality of halal food products in selected food processing plants in Malaysia. Four food manufacturers were recruited from the northern state of Malaysia. Swabbing tests were conducted to determine the cleanliness of working environment and workers’ hygiene. Food samples were collected and microbiological analyses were carried out. Based on the hygiene and microbiological results, a generic Halal Control Point (HCP) plan and specific HCP plans were developed for the participating food manufacturers. The swabbing tests of food contact surfaces (tabletops) revealed that only Company C (oat products) and Company D (coffee powder) passed the Adenosine Triphosphate (ATP) hygiene test (≤ 10 Reflective Light Unit [RLU]). The ATP swabbing tests conducted on all workers’ hands and aprons from three manufacturing plants indicated a fail result (> 30 RLU). 4.82 log CFU/ml bacteria (total plate count) were found in soy sauce and none were detected in other types of food products. One generic Halal Control Points (HCPs) plan and four specific HCP plans were developed for the manufacturing process of halal food products for each food processing plant. The HCP plans are of value for food industry seeking to identify potential halal control points in food production.

Acknowledgements: The researchers would like to thank the food manufacturers from Penang, Malaysia for participating in the study.
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**A FAST METHOD FOR SUGAR ANALYSIS OF INSTANT COFFEE SAMPLES**

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**Purpose**
To demonstrate a fast method for analyzing sugars in instant coffee using High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) and a High Concentration Carbohydrate Analysis kit.

**Methods**
Instant coffee sugars are ionized in a strong base, separated by High-Performance AnionExchange (HPAE) chromatography, and detected by Pulsed Amperometric Detection (PAD). Analysis is facilitated by the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

**Results**
Using this method, carbohydrates present in soluble, as well as total, carbohydrate extracts of instant coffee were quantified in less than six minutes. The results for the method linearity, precision, and robustness are presented.
Seafood fraud is a serious global problem. Selling fish products as fresh when they have actually been frozen-thawed is a common fraudulent practice. Moreover, fish intended for raw consumption must be previously frozen according to Regulations EC 853/2004 (1) and EU 1276/2011 (2), in order to protect consumers. Since 2008 we have been evaluating the most performing techniques in distinguishing fresh from frozen fish in order to make available reliable tools. A histological method, with performances assessed on 35 fish species (sensitivity 90.70% - C.I. 82.49-95.9%, specificity 92.59% -C.I. 75.71-99.09%) and showing the best predictive power when compared to spectroscopic techniques, was set up, validated and accredited (3), and is now applied in monitoring programmes, performed by regulatory authorities and food business operators.

Cold smoked salmon is a popular, ready-to-eat product among consumers in many European countries as well as a commodity of economic importance in the world market. The production and sale of cold smoked salmon boost around Christmas and Easter time. Very often the smoked fillet is frozen short-term to make it easier to slice the product (hardening treatment): products are maintained under these conditions until the right temperature, defined by each producer on the basis of the characteristics of the product and slicing technologies, is reached. Then, after packaging, there are three options for the storage of the product: refrigeration, chilling and freezing. The product can be maintained at refrigeration temperature (0/+4°C), or stored at -18°C for a maximum of 12 months, then thawed and marketed in a refrigerated condition for a period equivalent to the shelf-life of the refrigerated product. As a matter of fact the freezing of the fresh raw material or the smoked product is commonly carried out in order to control the supply for smoked salmon in relation to rapid and large changes in demand and to take benefits from periods with surplus of fresh salmon on the market. The third storage option is represented by the chilling treatment at -3°C for maximum 30 days, before the product is marketed in a refrigerated condition. This practice is well-known and popular in north European countries.

The aim of the study was to evaluate our histological method’s reliability in distinguishing between fresh, stored at -3°C and frozen-thawed raw smoked salmon. In particular, we wanted to establish if the product maintained at -3°C, that represents a storing procedure and not a freezing procedure, could be considered fresh from a histological point of view.
118 samples were the reference materials included in the study. Samples were divided into three groups: group A (n=46): fresh samples (stored at 0-4°C); group B (n=36): frozen at -18°C, group C (n=36): stored at -3°C. Ten fresh samples were prepared without the hardening phase. All samples were fixed in 10% neutral buffered formalin for 24 hours, placed in numbered plastic boxes, and routinely processed. Paraffin embedded blocks were cut on a microtome into 3±2 μm sections and stained with haematoxylin and eosin (HE). Two expert histopathologists independently examined the slide preparations by optical microscopy at increasing magnification (10X, 20X, 40X) and classified them as frozen-thawed or fresh, according to the standard operating procedure criteria in use at the IZSPLV laboratory. Frozen-thawed samples (positive) were identified when optically empty vacuoles of various dimensions, caused by ice crystals, were observed in the cytoplasm of muscle cells; fresh (negative) samples were identified when these microscopic changes were absent. Sensitivity and specificity of the histological method were calculated, with corresponding 95% confidence intervals. Statistical analysis showed 80.6% (C.I. 64%-91.8%) sensitivity and 95.6% (C.I. 89.1%-98.8%) specificity for the histological method applied to smoked salmon.

It is important to note that 11.1% (C.I. 3.1%-26.1%) of the samples stored at -3°C were interpreted as frozen, but this limit can be easily overtaken by the adoption of an improved sampling plan, in which results of an homogenous group of samples (n=6) contribute to the definition of a single final result; it will permit to considerably reduce false positives, allowing the correct histological differentiation between samples stored at -3°C and frozen samples. Furthermore we observed that the technological hardening phase does not determine microscopic alterations referable to those seen in frozen samples.

