

Programme & Book of Abstracts



5th FOODINTEGRITY CONFERENCE

Nantes, France
14-15 November 2018

Assuring the integrity of the food chain:
Delivering real world solutions



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Delivering real world solutions

EDITORS

JEAN-FRANÇOIS MORIN – MONIKA TOMANIOVA
JAMES DONARSKI - PAUL BRERETON

ORGANISED BY



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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.*



ISBN 978-2-9566303-2-6

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PROGRAMME

Programme at a glance

Wednesday 14 November		Thursday 15 November	
		Satellite events Vendor Seminar ScieX Room KL Vendor Seminar Agilent Room J	
8:15			
9:00	Setting the Scene and Outlying the issues Beginning of conference - Plenary sessions Strategic look from the big players Auditorium 450		Forward Look Parallel sessions Targeted versus non-targeted: problems and solutions Auditorium 450 Will Blockchain really solve our food fraud problems? Room 200
11:00	«Hot» topics Auditorium 450		Plenary session Forward look: future research and innovation needs for the food sector Auditorium 450
11:00			
12:30	Lunch break		Lunch break
13:30	Satellite events Vendor Seminar Bruker Room J FI Demo Corner Foyer haut Poster session Mezzanine		
14:30	Solutions Parallel sessions The lab comes to the factory Room KL Transparency and trust in the food chain Room 200 Standardisation: new initiatives Room J		Visit to Eurofins laboratories (pre-booked only)
16:30	Complex foods: tools for food authenticity assessment Auditorium 450 Available IT resources for food authentication Auditorium 450 Organic food authentication Room KL Tools and needs for the future: gap analysis, interactive workshop Room J		
18:10	Plenary session Young science session Auditorium 450		Satellite event Vendor Seminar G.A.S. Room J
18:50	Conference dinner Machines de l'île and Nantilius (pre-booked only)		
Evening			
			18:00

Full Programme – 14 November

Setting the Scene and Outlying the Issues			
Beginning of conference - Plenary sessions			
8:00-9:00	Reception of participants		Mezzanine
9:00-9:30	Official Opening and Welcome		Auditorium
9:05	Welcome by Eurofins	Rodolphe Labal (Eurofins)	
9:10	Welcome by Nantes Métropole	Nantes Metrople's President or her representative	
9:15	Welcome by European Commission, DG RTD	Razvan Anistoroaei (DG RTD, European Commission)	
9:20	Delivering real world solutions: the role of the FoodIntegrity project	Paul Brereton (Queen's University of Belfast)	
9:35-10:30	Session 1: Strategic look from the big players		Auditorium
9:35	Food Fraud at the sharp end – Reliance on science?	Peter Whelan (FSAI)	
10:00	Role of the European Commission's Joint Research Centre in countering food fraud	Elke Anklam (JRC, European Commission)	
10:30-11:00	Coffee Break		Mezzanine
11:00-12:30	Session 2: «Hot Topics»		Auditorium
11:00	The strategy of the flavour industry on vanilla and vanilla extracts	Antoine Kastler (EFFA)	
11:20	Assuring food integrity in the spice and herb supply chain	Clare Menezes (McCormick)	
11:40	Non-tariff barriers in global agri-food trade - Implications for food security	Michael Haverty (The Andersons Centre)	
12:00	New breeding techniques: science, regulation and analytical challenges	Mathieu Rolland (ANSES)	
12:30-13:30	Lunch Break		R2
12:30-14:30	Satellite Events		
13:45-14:15	Food Integrity with New Analytical Technologies: unlocking the truth	Amanda Manolis (Thermo Fisher)	Room KL
13:45-14:15	NMR-based tools for food authenticity and quality control	Andrea Steck (Bruker)	Room J
12:30-14:30	FI Demo Corner		Foyer
12:30-14:30	Poster Session		Mezzanine
Solutions			
Parallel sessions			
14:30-16:00	Session 3 : Complex Foods: tools for food authenticity assessment		Auditorium
14:30	Food proteomics as a novel tool to ensure the integrity of food: from targeted to untargeted approaches	Jens Brockmeyer (University Stuttgart)	
14:50	Using chemical and DNA markers to authenticate manuka honey	Claire McDonald (Ministry of Primary Industries of New Zealand)	
15:10	Authenticity and quality control of food by automated 1H-NMR spectroscopy combined with multivariate statistics	Andrea Steck (Bruker BioSpin GmbH)	
15:30	Evaluating the integrity of complex foods by combined methods. Case study: bakery products containing chia, flax and sesame seeds	Daniel Wunderlin (CONICET)	
14:30-16:00	Session 4: The lab comes to the factory		Room KL
14:30	F.I.S.H.U.B - A mobile app to verify fish species through a picture	Pier Luigi Acutis (IZSPLVA)	
14:50	Portable authentication technologies - Their place in detecting spirit drinks fraud	Ian Goodall (The Scotch Whisky Research Institute)	
15:10	PhasmaFOOD: a new generation optical food sensing device and an open software architecture delivering food-tech Innovation	Yannick Weesepeol (RIKILT - Wageningen University)	
15:30	On-site analysis of individual pork carcasses for the predictions of quality parameters in Iberian ham	Ana Garrido Varo (Universidad de Cordoba)	
14:30-16:00	Session 5: Transparency and trust in the food chain		Room 200
14:30	Data sharing as a solution to identify food issues early	Niels Lucas Luijckx (TNO)	
14:50	Finding the needle in the haystack: preventing food fraud by comparing production volumes with trade volumes	Gerald A. Herrmann (Organic Services)	
15:10	A systematic review into European consumer perceptions of food and authenticity	Helen Kendall (School of Natural & Environmental Sciences)	
15:30	Tracing Mediterranean high value food products: the REALMed approach	Carla Alegria (FCiència.ID - AIDC)	
14:30-16:00	Session 6: Standardisation: new initiatives		Room J
14:30	A standardised definition of terms relating to food authenticity and food fraud	Peter Olsen (Nofima)	
14:45	Guidance document towards food authenticity, an initiative of ILSI Europe	Bert Pöpping (ILSI)	
15:00	How can food safety standards help improve food fraud mitigation?	Bruno Séchet (IFS)	
15:30	Food fraud and food authenticity: new definitions and a new approach	Joanne Carter (IFAAO - True Foodies Only)	
16:00	Discussion panel		

PROGRAMME

16:00-16:30	Coffee Break		
16:30-18:00	Session 7: Molecular biology approaches to food integrity		Room 200
16:30	The future of NGS (Next Generation Sequencing) analysis in testing food authenticity	Ed Haynes (FERA)	
16:45	NGS-based metabarcoding for the analysis of plant food products	Maria Logacheva (Skolkovo Institute of Science and Technology)	
17:00	A novel metabarcoding approach to complex foods: using the Oxford Nanopore's MinION to fight fraud	Sarah Helyar (Queens University Belfast)	
17:15	Miniaturized device for isothermal DNA amplification	Joana Carvalho (Intl. Iberian Nanotechnology Lab.)	
17:30	Authentication of shrimp products in the UK	Amanda Naam (Queen's University Belfast)	
16:30-18:00	Session 8: Available IT resources for food authentication		Auditorium
16:30	Using the Food Authenticity Knowledge Base: case studies for food operators and regulators	Alain Maquet (JRC, European Commission)	
16:40	The Food Authenticity Research Network (FARNHub) for sharing and accessing information on food authenticity activities	Philippe Vermeulen (CRA-W)	
16:50	The Food Authenticity Network: the one-stop-shop that can help protect the integrity of your food	Selvarani Elahi (LGC)	
17:00	Food Integrity "Industrial Integration Tools"	Michele Suman (Barilla SpA)	
17:10	Food fraud Early Warning System	James Donarski (FERA)	
17:20	Development of a food fraud media monitoring system	Hans Marvin (RIKILT Wageningen University)	
17:30	Food authenticity risk assessment : 30 years of experience enhanced through data mining	Eric Jamin (Eurofins)	
16:30-18:00	Session 9 : Organic food authentication		Room J
16:30	Is it organic? What do existing analytical techniques have to offer and how close are we to implementing them?	Simon Kelly (IAEA)	
16:45	Enhanced fraud prevention through combining supply chain and satellite information – reducing vulnerabilities in organic certification	Annette Sutter (Organic Services)	
17:00	Authentication of organic vegetables from real farms using stable isotope ratios: a legislative initiative from the Andalusian government. Indirect impact on new fertilizers legislation.	Jose Manuel Moreno-Rojas (IFAPA)	
17:15	Bio-Fraud-Scan: concept for a method to check authenticity of organic foods via pesticide metabolites	Mikko Hofsommer (GfI Berlin)	
17:30	Discussion panel		
16:30-18:00	Session 10: Tools and needs for the future: gap analysis: Interactive Workshop		Room KL
16:30-18:00	Open discussion moderated by Saskia Van Ruth (RIKILT, Wageningen University)		
18:10-18:40	Satellite Event		
	Testing of flavours inducing volatiles and off smells in food and beverages without sample pre-treatment	Thomas Wortelmann (G.A.S.)	Room J
18:10-18:50	Plenary Session: Young science: short talks on food integrity		Auditorium
18:10	Key success factors for an information sharing system to prevent and detect food integrity issues: insights from a stakeholder consultation	Fien Minnens (Ghent University)	
18:15	Towards an NMR-based monitoring of coffee origin? An industrial case-study	Raphaël Recht (Aerial)	
18:20	MRM-MS of marker peptides distribution as a tool for authentication of single-cut meat products	Ilona Klosowska-Chomiczewska (Gdansk Uni. of Technology)	
18:25	New rapid GC-MS method versus conventional pycnometry: what is a real alcoholic strength of these spirits and liqueurs?	Michal Stupak (UCT Prague)	
18:30	Is it organic? Compound-specific stable isotope ratio analysis for authenticity testing of organically grown vegetables	Vlastimil Novak (University of Copenhagen)	
18:35	Using ambient mass spectrometry and chemometrics to rapidly determine poultry production system authenticity	Nicholas James Birse (Queen's University Belfast)	
18:40	A novel multi-platform high resolution mass spectrometry non-targeted approach facing extra virgin olive oil adulteration	Daniele Cavanna (Barilla)	
19:30-23:00	Conference Dinner		Nantilus