On the basis of the performances showed by the method, we can conclude that histology is a reliable tool to differentiate fresh from frozen-thawed fish, including smoked salmon subjected to storing procedures: the method was validated by the Authors and will be shortly accredited even for this matrix, becoming available for the needs of the food business operators as a valid analytical support.

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(2) Regulation (EU) 1276/2011
Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature. Honey is essentially a concentrated aqueous solution of different carbohydrates, including fructose, glucose, maltose, sucrose and other –oligo and –poly saccharides. Serbia has a very long tradition of beekeeping. Its favourable climate, good geographical conditions and a variety of botanical species provide great potential for the development of apiculture. When placed on the market as honey or used in any product intended for human consumption, honey shall not have any other food ingredient added, including food additives, nor any other additions to be made. One of the important assessment of authenticity is sugar content, considering that natural honey primarily consists glucose and fructose and may contain low levels of sucrose and/or maltose. Often, on the market some products could be found which are presented as honey, which consist exogenous sugars derived from cane, corn, millet or sorghum. That is one of the biggest frauds when it comes to honey. SP Laboratorija is accredited laboratory for honey analysis. For detection of sugars: glucose, fructose, sucrose and maltose we used Ion Chromatography with Electrochemical Detector with reference silver (AgCl/Ag) electrode and working gold (Au) electrode. We used CarboPac PA10 (250x4mm) column and a guard CarboPak PA10 (4x50mm). Also we determinated carbon and nitrogen stable isotope ratios (δ13Choney, δ13Cprotein, δ15N) by EA-IRMS Elemental Analyzer (FlashEA 1112 HT) coupled with Isotope Ratio Mass Spectometry (Thermo Finnigan DELTA V Advantage). This method is based on the 13C/12C carbon isotope ratio analysis and is able to detect, with a limit of detection of 7%, the presence of exogenous sugars derived from plants using the C4-photosynthetic cycle. Commercial samples of different types of honey: accacia, meadow, lime, floral and forest are collected and analyzed during past 5 years. In this period, we collected 24 different samples of honey, among which 6 of them were acacia type, 7 were meadow, 5 were lime, 5 were floral and 1 was forest origin. In these samples we analyzed sugar content - glucose, fructose, sucrose and the presence of C4 sugar. According to Serbian Regulation for honey, presence of sucrose can not be above 5g/100g for all type of honeys, except acacia honey, where is allowed content of 10g/100g and the sum of glucose and fructose must be above 60g/100g. During these analysis, 5 samples (20,83%) had increased sucrose content, of which 2 were meadow origin, 1 was lime, 1 was acacia and 1 was forest. According to results 4 samples (16,67%) had lower content glucose and fructose in the sum, of which 2 were meadow, 1 was lime and 1 was acacia type. When it comes to increased content of C4 sugar, 9 samples (33,33%) were above of 7%, among which 3 were meadow, 3 were floral, 2 were acacia and 1 was lime origin. Obtained results were processed by chemometric tools. According to results, we can notice that Serbian honey have appropriate quality and that generally is in accordance with Serbian Regulation. In the future, monitoring for the honey should be continued.
Keywords: non targeted analysis, Direct Analysis in Real Time, High Resolution Mass Spectrometry, food authenticity.

Authentication of food is a burning topic and the development of rapid and reliable analytical strategies to authenticate the origin of food has become a priority at global level to combat food frauds. Food authentication is typically attained by applying the classical targeted approach where a certain analyte defined by characteristic parameters is further confirmed by reference standards. The non targeted approach offering the advantage of rapidity of the analysis without requiring any knowledge about the composition of the food sample to be analysed, has emerged in the last years gaining increasing attention and has been taken up by European research projects such as Foodintegrity as objective of the WP18. Such approach allows to capture the highest number of information also referred to as features that are strictly correlated to the whole food matrix analysed. The comparison of food fingerprints to an authentic sample set will enable through the use of multivariate statistical models, detection of food sample adulteration or misdescription. However the bottleneck of such approach relies on the extensive data treatment required before submitting the pre-processed matrix to statistical analysis. In this work we present the optimization of a workflow based on the coupling between a Direct Analysis in Real Time (DART) ambient pressure source and a single cell Orbitrap™ based mass spectrometer applied to the non targeted analysis of foods to track the geographical origin and/or Foodintegrity/authenticity.

A typical workflow describing the main steps of a DART-HRMS analysis such as optimization of instrumental settings, sample preparation and post-acquisition data treatment to make the final data suitable for further statistical evaluation, will be presented along with the most critical steps. The pre-processing should include noise filtering, background subtraction, mass shift correction, mass alignment, spectra normalization, etc. Finally, an averaged spectrum representative of a certain food is typically generated from the analytical platform in use, that can be further converted into a format compatible for the subsequent statistical analysis. The duly optimization of all pre-processing steps on the acquired MS data is fundamental to produce reliable data prone to be submitted to multivariate statistic to generate trustworthy results. A case study reporting a typical DART-HRMS based workflow herein optimized will be shown applied to different food samples for food authenticity studies.