Full Programme – 15 November

8:15-9:00		Satellite Events	
8:15-9:00	Targeted proteomics to tackle the difficult ones: authentication of closely related species and semi-untargeted MS approaches	Jens Brockmeyer (University Stuttgart)	Room KL
8:15-9:00	Metabolomic profiling of Manuka honey	Emmanuel Sauvard (Agilent Technologies)	Room J
Forward Look			
9:00-10:30 Parallel Sessions			
9:00-10:30		Session 12: Industry is the main victim of food fraud? A debate on a new paradigm	
9:00	On the panel: Paul Brereton (Queen's University of Belfast), Shefalee Loth (Which?) , Beate Kettlitz (FoodDrink Europe), Eric Marin (DG Santé)		Room KL
9:00-10:30		Session 13: Targeted versus non-targeted: problems and solution	
9:00	Challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis	Michele Suman (Barilla SpA)	Auditorium
9:15	To target or not to target? Definitions and nomenclature for targeted versus non-targeted analytical food authentication	Kristian Holst Laursen (University of Copenhagen)	
9:30	Investigations on the comparability of fingerprinting data – Learn to walk before you run	Carolin Lörchner (German Federal Institute for Risk Assessment)	
9:45	Development of unbiased and multi users classification tools based on non-targeted analysis: the crucial role of the statistical equivalence of the "scaled NMR spectra" in the validation process	Vito Gallo (Politecnico di Bari)	
10:00	A guidance for the analytical validation of untargeted methods: results, criticisms and perspectives from the food integrity project	Marco Arlorio (Università degli Studi del Piemonte Orientale)	
10:15	Discussion panel		
9:00-10:30		Session 14: Will Blockchain really solve our food fraud problems?	
9:00	Introduction by the chair	Niels Lucas Luijckx (TNO)	Room 200
9:10	Will the use of blockchain technology ensure food traceability and authenticity?	Petter Olsen (Nofima)	
9:30	Blockchain / DLT and product authenticity in the agri-food sector - Sustainable, artisanal and other specialist categories	Fiona Delaney (Origin Chain)	
9:50	Laboratory 4.0 and Blockchain: hypes, buzzwords - Confusion or added Value? - Views of an instrument supplier	Christoph Jansen (Mettler-Toledo GmbH Schweiz)	
10:10	Discussion panel and polls		
10:30-11:00 Coffee Break			
11:00-13:00 Plenary Session			
11:00-13:00		Session 15: Forward look: future research and innovation needs for the food section	
11:00	Food research in Horizon Europe	Razvan Anistoroaei (DG RTD, European Commission)	Auditorium
11:15	EIT strategic agenda for innovation	Mercedes Groba (EIT Food)	
11:30	Authent-Net joint strategic research agenda	Lucy Foster (DEFRA, Ministry of Agriculture, Authent-Net)	
11:45	Outcome of future priorities workshop / gap analysis	Saskia Van Ruth (RIKILT, Wageningen University)	
12:00	Discussion panel		
12:20	Summing up of FoodIntegrity	Michèle Lees (FI advisory board)	
12:35	Awards		
12:45	Closing remarks	Paul Brereton, Jean-François Morin (Food Integrity)	
End of Conference			



KEYNOTE
SPEAKERS



Elke Anklam

European Commission's Joint Research Centre

Director of the JRC-Geel site and Director of JRC Directorate F: Health, Consumers & Reference Material

Elke Anklam is a chemist, with specialisation in food-, organic- and radiation chemistry. After obtaining her PhD from the University of Hamburg (Germany), she worked in various European Research Institutions and was a Teaching Professor at the Applied University of Fulda (Germany).

Since 1991, she has been working at the European Commission's Joint Research Centre (EC-JRC). Since 2006, she has a Director position in the JRC. At present, she is the Director of the JRC-Geel site and Director of JRC Directorate F: Health, Consumers & Reference Material, located at the JRC-Geel and JRC-Ispra site.



Lucy Foster

DEFRA, Ministry of Agriculture, Authent-Net

Lucy Foster began her Government career as a government scientist at the Ministry of Agriculture, Fisheries and Food in 1998 working on food labelling and standards. She joined the Food Standards Agency in 2000. She moved to the Department of Food, Environment and Rural Affairs in 2009 where she is currently Head of Food Science and responsible for broad portfolio of strategic research, evidence and analysis underpinning agriculture and food policy.

Gained throughout her Civil Service career, she has considerable experience across a wide range of food issues from both a science and policy perspective including agriculture, environment, food technology, microbiological foodborne disease, food hygiene, nutrition, food additives and food compositional and labelling standards.

She is a food scientist by training and gained research experience at Unilever (Colworth) and the Institute of Food Research, Norwich. Her PhD specialised in food composition and analysis. She is also a fellow of the Institute of Food Science and Technology.



Mercedes Groba

European Institute of innovation & Technology (EIT), Food

Innovation Programme Manager

Mercedes Groba is an agricultural engineer, with specialisation in agri-food, and also a degree in nutrition. She has been working in R&D and innovation for more than 10 years both in public and private organizations where She has been working on large consortiums with partners across Europe including, Greece, Portugal, France, German, Ireland, Spain, Netherlands, United Kingdom, involving industries, universities, research centres, and other organisms. After managing an EU project at the University of Oxford she joined EIT Food last summer with the ambition to make real a food system smarter and sustainable, supporting innovative and economically sustainable initiatives which improve our health, access to quality food, and the environment.



Michael Haverty

The Andersons Centre, UK

Senior Agricultural Economist

Michael Haverty is a Senior Agricultural Economist with The Andersons Centre in the United Kingdom. He is originally from the Republic of Ireland and has worked on numerous trade and Brexit-related projects concerning agri-food in recent years. These include studies on the impact of a No-Deal Brexit on the Northern Ireland beef and sheep meat industry, assessments of non-tariff barriers across a range of agri-food sectors, and studies on agricultural and labour policy.

He regularly contributes to conferences, seminars and news articles on agri-food trade and Brexit. In the past, Michael has led numerous market analysis projects covering Europe, the Americas and Asia as well as co-authoring peer-reviewed journal articles concerning market orientation and competitive benchmarking in food and farming. He has worked in research roles with Newcastle University, IDA Ireland and served as a trainee (stagiaire) with the European Commission.



Eric Jamin

Eurofins Analytics France

Head of the Authenticity Competence Centre

Eric Jamin has a PhD from University of Nantes (France). He has been working for Eurofins for 20 years in the field of isotopic analyses. He undertook Research & Development activities to set up new analytical methods for food authenticity and quality testing.

As an authenticity analyses expert, he has taken part in several professional committees such as Qualijus Executive Committee & SGF-IRMA Authenticity Experts Group (Fruit Juices), OIV analytical commission (wines & spirits) and the International Honey Commission (IHC). He is currently head of the Authenticity Competence Centre of Eurofins laboratories, based in Nantes (France). He has a team of ca 40 staff members, with expert skills in isotope analyses of beverages and food products.



Antoine Kastler

European Flavour Association

President

Mr. Kastler has been in the flavours industry for over a decade. He brings to the role his company and association experience as Vice President of Flavours at Robertet, a flavour house based in France and as representative of the French National Association (SNIAA) at the European Flavour Association (EFFA) Board. He also played an instrumental role for EFFA as its vice-President for four years and became President in 2017. Under his leadership, the EFFA has improved its positioning with the FlavourDays events, the newly created Public Affairs Committee, and taken advantage of its strong know-how with products such as the EFFA Guidance Document. He studied at École Supérieure de Technologie Électronique (ESIEE group) near Paris.



Beate Kettlitz

FoodDrinkEurope

Director for Food Policy, Science and R&D

Beate Kettlitz is the Director for Food Policy, Science and R&D at FoodDrinkEurope, the umbrella organisation, representing the European food and drink industries. She has a background in food chemistry and is an expert on technical regulatory matters in the food chain and runs the secretariat for the European Technology Platform FoodforLife. With the involvement in the ETP FoodforLife academia, food industry and other stakeholders identify research needs, which ideally lead to innovation, leading to the enhancement of competitiveness. She was also food policy adviser at the European Consumers' Organisation (BEUC). Prior to that, she held positions in industry and in German local authorities. Before moving to industry, she reached the position of head of department at the Regional Hygiene Institute of Potsdam (DE).



Shefalee Loth

Which?, UK

Principal Food Researcher

Shefalee Loth is Principal Food Researcher for the consumer organisation Which? in the UK. She has worked for Which? for 9 years and is a qualified Public Health Nutritionist. As part of her role at Which? she has focused on the prevalence of food fraud and its impact on consumer trust of the food industry. She also works on nutrition and food safety topics more widely. Which? has been campaigning for consumers since 1957 and currently has more than 1.3 million members and supporters making it the largest independent consumer body in the UK.



Clare Menezes

McCormick, UK

Director Global Food Integrity for the Global Quality & Food Safety Center of Excellence

Clare Menezes is Director Global Food Integrity for the Global Quality & Food Safety Center of Excellence, in this role one of her key areas of focus is herbs and spice integrity and specifically supply chain vulnerability management, as part of the McCormick global authenticity programme. As part of her leadership accountability she is responsible for directing Global Centers of Analytical Science and consequently heads McCormick's activity investigating Emerging Technologies for the detection and verification of product integrity challenges.

She has worked in the herb and spice Industry for more than 20 years holding a number of technical roles including microbiology, food safety, quality assurance, supplier quality and regulatory responsibilities. She then joined McCormick in 1998 and was appointed to her current position of Director Global Integrity for the Global Quality & Food Safety Center of Excellence in 2018. Prior to joining McCormick, Ms. Menezes was a Public Analyst with Severn Trent Water and Company Microbiologist at Tate and Lyle Sugars (UK). Ms. Menezes has a BSc degree in Microbiology, biochemistry and physiology from the University of Reading (UK) and is a Registered Food Safety Principle (RFoodSP) and recognized Fellow of the Institute of Food Science and Technology.



Mathieu Rolland

ANSES Plant Health Laboratory

Head of GMO team

Mr. Rolland is the current Deputy Head of Unit "Coordination of Reference Activities" & Head of the GMO Detection Team at the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES). Previously, he held the position of Head of the GMO and Pathogens Detection Unit at the Laboratoire de Biologie Moléculaire et de Biochimie du Geves (BioGEVES). He was also a Post doctoral associate at the Department of Plant Pathology and Plant-Microbe Biology of Cornell University, Ithaca, USA.

His education background includes a PhD on "The development of new molecular tools for the detection, quantification and characterization of PVY isolates. Estimation of the relative fitness of PVY genomes according to their necrotic properties" at the Department of Plant Health and Environment of the French National Institute for Agricultural Research. He also has studies of Plant Virology and Plant Protection at Agrocampus Ouest and of Plant Science at the University of Angers



Michele Suman

Barilla SpA

Head of Food Chemistry & Safety Research
Department within the Food Research Labs

Michele Suman's educational background includes: Analytical Chemistry Degree, Summa Cum Laude (University of Ferrara, 1997); National Prize for Young Researchers (Italian Chemistry Federation, 1998); Master in Science-Technology & Management working also at the Natta Research Center (Shell-Montell Polyolefins, Ferrara, Italy); PhD in Materials Science (University of Parma, 2005).

He is Vice-Chair of the ILSI Process Related Compounds & Natural Toxins Task Force and member of: Board of the European Technology Platform (Italian Section) "Food for Life"; Board of the Italian Chemistry Society – Mass Spectrometry Division; Working Groups within European Committee for Standardization (CEN); Editorial Advisory Boards of Food Additives and Contaminants Journal and World Mycotoxin Journal, Scientific Committees of International Events (e.g. World Mycotoxin Forum (WMF), Rapid Methods Europe (RME), Recent Advances in Food Analysis (RAFA). His scientific production is documented by 5 book chapters, 115 contributions at national and international conferences and 70 papers in international ISI journals.

Since 2006 he is the head of Food Chemistry & Safety Research Department within the Food Research Labs of Barilla SpA (Parma, Italy) working on research projects about food safety-quality-authenticity, food contact materials, sensors, mass spectrometry.



Peter Whelan

Food Safety Authority of Ireland

Director of Audit and Investigations

Peter Whelan qualified as an Environment Health officer in 1982. He worked as an EHO, Senior EHO and Principal EHO in the Eastern Health Board and Dublin Local Authorities before taking up the post of Contracts Manager and then Director of Service Contracts in the Food Safety Authority of Ireland (FSAI). Peter then took a 5 year secondment to the post of Executive Chairman of the newly formed Sea Fisheries Protection Authority.

Peter is now Director of Audit and Investigations in the FSAI where he is responsible for the investigation of food fraud. Further studies include a MSc in Food Science, University College Dublin; MSc in Health Services Management, Trinity College Dublin; a BA (Hons) degree in Psychotherapy, Dublin Business School and currently a MSc in Organisational Work Psychology/Behaviour, Dublin City University.



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Lab Products & Services, with its premium laboratory instruments, consumables and services, concentrates on serving the needs of laboratories performing research and quality assurance at pharma and biopharma companies and on those of academic research institutes. Founded in 1870, the company earned sales revenue of more than 1.3 billion euros in 2016. More than 6,900 people work at the Group's 50 manufacturing and sales sites, serving customers around the globe. In our portfolio: lab weighing (precision -analytical – and micro-balances, moisture analysers, calibration weights, mass comparators), Filtration (syringe filters, filter paper, vacuum filters, tangential filtering, ultra-filtration, centrifuges),

Microbiology (sterility testing, microbiological water/fluid controls, filters & media, pumps, air monitoring), Water systems (ultra-pure water systems, Type 1,2 &3 water, reverse osmose, de-ionized, stocking water in bags) Liquid handling (mechanical and electronic pipettes, bottle top dispenser, tips, filters), cell culture analysis with high throughput and with integration in incubators for live checks, and as important, our service which help you in installing and qualifying your material (DaKKS – COFRAC – USPmin – IQ/OQ ...), perform maintenance and help and repair if needed.

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Merck is a leading science and technology company in healthcare, life science and performance materials. Around 50,000 employees work to develop technologies that improve and enhance life—from biopharmaceutical therapies to treat cancer or MS, systems for scientific research and production, to liquid crystals for smartphones and LCD televisions. The life science business' purpose is to solve the toughest problems in the industry by collaborating with the global scientific community. Merck's extensive portfolio of more than 300,000 products includes innovations in lab water, gene editing, protein & cell biology, separations, chemistry, biomonitoring, IVD and bioprocess solutions.

Founded in 1668, Merck is the world's oldest pharmaceutical and chemical company. The founding family remains the majority owner of the publicly listed corporate group. Merck holds the global rights to the „Merck“ name and brand, except in the U.S. and Canada, where the company operates as EMD Serono, MilliporeSigma and EMD Performance Materials.