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Fish represents an important source of nutrients with many beneficial effects on human health. The high nutritional value is especially due to the richness in essential amino acids, highly digestible proteins, vitamins (A, D, and B), minerals and the high content of polyunsaturated fatty acids (PUFA). This latter represents the primary source of omega-3 (n-3) fatty acids, such as docosahexaenoic acid (DHA; C22:6 n-3) and eicosapentaenoic acid (EPA; C20:5 n-3), whose importance in prevention of certain human diseases is widely documented in literature. However, factors such as the living conditions of the salmons or the non appropriate storage conditions applied during food dispatch to the local retailers might affect considerably fish quality, also compromising the availability of some beneficial compounds. Freshness is one of the most principal attribute to control because fish represents a perishable and vulnerable food item. It is well-known that the internal and intrinsic characteristics such as the fragile muscle tissue and the activity of endogenous protease or inappropriate handling methods and storage conditions can be prone to resulting in physical, chemical, biochemical and microbial changes and contaminations of fish, thus affecting its freshness quality. In addition factors such as the living conditions of salmons whether farmed or caught or the non appropriate storage conditions might impair fish quality with significant changes in the lipid profile. In the last years, different analytical methods have been applied to assess fish quality by using conventional hyphenated techniques. In this study, we report the optimization of a rapid method based on the ambient ionization source DART (Direct Analysis in Real Time) coupled to an OrbitrapTM based High Resolution mass spectrometer applied to study the change of the lipid profile of salmon during storage. DART source is considered very efficient for soft ionization of both polar and apolar compounds without requiring any chromatographic separation prior to sample ionization and thanks to the easy in execution, rapidity of analysis and minimal sample preparation required this approach is gaining increasing attention especially in the assessment of food authenticity. The DART-HRMS method developed has been applied to the analysis of Salmo salar samples stored under different conditions and results show significant changes highlighted along the lipid profile, with special regard to the levels of fatty acid and triacylglycerols. Thanks to the high mass resolving power offered by the instrument in use, providing a mass accuracy better than 5 ppm, the composition of some lipids could be also inferred by entering the elemental composition predicted by the XCalibur™ software into the Lipid Maps database.

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STABLE ISOTOPE RATIOS OF H, C, O, N AND S FOR THE GEOGRAPHICAL TRACEABILITY OF ITALIAN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Keywords: Isotope Ratio Mass Spectrometry, rainbow trout (Oncorhynchus mykiss), diet, traceability.

Multielement isotope ratio was assessed in fat and defatted (protein) fillet of 130 rainbow trout, reared with feed incorporating a high or low fish content in 20 Italian farms, focusing on two northern Italian regions (Friuli Venezia Giulia and Trentino) . The aim was to investigate the ability of isotopic analysis to trace the geographical origin of trout, also according to the type of feed. The carbon, nitrogen and sulphur isotope ratios of feed and fillet were highly positively correlated both within each matrix (feed or fillet) and between the two matrices and negatively correlated with the δ2H and δ18O of feed and the δ2Hfat. The δ2Hprotein and δ18Oprotein were positively interrelated with the δ18O of tank water. By applying the tested Partial Least Squares – Discriminant Analysis multiclass model (85 fillets) to the validation dataset (45 fillets), 91% accuracy was obtained for the two Italian regions.

Keywords: allergens, β-lactoglobulin, western blot, milk.

Milk allergy is the most common and known food allergy, and β-lactoglobulin is the main allergen, especially in children (1,2).

β-lactoglobulin is a globular whey protein composed of 120 amino acids with a molecular weight of 18 and 36 kDa in the monomeric and dimeric form, respectively. Currently the detection of β-lactoglobulin in food is performed by screening methods such as ELISA, that search a protein fraction, directly or indirectly, or through the detection of the DNA encoding the allergenic protein (PCR). Most of the ELISA assays are able to detect the presence of allergens at very low levels, but they can lack some specificity, leading sometimes to the identification of false positive samples. At present, there are no methods to confirm positive samples.

In an our previous study, we developed a Western Blot (WB) protocol for the detection of β-lactoglobulin on fresh milk samples belonging to different species (cow, sheep, goat, donkey), with a sensitivity of 1 ng of total loaded proteins.

The objective of this study was to assess the validity of the WB protocol for the β-lactoglobulin detection in foods, i.e. biscuits, subjected to technological processes such as cooking.

Extraction was carried out on four different commercial biscuits containing variable amount of milk. Briefly, two grams of minced biscuits were incubated overnight with high-salt buffer with sodium dodecyl sulphate and β-mercaptoethanol (HSB/SDS/β-ME) and centrifuged at 20000 rcf for 30 minutes at 4 °C. Supernatant was filtered and protein content was quantified by Qubit method. 10 µg of total proteins was equilibrated in a buffer (10 mM Tris-HCl, 5% SDS, pH 7.6, glycerol and bromophenol blue) and subjected to SDS-PAGE (12% acrylamide pre-cast gels). Proteins were transferred onto PVDF membrane and immunodetection was carried out with a rabbit anti-bovine β-lactoglobulin antibody and an alkaline phosphatase–conjugated goat anti-rabbit immunoglobulin. Blot images were captured using a CCD camera.

Furthermore, the same protocol was applied to “Vegan” biscuit, as negative control and a β-lactoglobulin standard (Sigma, 10 ng/well) was used as a positive control.