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Eurofins is the world leading food and feed testing laboratory group, deploying a comprehensive range of state-of-the-art analytical techniques in order to support its clients' increasingly stringent quality and safety standards. The Group has built up a global network of food testing laboratories and Competence Centres that perform more

than 150 million assays per year to establish the safety, composition, authenticity, origin, traceability and purity of food. Since its creation 30 years ago Eurofins is recognized as an expert in food authenticity and integrity.

Our authenticity analyses are « tailor-made » for each product and include basic methods and specific tests, selected to check for likely adulteration practices. Eurofins' specific expertise is the use of isotopic techniques and in particular the Site Specific Natural Isotope Fractionation Studied by Nuclear Magnetic Resonance (SNIF-NMR®) method of authentication. This is one of the most powerful techniques for detecting the adulteration of natural products. Isotope Ratio Mass Spectrometry is also used as a complementary technique. Eurofins also carries out molecular biology techniques and a large number of classic physical, chemical and biological methods: liquid or gas chromatography, spectroscopy (atomic absorption, ultraviolet) and has a very wide range of analytical tools to control product authenticity. A recent breakthrough was introduced through NMR-profiling, a new holistic approach combining targeted and non-target analyses for a fast & complete authenticity screening of whole matrices.

Fraud is becoming more and more sophisticated and increasingly difficult to detect by basic analyses since it is often designed to get round the tests in use. It is therefore necessary to resort to advanced analytical techniques to detect the non-compliant products. A considerable investment in research and development enables Eurofins to apply the most suitable methods to each specific case when evaluating the authenticity of a product.

Visit us on our stand during the 5th FoodIntegrity Conference or on our website

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G.A.S. is an instrument manufacturing high-tech company founded in 1997 coupling gas chromatography to ion mobility (GC-IMS) to measure traces of volatile organic compounds (VOC) in air, human breath and the headspace of liquid and solids.

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Through the maintenance of a close collaboration with the industry, regulators and academia in the areas of safety, quality control and scientific innovation, New Food understands the necessity of cooperation and collaboration between all parties to ensure that the global food and beverage community moves forward together. To achieve our goal, New Food provides a bi-monthly print publication, a digital platform that includes regular webinars, as well as a variety of events throughout the year.

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VENDOR SEMINARS

Food Integrity with New Analytical Technologies: Unlocking the Truth

ThermoFisher
S C I E N T I F I C

Date: **November 14 2018, 13:45**

Speaker: **Amanda Manolis**

Thermo Fisher Scientific, Austin, USA

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 techniques 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 next generation sequencing and stable isotope fingerprints because these technologies can differentiate between food and beverage samples which otherwise share identical chemical or similar genetic composition. We briefly explore how these technologies really detect food and beverage fraud based on the unique problem you are trying to solve.

Isotope Fingerprints Interactive Room



Enter the Isotope fingerprints interactive room

Follow the Isotope Hunter on their journey to understand food and beverage samples stories and confirm their identities by investigating their isotope fingerprints. Are you ready to become an Isotope Hunter? Visit this interactive experience.

Where: in room M / Level 4

When: open the whole conference

¹H-NMR Spectroscopy, Food Screener, Food Authenticity, Food Quality



Date: **November, 14 2018, 13:45**

Speaker: **Andrea Steck**

Bruker BioSpin GmbH, Ettlingen, Germany

Cases of food adulteration hit the headlines worldwide on a regular basis. Mislabelling of content or origin, substitution of high value ingredients by low-cost imitates, and additions of fillers or even potentially harmful components are typical fraudulent practices. By now, a majority of them is elaborate in order to circumvent conventional (targeted) analytical methods, and/or veiled within complex and global supply chains.

Analytical tools, suitable for effective prevention and enforcement of food fraud beyond single spot tests, optimally combine non-targeted fingerprint analysis and targeted quantification in one run, and are rapid, cost-effective and reliable likewise.

With its high-resolution ¹H-NMR based food screening solutions, Bruker BioSpin GmbH provides unique solutions for authenticity and quality control developed in a joint effort with experts in the fruit juice, wine, and honey sector. The methodology uses comprehensive ¹H-NMR spectra databases of authentic samples and multivariate statistics to generate models e.g. for geographical origin, botanical variety and different type of adulterations. Simultaneously, absolute quantification of a multitude of parameters (e.g. sugars, amino acids, fermentation markers) relevant for quality and authenticity are performed.

We invite you to learn more how this sophisticated technology is transformed into an automated push-button solution, and how it is applied in real life of food screening.

Testing of Flavour Inducing Volatiles and Off-Smells in Food and Beverages without Sample Pre-Treatment



Date: **November, 14 2018, 18:10**

Speaker: **Thomas Wortelmann**

G.A.S. mbH, Dortmund, Germany

Respecting the dramatically increasing demand in food quality control same as customer demands in a straightforward workflow same as in rugged analytical equipment with a high throughput; this presentation highlights the unique and enhanced capability of combining gas chromatography with ion mobility spectrometry in static headspace as hyphenated analytical metrology. The applied physical working principles of both technologies assure maximum reliability and the second dimension of separation realized through the TOF-IMS allows full orthogonality so that co-eluting compounds and even isomers can be separated. The sensitivity of the detector lies in the low ppb-range for (off-) flavour inducing volatiles facilitating a direct sampling. By this sensory panels can be supported by a machine that works 24/7 providing impartial flavour documentation. This presentation gives an introduction into the technological set-up and insight into successful exemplary applications in the field of food & beverages quality control, authenticity and integrity.

Targeted Proteomics to Tackle the Difficult Ones: Authentication of Closely Related Species and Semi-Untargeted MS Approaches



Date: **November, 15 2018, 08:15**

Speaker: **Jens Brockmeyer**

Sciex, Villebon-sur-Yvette, France

Species authentication using DNA-based methods often still shows limitations when closely related species and mixtures thereof have to be analyzed. A relevant example is the differentiation of tuna species that, despite their close phylogenetic relationship, show significant differences in price and are therefore especially prone to fraud. To showcase the potential of food proteomics, we employed a bottom-up approach to identify specific marker peptides covering all eight relevant *Thunnus* and *Neothunnus* species as well as marker for *Katsuwonus pelamis* (bonito). In addition, several “group marker” covering different branches of the phylogenetic tree were identified to allow further confirmation.

Besides the identification of marker peptides specific for different species we detected peptides that are widely distributed in numerous different mammalian, bird or fish species (“global marker”). The relative quantitation of specific marker peptides and global markers allows for the identification of fraudulent blending e.g. in meat products without knowledge of the species used for adulteration and is in our opinion a very promising approach for future testing routines.

Metabolomic Profiling of Manuka Honey



Date: **November, 15 2018, 08:15**

Speaker: **Emmanuel Sauvard**

Agilent Technologies, France

Consumers are increasingly concerned about the quality, authenticity and impact on their health of the food they consume. Manuka honey is very popular in the UK because of its many properties, such as its antimicrobial power, and is not immune to consumer concerns. The quantity consumed today is not consistent with the production, hence growing concerns about its authenticity. There is also no methodology to characterize its benefits and there is no evidence that its integrity is preserved between production and consumption.

The work carried out aims to perform multivariate statistical comparisons of data from reference Manuka honeys and from different production sites. A Principal Component Analysis -PCA- was selected to conduct the study. Metabolomic profiles are taken into account as a source of data. The goal is to highlight the differences and establish a relevant predictive model.

A first selection of honeys is made -Gales, Manuka 24 and Nelson 100- in consideration of the observed antimicrobial activity, after a test in 10 replicates. The advantage of this approach lies in the fact that sample preparation is extremely limited and therefore of minimal inherent bias.

A method of analysis is developed, considering the complexity of the matrix, both in the number of its constituents and in its variability. The choice was HILIC stationary phase chromatography separation and time-of-flight mass spectrometry detection for the non-targeted and quadrupole tandem detection for the targeted approach. The separation tests in reverse phase chromatography prove to be insufficient for the characterization of the polar fraction and fewer than 30 characteristics unique to a group are found under these conditions.

Similarly, it does not make it possible to highlight all known markers of honeys as reported in the literature.

The data acquired using High Performance Liquid Chromatography / Quadrupole Mass Spectrometry and Time of Flight (HPLC MS QTOF) are processed automatically and locally with the tools of the Mass Hunter platform. The « Pro-finder » algorithm allows to isolate 2300 unique entities to honeys. Chemometrics then allows the relevant extraction of information and leads to the realization of a model. 40 tests in positive and negative mode were done. Honeys are then differentiable via the « Mass Profiler Professional » software platform.

However, the study must be completed to determine the « true » honeys of the « false », to say those that have undergone alteration and / or modifications. Targeted analysis is used to search for known markers of honeys. Methyl-gyloxal, 5-Hydroxymethyl Furfural, Methyl-

Syringate and Leptosperin are analyzed using High Performance Liquid Chromatography / Tandem Quadrupole Mass Spectrometry (HPLC / MS TQ). The data are added in the established model. Their treatment shows a correlation between the presence of the markers and some of the honeys tested. Honeys are differentiated, and their quality checked against markers.

- The predictive character of the extended model – targeted and untargeted data – is evaluated on 9 sets of samples. The results show that:
- The authenticity of honey is established with a risk of error of 2%;
- The origin of the places of production is differentiated between New Zealand, Europe, Argentina and Mexico;
- The risk of error on the brand of honey is 32%.

This first study should be completed with a larger population of samples to test and validate the model and increase its accuracy by taking into account a larger number of replicates.



DEMO CORNER & WORKSHOPS

The role of modern analytical approaches in fighting with food fraud

Organised as satellite event of the 5th FoodIntegrity Conference:
Assuring the integrity of the food chain: Delivering real world solutions

Date: 13th November 2018

Time: 10:00 – 13:00

Venue: La Cité – Nantes Congress Centre, Nantes, France

Participation in the workshop is free for all delegates of the FoodIntegrity 2018 conference.

To save your seat, please send your preliminary interest at monika.tomaniova@vscht.cz

The workshop is intended to scientists and representatives of industry who are interested in modern analytical approaches used in fighting with food fraud that have been advanced by the FoodIntegrity partners and successfully implemented into practice. It aims to deepen knowledge in applications of modern analytical techniques, employing profiling, fingerprinting, metabolomics strategies, and data mining by chemometrics, for food authentication.

10:00	Welcome to the workshop
10:00-10:20	Peptides as a tool for meat speciation using liquid chromatography coupled to mass spectrometry Michele Suman, Barilla, Parma, Italy
10:20-10:40	NMR based profiling for food authentication James Donarski, Fera Science Ltd, York, UK
10:40-11:00	IRMS for food authentication Federica Camin, Fondazione Edmund Mach, San Michele All'Adige, Italy
11:00-11:20	Advanced mass spectrometry based approaches for authentication of complex foods Daniel Wunderlin, National University of Córdoba, Córdoba, Argentina
11:20-11:40	Coffee break
11:40-12:00	Assuring olive oil integrity: the needs for innovative approaches Diego Luis García González, Instituto de la Grasa, Spanish Council for Scientific Research, Sevilla, Spain
12:00-12:20	A novel approach to assess the quality and authenticity of Scotch Whisky based on gas chromatography coupled to high resolution mass spectrometry Michal Stupak, University of Chemistry and Technology, Prague, Czech Republic
12:20-12:40	Non-targeted detection of adulteration - from analytical chemistry to chemometrie Janet Riedel, German Federal Institute for Risk Assessment, Berlin, Germany
13:00	Close of the workshop & lunch

FoodIntegrity Demo Corner

Demonstration of approaches developed by the FoodIntegrity for assuring the integrity of the food chain

14-15 November, 2018/Nantes, France

La Cité–Nantes Congress Centre, Foyer

9:00–17:00 (14 Nov.) and 9:00–13:00 (15 Nov.)

Explanations on-site during coffee and lunch breaks on 14 Nov.

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



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

App & Infographics using 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



Improving Supply Chain Integrity through Data Sharing

Video on integrity management solution Check X helping to prevent fraud in food supply chains with food fraud vulnerability



Providing assurance in the spirit drinks sector "Hands on" demonstration of authentication of spirit drinks



Scientific Opinions on issues that concern food fraud



FoodIntegrity Handbook: A guide to food authenticity issues and analytical solutions



Do you wish to receive information about the FoodIntegrity results?

Join us at the Demo Corner and learn more!

BECOME INVOLVED AND SIGN-UP AS A STAKEHOLDER!

You may register on-site for the project COMMUNICATION or on www.foodintegrity.eu/ page Contact us

Join us and discuss with FoodIntegrity experts the latest developments and strategies in the field of food integrity: safety, quality, authenticity and traceability!

FoodIntegrity is a European five-year project, which will draw from a well of experience consisting of 60 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 multifaceted project and let you know how you can get involved. We hope you find it useful.



ORGANISING COMMITTEES

Scientific Committee



Pier Luigi Acutis

Istituto Zooprofilattico Sperimentale Piemonte (IZSPLVA)

Senior Veterinarian, Head of the Genetics and Immunobiochemistry Laboratory



Marco Alorio

Università del Piemonte Orientale

Full Professor of Food Chemistry



Paul Brereton

Queen's University Belfast

Director of Strategic Alliances (Professor of Practice)



Federica Camin

Fondazione Edmund Mach

Head of the Traceability Unit



James Donarski

Fera Science Ltd

Head of Food Authenticity



Chris Elliott

Queen's University Belfast

Professor of Food Safety and Founder of the Institute for Global Food Security



Lynn Frewer

Newcastle University

Professor of Food and Society



Diego Luis García González

Consejo Superior de Investigaciones Científicas Sevilla

Researcher



Ana Garrido

Universidad de Córdoba, Spain



Ian Goodall

The Scotch Whisky Research Institute

Senior Scientist for Product Protection research



Jana Hajslova

University of Chemistry and Technology Prague

Head of the Accredited Laboratory, Department of Food Analysis and Nutrition



Gerald Herrmann

Organic Services GmbH

Founder



Simon Kelly

Joint FAO/IAEA Division of Nuclear Applications in Food and Agriculture
Food Safety Specialist (Traceability)



Rolando Lorenzetti

Consorzio Italbiotec
Scientific Director



Niels Lucas Luijckx

TNO Innovation for Life
Senior Consultant and Researcher in Risk Management Food Safety



Elena Maestri

University of Parma, Siteia.Parma
Full Professor in Applied Biology in the Department of Chemistry, Life Sciences, Environmental Sustainability.



Jean-François Morin

Eurofins Analytics France
Director Collaborative Research



Petter Olsen

Noforma AS Tromsø
Senior Scientist



Dolores Pérez Marín

Universidad de Córdoba, Spain
Full-Professor in Fundamentals and Technology of Livestock Production and in Non-destructive Spectral Sensors for Quality, Safety and Traceability of Agro-Food Products



Saskia Van Ruth

Wageningen University and Research / Queen's University Belfast
Professor of Food Authenticity and Integrity



Michele Suman

Barilla SpA
Head of Food Chemistry & Safety Research Department within the Food Research Labs



Monika Tomaniova

University of Chemistry and Technology Prague
Senior Researcher



Andrew Watson

Institute of Food Research UK
Research Scientist



Daniel Wunderlin

Universidad Nacional de Córdoba, Argentina
Full Professor and Principal Researcher From the National Research Council of Argentina

Consortium Organising Committee



Paul Brereton

Queen's University Belfast

Director of Strategic Alliances (Professor of Practice)



James Donarski

Fera Science Ltd

Head of Food Authenticity



Claire Sykes

Fera Science Ltd

EU Project Manager



Jean-François Morin

Eurofins Analytics France

Director Collaborative Research



Béatrice Bouchard

Eurofins Analytics France

Project Assistant



Edgar Morales Ortega

Eurofins Analytics France

Events Trainee

Local Organising Committee



Jean-François Morin
Eurofins Analytics France
Director Collaborative Research



Guillaume Naret
Eurofins Analytics France
Marketing Project Manager



Joséphine Renaud-Huet
Eurofins Analytics France
Event Manager



Béatrice Bouchard
Eurofins Analytics France
Project Assistant



Edgar Morales Ortega
Eurofins Analytics France
Events Trainee



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- P7.3 A novel DNA-based approach for argan oil authentication: detection of olive oil as a potential adulterant** Mafra, I., Raja, F.Z., Costa, J., Amaral, J.S., Grazina, L., Villa, C., Kartah, B.E., Charrouf, Z., Oliveira, M.B.P.P.
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- P7.10 Bioactive peptides in food of plant and animal origin** Maestri, E., Pavlicevic, M., Montorsi, M., Imperiale, D., Marmiroli, M., Marmiroli, N.
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- P7.16 Genetic tools for identification of flaxes, chia and sesame ingredients in seeds and processed foods** Bruno C., Posik D.M., Zappa M.E., Wunderlin D., Giovambattista G., Peral García P.
- P8.1 What about the progress of Non-Targeted Screening Mass Spectrometry and chemometrics in verifying the geographic origin of tomatoes?** de Dominicis, E., Gritti, E., Piva, M., Menegon, V., Saner, S., Dameno, F.
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- P13.4 Enhancing the detection of olive oil illegal blends: short liquid chromatography (LC) and HRMS fragmentation for TAG fingerprinting** Tres, A., Quintanilla-Casas, B., López, J., Bustamante, J., Simón, M., Guardiola, F., Barrón, D., Vichi, S.
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ORAL SESSIONS ABSTRACTS

O1.1 FOOD FRAUD AT THE SHARP END – RELIANCE ON SCIENCE?