Western Blot analysis of different biscuits showed intense signals corresponding to the monomeric and dimeric β-lactoglobulin form, with molecular weight slightly higher than 18 and 36 kDa, respectively. No signal was detected in the “Vegan” biscuit sample.
The application of the protocol described above has shown promising results in β-lactoglobulin detection in heat-treated foods. Among the different extractive methods initially tested, HSB/SDS/β-ME buffer has given the most effective results, showing a high extractive power without affecting the antigenic properties of β-lactoglobulin, which is the critical point in this analysis. Furthermore, the use of a polyclonal antibody seems most suitable for heat-treated foods, while a monoclonal antibody may be negatively influenced by epitope modifications (3). Also in terms of sensitivity, the method is promising and presumably able to detect very low amount of β-lactoglobulin, at least comparable with the ELISA detection limits. Further analysis, currently ongoing, will allow to verify these preliminary results and the related sensitivity, and investigations on other foods (baby food) will be conducted.

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Acknowledgements: This research was funded by the Italian Ministry of Health [grant number IZS PLV 01/14RC].
The contamination of food and feed with harmful fungal derived mycotoxins is a problem worldwide. Over 400 different potential mycotoxins are now known, some of them have been studied in great detail, while the data about toxicity and occurrence for many others are scarce. The fast elimination of contaminated food and feed from the supply chain is crucial for the protection of human and animal health as many of these toxins can cause serious diseases both after acute and chronic exposure. Fast and easy detection methods are needed for this purpose. One of the aims of MycoTest project was to deliver new commercial antibody-based (ELISA) screening methods for the detection of three important mycotoxins: T-2/HT-2 toxins, ochratoxin A and sterigmatocystin.

T-2/HT-2 toxins belong to the trichothecenes group of mycotoxins produced by Fusarium ssp. that can contaminate a wide range of agricultural crops. Their toxic effects include inhibition of protein synthesis and alteration of cell membrane functions. There are currently indicative levels in food (between 15 and 1000 µg/kg) for these toxins in the EU and the repetitive findings above these levels trigger investigations into the reasons for contamination. In MycoTest project a new ELISA for T-2/HT-2 was developed based on a monoclonal antibody with good cross-reaction with both T-2 and HT-2 toxins. The ELISA was validated according to Commission Regulation 519/2014 for the detection of these mycotoxins at a level of 12.5 ppb and above in cereals and 7.5 ppb in baby food. The assays is currently offered to the customer worldwide.

Ochratoxin A is produced by fungi from Aspergillus and Penicillium and it is a potent renal toxin, immunotoxic, neurotoxic and teratogenic. The maximum levels for ochratoxin A in cereals, dried vine fruit, coffee, wine, grape juice and baby food have been set to 0.5-10 µg/kg in the EU by Commission Regulation (EC) 1881/2006. In MycoTest project a new ELISA for the detection of ochratoxin A was developed based on a monoclonal antibody with good cross-reaction with both T-2 and HT-2 toxins. The ELISA was validated according to the Commission Regulation 519/2014 for the detection of these mycotoxins at a level of 12.5 ppb and above in cereals and 7.5 ppb in baby food. The assays is currently offered to the customer worldwide.

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Aflatoxins (B1, B2, G1, G2 and M1) are a group of the most potent carcinogens found in nature and sterigmatocystin is one of the precursors in aflatoxin biosynthesis. Also sterigmatocystin has been shown to be genotoxic and carcinogenic. The regulation in the EU requires many types of foods such as cereals, nuts and milk to be tested for aflatoxins, but not for sterigmatocystin. Data about sterigmatocystin occurrence are limited. In MycoTest project a new monoclonal antibody specific to sterigmatocystin was produced and then used to develop an assay for a sensitive detection of sterigmatocystin in cereals. The final validated sterigmatocystin ELISA will be the first commercial test for the detection of this harmful toxin.

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UTILIZATION OF HIGH RESOLUTION TANDEM MASS SPECTROMETRY FOR DETERMINATION OF 59 ANALOGUES OF PDE5 INHIBITORS AS POSSIBLE ADULTERANTS OF BOTANICAL-BASED DIETARY SUPPLEMENTS

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Keywords: phosphodiesterase type 5 inhibitors; erectile dysfunction; dietary supplements; food fraud; U-HPLC-HRMS/MS.

The popularity of natural preparations supporting the sexual potency have significantly increased during the recent years, what also has led to an increase of illegal use of various phosphodiesterase type 5 inhibitors (PDE-5) in these herbal-based sexual performance enhancement products. These fraudulent practices can be very dangerous for consumers, due to the fact that a presence of non-declared biologically active may result in interferences with other medicaments used by users. The main contraindications are with antihypertensives, antidepressives, sedatives, anxiolytics, and can lead to a stroke and/or heart attack. Although erectile function supporting dietary supplements are frequently consumed, there is still lack of proper analytical methods usable for its sensitive chemical analysis. In this study, rapid ultra-high pressure liquid chromatography coupled with high resolution tandem mass spectrometry (U-HPLC-HRMS/MS) method has been developed for simultaneous determination of 59 phosphodiesterase type 5 inhibitors, which can be used for the treatment of erectile dysfunction.