Whelan, P.^{1,*}

1-Food Safety Authority of Ireland

*corresponding author e-mail: pwhelan@fsai.ie

Food Fraud is not a scientific, regulatory or academic issue. It is an issue of People and Profit. People gaining advantage in fraudulent ways using food and food materials. The fraud opportunities across both complex and simple food supply chains seem to be endless and the consumer is being deceived.

Food fraud is difficult to investigate and is one of the few crimes where people unknowingly consume the evidence. Therefore food fraud investigations depend on an array of supports and are often conducted in a forensic manner. Food fraud investigations require the establishment of multidisciplinary teams to tackle every aspect of illegality encountered. Investigations must comply with all of the legal process requirements of criminal law. Investigations can be strongly supported by good science and reliable and valid analytical results however, investigations can also be hampered or completely undermined by unreliable scientific analysis and contradictory results.

Many food fraud investigations are conducted in a forensic way and include the use of open source surveillance, covert surveillance, human intelligence sources, whistle-blowers, forensic accountants, forensic IT specialists, inspections, audits, mass balances, sophisticated traceability exercises, witness interviews, suspect statements, search warrant applications to court, execution of the warrant, sampling and analysis, collaboration with other regulatory agencies within and outside the investigating country, Europol support etc. Investigating teams spend long hours attending case conferences where information and intelligence is examined and all actions required are agreed and executed. Detailed instructions are outlined before the investigating teams go onsite.

Generally consumers are not aware that they are being deceived. Much of the available information in relation to food fraud lies with industry sources. To win the fight against food crime it is important that full and frank exchanges of information are taking places between all parties concerned. Trust, transparency and a united approach to the fight against food fraud is needed.

O1.2

ROLE OF THE EUROPEAN COMMISSION'S JOINT RESEARCH CENTRE IN COUNTERING FOOD FRAUD

Anklam, E.^{1,*}, Ulberth, F.

1-European Commission, Joint Research Centre, Directorate Health, Consumers & Reference Materials

*corresponding author e-mail: elke.anklam@ec.europa.eu

Keywords: food fraud, food quality, EC Knowledge Centre

Consumers expect that the food they buy is genuine, i.e. is not subject to fraud and is of as high quality as possible. This holds especially true for high priced products. Unfortunately, the recent fraud cases have shattered the confidence consumers have in their food supply. Moreover, this has also serious effects on the reputation of honest food business operators. There prevention of fraud in the agri-food chain and promotion of authentic products is a major element to assure the commercial success of European high-value food products on the internal as well as on international markets. Marketing standards and EU quality schemes such as geographical indications and traditional specialties shall ensure that consumer expectations are met and enable the functioning of the EU Single Market and the global trade. Moreover, the detection and prevention of food fraud requires strategic planning and investment at the National and European level with a proportionate and sustainable budget. Concerted measures at the EU level are needed to address the problem and restore citizens' confidence.

For this reason, the European Commission has recently created a Knowledge Centre for Food Fraud and Quality (<https://ec.europa.eu/jrc/en/food-fraud-and-quality>) to supply EU institutions and Member States' authorities with the best available and validated practices to fight fraud in the food chain and create a level playing field to give consumers the freedom of making informed decisions when purchasing food.

The EC Knowledge Centre for Food Fraud and Quality produces, collects and collates information, makes sense of it, and transforms it into knowledge, which shall inform policy making to ensure and protect the authenticity and quality of food supplied in the EU. It is collectively operated by the EC Directorate-General Joint Research Centre (JRC) and the Directorates-General regulating the feed-food chain and protecting consumer rights. It aims at:

- Creation of a formalised science/policy interface which facilitates the flow of scientific evidence into the policy making cycle to support initiatives for safe-guarding the quality of agri-food products and protecting the integrity of the food chain;
- Community of Practice linking policy makers from different Commission services, scientists and competent authorities in the Member States to access and make best use of shared knowledge;
- Building collaboration with authorities in Third Countries.

It complements the activities of the EU Food Fraud Network, which is operated by the European Commission Directorate-General for Health and Food Safety.

The EC Knowledge Centre for Food Fraud and Quality is hosted by the JRC and its main laboratories are based at JRC's Geel site. The JRC has long-standing expertise in food

science including research into the authenticity of foods and expertise in developing, applying and validating analytical test methods to detect fraud in the food chain.

This presentation will give an overview on the services offered by the JRC hosted EC Knowledge Centre and will describe the current scientific activities in the area of food fraud and food quality.

O2.1

THE STRATEGY OF THE FLAVOUR INDUSTRY ON VANILLA AND VANILLA EXTRACTS

Kastler, A.^{1,2,*}

1-EFFA President, Brussels, Belgium

2-Deputy Manager Flavor Division, Robertet, Grasse, France

*corresponding author e-mail: Antoine.kastler@robertet.fr

Keywords: vanilla, authenticity, EFFA Guidance Document, compliance

Mr. Kastler will present the strategy of the flavour industry regarding vanilla and vanilla extracts. While vanilla is facing scarcity and growing prices, the flavour industry keeps an eye on Food Integrity and supports the authorities, ensuring the authenticity and the compliance of the finished products.

O2.2

ASSURING FOOD INTEGRITY IN THE SPICE AND HERB SUPPLY CHAIN

Menezes, C.^{1,*}

*1-Director Global Food Integrity – Global Quality & Food Safety Center of Excellence.
McCormick & Company*

*corresponding author e-mail: clare.menezes@mccormick.co.uk

Keywords: herbs & spices, food integrity, best practice

Over the past 10 years the global demand for herbs and spices has grown steadily and a recent report of the CBI, citing a Eurostat study of 2017, claims that the EU herbs and spice market will grow another 25% by 2020. The demand for herbs and spices seems to be driven by two main factors: 1) an increasing trend to adopt healthy diets and avoid salt and sugar as well as red meat and 2) the increased demand for exotic and ethnic tastes and cuisines. There are several reports suggesting this increase in demand has not been matched by a proportional surge in supply. This is driving higher prices and a raising concern over the potential vulnerability to food fraud within this market.

This presentation will take you through the leading practices embedded within our business to deliver the integrity expected of all McCormick products including an overview of how our Supply Chain Controls, long term alliances and Global Standards drive prevention.

At the heart of these programs is a positive control based approach, designed to prevent the entry of adulterated products into any node of our supply chain.

Our approach is formulated on the premise that the most effective way to assure the absence of economic adulterants and contaminants is through control of the supply chain back to source.

The three essential elements of the approach are:

- Strategic vendor alliances
- Source material control, and
- Manufacturing process control

These in combination, form the cornerstone of the management of the supply chain, from the plants growing in the field, through our manufacturing facilities ultimately assuring that our customers and consumers only receive adulterant free product.

O2.3

NON-TARIFF BARRIERS IN GLOBAL AGRI-FOOD TRADE – IMPLICATIONS FOR BREXIT AND FOOD SECURITY

Haverty, M.^{1,*}

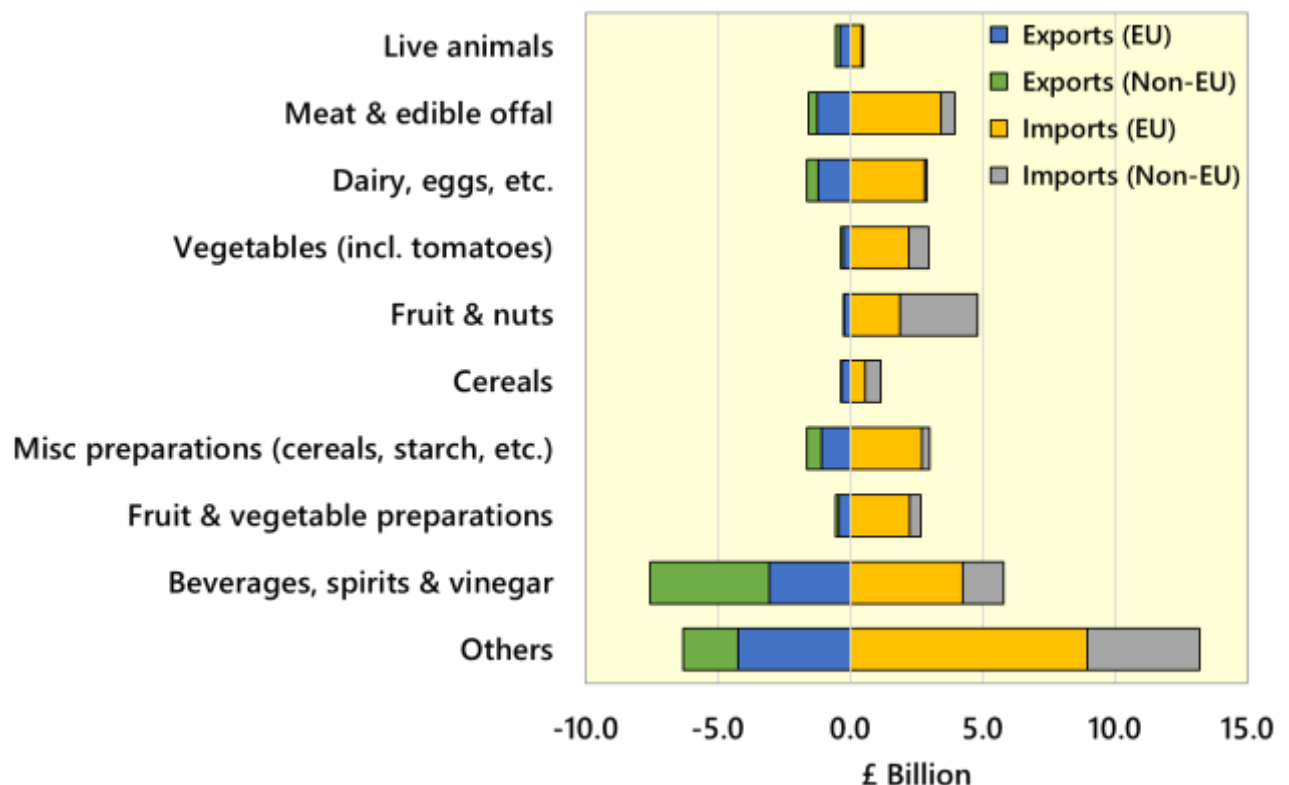
1-Senior Agricultural Economist, the Andersons Centre, Leicester, United Kingdom

*corresponding author e-mail: mhaverty@theandersonscentre.co.uk

Keywords: non-Tariff barriers, trade, agri-food, Brexit.

This presentation will provide an overview of non-tariff barriers (NTBs) in global agri-food markets and will include an overview of previous studies' estimates of the impact of NTBs on trade. It will focus on some of the key issues which have influenced global markets recently including trade disputes between the US and China and its implications using the example of the soybeans sector. The implications of Brexit will also be analysed from both a UK and EU perspective. This will also encompass coverage of recent research which attempts to quantify the impacts of Brexit; particularly in terms of NTBs. Consideration will also be given to the potential impact of NTBs on global food security as well as the scope for increased fraud in terms of smuggling.

Figure 1: UK Agri-Food Trade Situation 2017



Source: UK HMRC, analysed by The Andersons Centre

O2.4

NEW BREEDING TECHNIQUES: SCIENCE, REGULATION AND ANALYTICAL CHALLENGES

Rolland, M.^{1,*}, Pornin, D.¹, Le Gallo, M.¹, Boutigny, A.-L.¹

1-Anses, Plant Health Laboratory, Angers, France

*corresponding author e-mail: mathieu.rolland@anses.fr

Keywords: CRISPR, GMO, SNP, genotyping

The term of “New Breeding Techniques” covers several genetic engineering technologies, some of which have been available for many years. Among these, technologies based on site directed nuclease allow the edition of genome on targeted positions, either to insert a portion of exogenous DNA, or to apply a modification of one or a few nucleotides. The most recently described site directed nuclease technology is based on CRISPR/Cas (an adaptive immunity mechanism of Bacteria and archaea). The use of this technique made genome editing both easier and more accessible. The increasing number of point mutations obtained using new breeding techniques raised the question of regulation of these products. In July 2018, the European court of justice ruled that, within Europe, organisms obtained by new mutagenesis plant breeding techniques should be considered as genetically modified organisms (GMOs) and that they should fall under the GMO regulatory framework. Enforcement of this regulation requires the ability to distinguish unequivocally a mutation obtained by new breeding techniques from a natural mutation and raises many questions and analytical challenges.

O3.1

FOOD PROTEOMICS AS A NOVEL TOOL TO ENSURE THE INTEGRITY OF FOOD: FROM TARGETED TO UNTARGETED APPROACHES

Brockmeyer, J.^{1*}, Viet, L.¹, Brümmer, I.¹, Watson, A.², Kemsley, K.², Gunning, Y.², Macierzanka, A.³

1-University Stuttgart, Institute of Biochemistry and Technical Biochemistry, Department of Food Chemistry, Allmandring 5b, 70569 Stuttgart, Germany

2-Quadram Institute Bioscience, Colney Lane, Norwich, NR4 7UA, UK,

3-Gdansk University of Technology, Faculty of Chemistry, Department of Colloid and Lipid Science, Narutowicza 11/12, 80-233 Gdansk, Poland

*corresponding author e-mail: jens.brockmeyer@lc.uni-stuttgart.de

Keywords: mass spectrometry, authenticity, complex Foods, peptide biomarker

Proteomics approaches have emerged recently as novel and powerful tools to address different aspects of food authenticity such as species differentiation, authentication of plant breeds or the detection of adulteration/contamination with undeclared species. The identification and evaluation of specific marker peptides is key for the subsequent development of targeted mass spectrometry-based detection methods. As an emerging new discipline, food proteomics often still lacks suitable marker peptides or targeted methods that can be applied in routine laboratories. This is especially true for complex foods and we have therefore addressed several different relevant aspects of food authenticity. This includes a method to differentiate mozzarella from cow and buffalo, the differentiation of tomato varieties, the identification of animal species in processed meat products and the development of the first untargeted proteomics approach to identify fraud in meat products without prior knowledge (or suspicion) of the fraudulently blended species. Besides the identification of marker peptides specific for different species or varieties, we were able to identify peptides that are widely distributed in numerous different mammalian, bird or fish species (so-called “global marker”). The relative quantitation of specific marker peptides and global markers allows for the identification of fraudulent blending in meat product without knowledge of the species used for adulteration.

This presentation gives an overview on the general aspects and strategies of targeted and untargeted food proteomics and highlights some of the results generated in the Food Integrity project.

O3.2

USING CHEMICAL AND DNA MARKERS TO AUTHENTICATE MANUKA HONEY

McDonald, C.^{1,*}, Keeling, S.¹, Brewer, M.², Hathaway, S.¹

1-Ministry for Primary Industries, Wellington, New Zealand

2-BioSS, Aberdeen, Scotland

*corresponding author e-mail: Claire.mcdonald@mpi.govt.nz

Keywords: honey, Manuka, CART, authentication

With an increasing diversity and complexity in global food supply chains, it is important that consumers and regulatory authorities have full confidence in the integrity and authenticity of food. Manuka honey is a high valued export product from New Zealand that has come under scrutiny due to offshore claims of fraud, adulteration and mislabelling after the product has left New Zealand. There are several industry approaches for defining Mānuka honey but until recently, a scientifically robust definition suitable for use in regulation was not available.

Here we present the results of a 3 year science programme which developed definitions for monofloral and multifloral Manuka honey produced from New Zealand. We used a multi-disciplinary approach to identify and trace markers found in Mānuka honey back to the source plant *Leptospermum scoparium* (Manuka). The suitability of 16 markers (chemical and DNA-based) was evaluated for use in regulatory definitions for Manuka honey. This involved selecting potential markers for investigation (found in the nectar and pollen), establishing plant and honey reference collections, developing and validating test methods and analysing test results to develop the final definitions.