Within the development of a new sensitive method for analysis of 59 PDE-5 inhibitors, both sample preparation procedure and separation/detection conditions were optimized. Extraction efficiency of particular extraction solvents, the influence of different mobile phase additives on target analytes separation, as well as the impact of various settings of mass analyzer on sensitivity of detection were examined. The data were collected in a full MS/data dependent MS/MS acquisition mode (full MS-dd-MS/MS). Before the U-HPLC-HRMS/MS method was used for analysis of real life samples, a comprehensive validation was performed. Repeatability with overall relative standard deviation (RSD) was <8 % (N=6), measured at 1.0 and 5.0 µg/g, limits of quantification were in the range 0.25 -- 0.05 µg/g and the recovery was 71-90 %. U-HPLC-HRMS/MS method for PDE5 inhibitors was successfully applied to 64 real samples, which were continuously collected in cooperation with the Czech Agriculture and Food Inspection Authority (CAFIA) during 2009 and 2015 as a part of official control activities. As can be
seen on Figure 1, the ultra-high resolution of orbitrap mass analyzer (70 000 FWHM at m/z 200) enabled measurement of ions with very high mass accuracy, when the mass detecting error usually does not exceed 2 ppm and confirmation of fragment ions acquired during the parallel MS/MS experiment. The assessment of a compliance of fragmentation pattern of suspicious analyte with those in the ‘in-house created’ MS/MS library (i.e. MS/MS mass spectra of PDE-5 inhibitor standards acquired within the method development) could be carried out. From the 64 samples analyzed, 10 samples were found to be positive for at least 4 up to 9 analytes. Altogether, 20 different analytes were identified in tested samples. Sildenafil, imidasosagatrizinone, sildenafil-N-oxide, and propoxyphenylsildenafil represented the most frequent PDE-5 inhibitors.

*Figure 1: Example of sildenafil identification in real sample by developed U-HPLC-HRMS/MS method*

In addition to that, the acquired U-HPLC-HRMS/MS fingerprints were demonstrated to serve as a powerful tool for revealing of other types of possible frauds in products labeling. Retrospective mining of markers of herbs declared on dietary supplements packaging allowed to assess the trueness of declaration (i.e. medical herbs composition), what is very useful for efficient fight with food fraud.

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MULTIDISCIPLINARY APPROACH FOR AUTHENTICATION OF CHICKEN MEAT AS FRESH OR FROZEN-THAWED

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Keywords: chicken, fresh, frozen/thawed.

According to EC Regulation 1234/2007 (1) poultry can be marketed either as fresh or frozen, but previously frozen meat can not be sold as “fresh”. There is a potential for fraud and a suitable analytical method, capable of distinguishing between fresh and frozen meat, is required for Official Control. In literature there are numbers of reports on studies for the discrimination of fresh vs. thawed meat. Nevertheless none of the analytical methods proposed can be considered adequate in terms of analytical performances. The most widely used enzymatic method is the HADH assay, that has recently been subjected to a European inter-laboratory study organized by LGC Laboratory of the Government Chemist (2). This method relies on measuring the β-hydroxyacyl-CoA-dehydrogenase (HADH) activity of intracellular juice obtained from prepared test samples. The ratio of the HADH activity of sub-samples tested before and after laboratory freezing is compared to a reference cut-off limit to determine whether the sample has previously been frozen.

In the present study the applicability of histology and determination of HADH activity as tools for detection of frozen poultry meat was evaluated.

Chicken carcasses were sampled at the slaughterhouse. For histology, 36 fresh breast samples and 36 frozen at -18°C were obtained; also 20 thigh samples (10 fresh and 10 frozen at -18°C) were prepared. Frozen samples were then thawed at 4°C. All samples were formalin fixed and routinely processed. Paraffin embedded blocks were cut on a microtome into 5±2 µm slides, stained with haematoxylin and eosin (HE) and examined by light microscopy, independently by two expert histopathologists. Samples were classified as frozen when optically empty vacuoles of various dimensions, caused by ice crystals, were observed in the cytoplasm of muscle cells; otherwise were classified as fresh.

The HADH study was carried out on 40 breasts samples out of the 76 tested with histology: 20 fresh and 20 samples frozen. For each sample the test was conducted on two sub-samples: X0 was analyzed “fresh”, X1 after a freezing treatment at -18°C. All samples were pressed and the juice, following dilution and filtration, according to the test procedure, was mixed with phosphate buffer, EDTA solution, NADH solution and acetoacetylCoA solution. The HADH activity was determined using a spectrophotometric procedure: the reaction was subjected to a continuative monitoring, measuring absorbance values in intervals of 3 minutes. The absorbance was read at 340 nm. The decrease in the absorbance registered in the interval time was used in order to calculate the activity of the exudate of X0 e X1: in absence of decrease the sample is considered fresh, whereas in case of decrease the sample is interpreted as frozen. A value of R1 = X0/X1 was calculated. Values of R< 0.5 were considered indicative of freshness, R1 ≥ 0.5 of frozen-thawed samples.
Histology and HADH tests require respectively 3 and 5 days. Sensitivity and specificity of the methods were calculated; Cohen's K was calculated as measure of inter-rater agreement for the histological method. The cut-off value of HADH test for a correct differentiation between fresh and frozen samples was calculated.

Histology resulted in 97.8% sensitivity (CI SE 95%: 88.7-99.6%) and 100% specificity (CI SP 95%: 92.3-100%); the combined Cohen's kappa was 1 (CI 95%: 0.9-1), showing optimal inter-rater agreement. For HADH test, the calculated cut-off value of 0.55 scarcely differs from the value obtained from the European inter-laboratory study. The HADH method proved to be robust and resulted in 100% sensitivity (CI 95%: 83-100%) and 100% specificity (CI 95%: 83-100%). We have validated and accredited the HADH assay and accreditation for the histological method is in progress.

Our results show the suitability of both tests for the authentication of chicken as fresh or frozen. Fast and reliable methods that would differentiate between fresh and frozen/thawed meat are being sought because customers are sometimes deceived, when buying frozen and thawed meat as fresh meat. Specifically, a simple and cost-effective control strategy that is capable of distinguishing between fresh and previously frozen chicken meat is required. The histological method could represent a screening tool in the framework of a monitoring plan, because of its rapid execution and cost effectiveness. In addition, it can be applied to thigh samples, Otherwise the HADH assay can be adopted as confirmatory test, having maximal values of sensitivity and specificity, but it could be applied only on a limited number of samples, considering the time required, its costs and the problematic availability of certified reference materials. Based on all the above considerations, an effective control strategy, made of histological test as a screening test and HADH assay as a confirmatory test, would be suitable to be implemented at a national level and then, hopefully, at a European level, in order to monitor food frauds related to storage conditions of poultry meat.

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WEBAPP “FOOD FRAUDS”: A NEW TOOL OF KNOWLEDGE SHARING IN THE FRAME OF THE SCIENTIFIC COMMUNITY

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Keywords: food frauds, webapp, microscopy.

Food fraud is a serious problem worldwide notwithstanding there is not yet a definition of food fraud in the EU legislation. Nowadays fraud can be defined as “a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients or food packaging; or false or misleading statements made about a product, for economic gain” (Spink & Moyer, 2011). All consumers are at risk of fraud due to the increasingly globalized food supply chains. In this scenario, research institutes involved in public health are called to fill the gaps in lab analysis to detect frauds.

Among them, the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta (IZSPLVA), an health public body located in Italy, whose tasks are to guarantee animals and consumer’s health, is involved in an European project and in many Italian projects aiming to harmonise frauds controls. In particular the Histopathology Laboratory has been working for years to fight food frauds, standardizing and validating histological and immunohistochemical methods applied to meat and fish with the aim of detecting sanitary and commercial food frauds. The histological test for differentiation of fresh and frozen/thawed fish and an HADH method to distinguish fresh from frozen-thawed chicken meat have been validated and accredited. The immunohistochemical detection of undeclared soy proteins and caseins in meat preparations and products, an histological method to distinguish between swine and bovine fresh and frozen/thawed meat and a standardized microscopical protocol to differentiate between mechanically separated meat (MSM) and non-MSM and between high and low-pressure-MSM are in development. Furthermore, the Laboratory is also Reference Centre for Biological Methods for the Detection of Anabolic Substances in Animals (CIBA). CIBA has been established in 2013 in order to test and validate biological methods to highlight illegal anabolic practices.

In the framework of a research project funded by the Italian Ministry of Health (IZSPLV 17/12 RC), the lab has designed and developed, with the collaboration of the Informatics Office of IZSPLVA, a web application called “Food fraud” in order to collect and share microscopic representative images. The webapp has been designed in order to represent a valid support for everyone who carries out analysis and controls on foods of animal origin, constantly updatable to follow the evolution of food frauds and of the analytical methods developed to detect them.
The webapp takes into account both frauds with possible impact on consumers’ health (e.g. the selling of fish intended for raw consumption, not subjected to freezing treatment in order to kill viable parasites other than trematodes) and commercial frauds (e.g. the selling of frozen-thawed meat as fresh). It is important to note that in no case it is possible to automatically exclude possible negative effects on health even for commercial frauds. For this reason, particular attention must be focused on all food frauds.

The web application has been implemented using the “Responsive Design Pattern”, therefore it can be used by desktop, tablet and mobile devices and even if at present it is in Italian in the future it will offer the language choice also for English. The consultation of the webapp is available for free for everyone but only the administrators as well as users authorised by administrators can upload images and insert “new frauds”.

Each fraud issue is reported with animal species, type of food and type of fraud and for each one uploaded pictures are labelled with a description which include magnification, staining (e.g. haematoxylin-eosin, immunohistochemistry) and status of the analytical methods (applied for research - validated - validated and accredited). Users can enlarge pictures by clicking on them.

Analytical detection methods are an important first line of defence for both detecting and deterring food frauds. The final goal is developing and validating microscopy-based methods in order to give an efficient support to the official control in revealing sanitary and/or commercial frauds and other issues with possible negative implications for consumers’ health. In addition, the sharing of scientific knowledge is fundamental to address efforts towards optimization of resources in terms of development and validation of new methods.

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GAMING SIMULATION AS RAPID METHOD FOR RISK BASED FOODINTEGRITY KNOWLEDGE ENGINEERING AND EVIDENCE BASED DECISION MAKING – A CASE STUDY

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Keywords: Foodintegrity, Gaming Simulation, Knowledge Engineering, Risk-based auditing.

Introduction
Multi-layered national and international requirements in form of laws, standards, guidelines, and Good Practices on all levels shape the world-wide food sector as we know it today. Auditing of Foodintegrity management systems is the focal point to guarantee a baseline of safety- and quality promises and to ensure developments and trends. The basis of effective and efficient auditing – risk-based auditing - is to know the relations, patterns, principles, and dynamics of the interplay between food, people, and technology. Current approaches and systems in the food sector seems to be not capable of taking into account the increasing variety of factors and their various interdependencies, which are required for evidence based decision-making in risk-based auditing.

Objectives
In case studies with Flo-Cert (certification body of Fairtrade), Gaming Simulation is used as a research instrument to analyse and validate the dynamic cause and effect relationships of two complex food systems. Stakeholders of the food chains in question simulate their reality, triggered, and facilitated by a game design. Key risk factors and their interactions will be determined for two specific food chains and a continuous improvement cycle for these indicators will be established. Based on these validated insights, evidence based decisions for risk-based auditing shall be enabled. Gaming simulation will be compared to current approaches mentioned in the ISO 31010.

Results, Discussion and Conclusion
Gaming Simulation is used to solve complex problems in other sectors including finance, energy, and environment. The ability of gaming simulation to integrate cognition, emotion, and action, simulate socio-technical interactions, to show current rules and interdependencies and gathering empirical data seems ideal to analyse food systems. A standardised process of knowledge engineering shall be suggested for further research and testing for comprehensible, evidence based decision making for risk-based auditing.

Acknowledgements: The Authors thank INTACT Consult GmbH in Lebring, Austria for enabling this research and IFS and Flo-Cert for their willing to participate.
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CASE STUDY FOR THE ASSESSMENT OF NEAR INFRARED HYPERSPECTRAL IMAGING TO DETERMINE FRAUDULENT ADULTERATION OF DURUM WHEAT

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Keywords: wheat, fraud detection, morphology, NIR hyperspectral imaging.

Italian industrial pasta must be prepared using exclusively durum wheat - DW (Triticum durum). The use of common wheat - CW (Triticum aestivum), should be considered as fraud. According to current Italian rules, only a maximum of 3 % CW is allowed to account for cross-contamination that may occur during the agricultural process. However, mixtures of both wheats can be found due to delivery problems or to reduce prices. For this reason, efficient methods for the detection of accidental or intentional contamination of CW to DW are therefore required. The current work aims to develop a fast method for the at-line and on-line detection of CW grains in a lot of DW grains by NIR hyperspectral imaging.

77 samples of DW and 180 samples of CW were collected in Belgium and Italy in 2015 and 2016. The aim was to cover the quality variability of DW at the reception of the Barilla Company as well as the large variability in terms of varieties for CW. All the samples were measured using a line-scan NIR hyperspectral imaging system in order to get individual images of 16 grains by sample. The Chemometric tool Partial Least Squares Discriminant Analysis (PLS-DA) was used as classification method. To discriminate DW from CW, two approaches were studied. The first based on NIR spectral profile and the second on 8 morphological criteria. Models were developed with samples collected in 2015 and were validated with samples collected in 2016. The models were applied to all the individual pixels in all the images of 16 grains. The results are presented at the grain level based on the individual approaches or by combining both approaches.

For the time being all the studies dealing with the detection of CW in DW used macroscopic, microscopic or molecular biology based methods performed at laboratory. The study shows the potential of NIR hyperspectral imaging combined with chemometrics to propose solutions for sorting grains and for detection of adulteration at the entrance of the production chain.

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STABILITY STUDY OF EDIBLE OILS BY MESH CELL FTIR UNDER MODERATE CONDITIONS OF LIGHT AND TEMPERATURE

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Keywords: FTIR spectroscopy, Mesh cell, moderate conditions, Oil stability index.

Mesh cell is a rapid tool designed to monitor chemical changes that occur as a consequence of oxidation at moderate conditions (García-González and Van de Voort, 2009; Tena et al, 2017). In this study the mesh cell accessory has been proposed for assessing edible oil stability under different temperatures (at 23, 35, 65°C) simulating the real conditions during storage and transport (<60°C) and under different light intensities (400, 1000, 7000 lux) to evaluate the resistance of the samples to photooxidation (Tena et al, 2017). Samples of edible oils, with a particular attention to extra virgin olive oils from different cultivars, have been stored in mesh cells (Figure 1). The oil stability of the samples determined by using this accessory has been compared with the oil stability determined with the common methods typically applied for this purpose (e.g. Rancimat) (Morales and Przybylski, 2013). Despite the moderate conditions applied, mesh cell-FTIR spectra have revealed the formation of hydroperoxides and the subsequent formation of alcohols and aldehydes (Tena et al, 2013). The combination of different pro-oxidant factors has allowed determining the relative influence of each factor in the degradation of the oil during its shelf-life depending on the composition of the sample.

Unlike other methods that require high temperature to accelerate the oxidation rate, mesh cell-FTIR has allowed differentiating the oil stability of the samples from a multi-factor perspective that includes several properties (temperature and light) and chemical species (primary and secondary oxidation products). The information obtained with this method can be relevant for optimizing handling (e.g. packaging and storage temperature) of edible oil samples during their shelf life, which is one of the challenges today (Aparicio-Ruiz et al, 2014).
Figure 1. Scheme for the analytical procedure followed to load the mesh cell with the oil samples and to acquire the FTIR spectra.

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DEVELOPMENT OF A COMPREHENSIVE ANALYTICAL PLATFORM FOR THE DETECTION AND QUANTITATION OF FOOD FRAUD USING A BIOMARKER APPROACH. THE OREGANO ADULTERATION CASE STUDY

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Keywords: food, fraud, oregano, adulteration, mass spectrometry.

Due to increasing number of food fraud incidents, there is an inherent need for the development and implementation of analytical platforms enabling detection and quantitation of adulteration. In this study a set of unique biomarkers of commonly found adulterants became the targets in the development of a LC-MS/MS method which underwent a rigorous in-house validation. The method presented very high selectivity and specificity, excellent linearity ($R^2>0.988$) low decision limits and detection capabilities ($<2\%$), acceptable accuracy (intra-assay 92-113%, inter-assay 69-138%) and precision ($CV<20\%$). The method was compared with an established FTIR screening assay and revealed a good correlation of quali- and quantitative results ($R^2>0.81$). An assessment of 54 suspected adulterated oregano samples revealed that almost 90% of them contained at least one bulking agent, with a median level of adulteration of 50%. Such innovative methodologies need to be established as routine testing procedures to detect and ultimately deter food fraud.

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TARGET PEPTIDE IDENTIFICATION FOR THE MASS SPECTROMETRIC DETECTION OF SOY PROTEINS IN FOOD MATRICES

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Keywords: allergens, soy proteins, LC-MS/MS, peptides.

Introduction
Soybean is one of the “big eight” allergenic foods in the US, and allergic consumers rely on labeling to practice complete avoidance. However, unintentional cross-contact can result in the presence of undeclared soy. Allergen detection methods are important tools for industry and regulators, and mass spectrometry (MS) may offer advantages over ELISA such as higher accuracy and high throughput. The objective of the current study was to identify and evaluate target peptides for the parallel reaction monitoring (PRM) detection of soy protein in foods.

Methods
Six soy ingredients were extracted with 50 mM Tris-HCl, pH 8.6, with 6 M Urea, 20 mM DTT, and 1% PVPP, and then prepared for LC-MS/MS by in solution tryptic digestion and C18 spin column cleanup. Samples were separated by liquid chromatography and analyzed in top 10 discovery MS/MS mode using a Q Exactive™ Orbitrap™ mass spectrometer. Label-free quantification was utilized to compare the peptide abundance in six commercial soy ingredients. Peptides that satisfied a set of predefined criteria were selected as preliminary target peptides. Performance of the selected peptides and instrument sensitivity were evaluated in serial soy flour digest dilutions (480 - 0.0048 ng on-column peptide content), other soy ingredients, and food matrices using a PRM method.

Preliminary data
From the discovery LC-MS/MS analysis, among 541 high confidence peptides, 316 peptides were from currently annotated proteins available in the soy protein sequence database, the majority of which are from beta-conglycinin, glycinin and lipoxygenase. Fifty-seven species-specific and high confidence peptides with relatively consistent abundance in all soy ingredients, from 14 soy seed storage proteins, were identified as potential target peptides. Abundances of these peptides varied among soy ingredients: one of the soy protein isolates showed relatively higher peptide abundances than the other ingredients, whereas a hydrolyzed protein isolate had generally lower peptide abundances. Further target peptide selection was made based on their performance in serial dilutions of soy flour digests and in other soy ingredients. The most robust peptides could be detected with 4.8 ng digested soy protein loaded on-column. Only peptides with high and consistent detectability in all soy ingredients were kept for food matrix tests. Consequently, 20 species-specific, high confidence, consistently detectable peptides, with 20 precursors, were selected as potential target peptides from six commercial soy ingredients for further targeted MS analysis.

Novel aspect
This study is the first to develop a PRM method on various soy ingredients, which may offer wide applicability.
ASSESSMENT OF CARROTS PRODUCTION SYSTEM USING BIOLOGICALLY ACTIVE COMPOUNDS AND METABOLOMIC FINGERPRINTS

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Keywords: carrot; organic, integrated and conventional production system; UHPLC-QTOFMS; fingerprinting/profiling.

Carrot (Daucus carota subsp. sativus) is a member of the Umbeliferae family. Carrot contains high quantities of carotenoids, particularly beta-carotene, and is also a good source of dietary fiber, manganese, potassium, ascorbic acid and other vitamins.

The aim of this study was an investigation of the influence of farming conditions on the carrot quality. Two carrot varieties, Afalon and Cortina, were grown at locality Troja in the Czech Republic in two different planting densities (600 and 900 pieces per hectare), by organic, integrated, and conventional way of farming.

Biologically active compounds in carrots such as carotenoids and ascorbic acid were quantified using LC based techniques with DAD detection and expressed on dry matter content. The higher content of vitamin C was found in variety Cortina. The main carotenoid detected in samples was beta-carotene, but alpha-carotene was also present in significant concentrations. The ratio of alpha- and beta-carotene was constant (44:56). Higher carotenoid levels were detected in samples from conventional farming. The effect of mold infestation by Alternaria dauci was investigated as well, the induction of phytoalexine 6-methoxymelein was observed in organic samples.

Metabolomic fingerprinting/profiling was used to assess authenticity of carrots. Characterization and classification of carrot samples according to their production system, variety and plant density was carried out using ultra-high performance liquid chromatography coupled to a quadrupole-time-of-flight mass spectrometry (UHPLC–Q-TOF-MS). Metabolomic fingerprints/profiles were obtained in both positive and negative ionisation mode; for chromatographic separation reverse phase and/or HILIC analytical column were used. To create robust statistical models, the obtained data were processed by advanced chemometric tools. Examples of PCA and OPLS-DA plots are presented in Figures 1 (way of farming) and 2 (variety and planting density).

UHPLC-QTOFMS procedure followed by multivariate data analysis enabled differentiation between organic, integrated, and conventionally produced carrots. Developed statistical models can be also used to distinguish carrot varieties and planting density. Metabolomic data thus were successfully used to predict the production system of the analyzed samples. Additionally, several important marker compounds were tentatively identified.
Acknowledgement: This work was supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601 - No.: MSMT-43760/2015). This work was also supported by the Ministry of Agriculture of the Czech Republic (Project no. QJ1210165).

Figure 1: OPLS-DA model of 48 samples (methanolic extracts, reverse phase, positive ion mode). The samples are clustered according to the way of farming: Conventional, Integrated and Organic (BIO).

Figure 2: PCA model of 48 samples (methanolic extracts, reverse phase, positive ion mode). The samples are clustered according to the A) variety and B) planting density.
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