Plant samples were collected from two flowering seasons representing Manuka and non-Manuka species from New Zealand and Australia. Honey samples, also representing Manuka and non-Manuka floral types, were sourced from seven New Zealand production seasons. Additionally, honey samples were sourced from another 15 countries to enable comparisons. All samples were tested for the markers being evaluated using test methods that were specifically developed for the science programme. The method of CART (Classification and Regression Trees) was then used to develop monofloral and multifloral mānuka honey definitions. A combination of 4 chemicals and 1 DNA marker effectively separates monofloral and multifloral mānuka honey from other honey types. The practicalities of developing and implementing a quantitative science-based definition for a variable natural food for use in regulation are also discussed. This novel approach for authenticating Manuka honey could be applied to other foods of mixed content.

Acknowledgements

Authors would like to acknowledge the technical experts who were involved at various stages of this programme including those who collected the plant and honey samples, developed the test methods and helped analyse the results. We are also grateful to industry who contributed their samples for testing.

O3.3

AUTHENTICITY AND QUALITY CONTROL OF FOOD BY AUTOMATED ¹H-NMR SPECTROSCOPY COMBINED WITH MULTIVARIATE STATISTICS

Steck, A.^{1,*}

1-Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany

*corresponding author e-mail: andrea.steck@bruker.com

Keywords: ¹H-NMR spectroscopy, multivariate statistics, food profiling, food adulteration

Global food supply networks have reached a state where numerous actors are involved in complex and fragmented steps, accompanied by emerging risks for deliberate food fraud driven by the prospect of economic gain. High-priced food products, but also those with high volume of sales, are preferential targets of malpractices. As certain fraudulent food manipulations elude detection by classical analytics, the goal is to develop methodologies able to filter out minor non-natural variations in complex matrices, and to identify and quantify single components within the matrices alike. Automated high-resolution ¹H-NMR spectroscopy, combined with multivariate statistical chemometrics, provides unique "all-in-one" capabilities for food quality and authenticity control^[1].

As ¹H-NMR is intrinsically quantitative; only one quantification reference for all NMR-detectable components in a mixture is required. Yielding targeted quantification of selected compounds as well as untargeted fingerprinting in a single run, NMR is a specific and holistic method likewise. Its supreme reproducibility enables worldwide lab-to-lab spectra comparison and collective database buildup. Unlimited data re-processing is given and allows applying future statistical algorithms, re-modelling of more or different parameters, or retrospective quantification of mixture components not in the focus of interest at present. This methodology, yet commercially applied and ISO-17025 accredited for fruit-juice, wine and honey profiling, is now under development for further matrices.

Additionally, dedicated sample preparation techniques are elaborated, integrating latest liquid dosing solutions^[A]. The principles behind this NMR methodology as well as recent applications and results are presented.

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Acknowledgements

[A] The author wishes to thank Mettler-Toledo GmbH (Gießen, Germany) and Mettler-Toledo AG (Greifensee, Switzerland) for many valuable contributions and discussions.

O3.4

EVALUATING THE INTEGRITY OF COMPLEX FOODS BY COMBINED METHODS. CASE STUDY: BAKERY PRODUCTS CONTAINING CHIA, FLAX AND SESAME SEEDS.

Wunderlin, D.^{1,2}, Baroni, M.V.^{1,2}, Kopka, J.³, Peral García, P.⁴, Brigante, F.^{1,2}, Lucini Mas, A.^{1,2}, Pigni, N.^{1,2}, Ribotta, P.¹, Erban, A.³, Alt, C.³, Posik, D.M.⁴, Zappa, M.E.⁴, Bruno, C.⁴, Lyall, V.⁴, Giovambattista, G.⁴

1-CONICET-ICYTAC: Institute of Food Science and Technology Córdoba, Argentina.

2-Department of Organic Chemistry, Faculty of Chemical Sciences, National University of Córdoba.

3-Max-Planck-Institute of Molecular Plant Physiology, Department of Molecular Physiology: Applied Metabolome Analysis, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany.

4-CONICET-IGEVET: Institute of Genetics and Veterinary, La Plata, Argentina.

Outstanding nutritive seeds, including chia, sesame and flax, are highly appreciated by consumers looking for nutraceuticals. Thus, the development of methods to verify the integrity of such seeds as well as processed foods containing them is of public interest.

We hypothesized that a tool-kit, constructed with chemical, genetic and metabolomic markers could be used to verify the integrity of both seeds and foods containing them. Thus, the main goal of this work was constructing a tool-kit with markers from different methods to identify chia, sesame and flax seeds, verifying if such markers remain stable from raw to processed foods.

Tool-kit development was based on both target and non-target methods to identify suitable metabolites to be included in a model. Additionally, genetic markers were also investigated as a third tool to be integrated in the kit. Results show that chemical markers can be obtained by a target analysis of polyphenols profile with the help of chemometrics, while non-target analysis, coupled to bioinformatics machine learning technology, lead to non-biased marker discovery. A robust DNA extraction method was developed, in addition to multi-specie PCR-real time assay, based on *rbcl* gene and real time melt curve plots, which provide with an accurate evaluation on the presence of nutritive seeds in both raw and processed foods. The final step is integrating these three different tools in a unique, combined, tool-kit to full assess the integrity of chia, sesame and flax from seeds to complex foods containing them. Bakery products supplemented with variable amounts of whole seeds (single or mixtures) were used to develop the tool-kit, which was tested with commercial products containing such seeds.

Acknowledgement:

This study was supported by FOODINTEGRITY grant FP7-KBBE-2013-7-613688.

O4.1

F.I.S.HUB – A MOBILE APP TO VERIFY FISH SPECIES THROUGH A PICTURE

Acutis, P.L.^{1,*}, Rossi, F.², Gkoumpili, C.³, Sciuto, S.¹, Esposito, G.¹, Ubaldi, P.³, Coscia, I.³, Mariani, S.³, Benso, A.²

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

2-Politecnico di Torino, Turin, Italy

3-Salford University, Manchester, UK

4-Esselunga SpA, Biandrate, Italy

*corresponding author e-mail: pierluigi.acutis@izsto.it

Keywords: fish, species, identification, software

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. Usually this fraud implies the substitution of valuable species with less valuable ones, but this commercial fraud can have other consequences, like health implications, in case of toxic or allergenic species. Currently fish species identification is based on mainly two different methods: identification by the naked eye, on the basis of morphological characters, and DNA analysis. The first method has the advantage of being performed directly at selling points, but it needs experienced personnel, like veterinary officers, and it relies on a subjective judgement and only on features clearly distinguishable by an experienced human eye. For these limitations not many controllers are confident enough to claim a substitution fraud just relying on their own evaluation. DNA analysis, on the other hand, is a very accurate method for species identification, but it has to be performed in a laboratory, implying costs and a limited throughput.

Objective of the F.I.S.HUB project was to develop a software framework to be used on the field to detect species substitution. The software is able to identify the species of a fish from its picture; it is composed of a photo database and a machine-learning server for image analysis and classification, and it is accessible through an App for mobile phones and other portable devices.

The work plan of the project was organized in four work packages. The first three define the main components of the project: 1) the creation of the photo database that will be used to train the fish identification classifiers; 2) the design and development of the server-side classification software, ad-hoc for each of the selected fish families; 3) the design and development of the mobile App; 4) the validation of the technology.

The F.I.S.HUB software has been designed with architecture able to guarantee a seamless communication among the classifier, the database and the mobile application. The F.I.S.HUB classifier has been designed and developed with the adoption of the state of the art of image processing and machine learning techniques applied to image/object recognition tasks. It compares the photo received from the users with the reference photos stored in the Photo Database.

The app is now ready to be downloaded and it is available on Apple and Google Play stores. In the database there are 26 species. The user, once entered in the app, has to decide whether he wants to directly take a picture of a specimen or to select a picture from the

gallery, then he has to select the species declared on the label and start the analysis. The software will verify whether the specimen belongs to the declared species giving a percentage of matches. The user can also find additional information on the morphological patterns of the species, e.g. spots, colour, shape, lines. The app is in two versions, English and Italian. In the last phase of the project, it has been tested on field, in fish markets. It resulted to be easy to use and the accuracy resulted to be dependent on the number of pictures present in the database: the higher the number of pictures the higher the power of identification and discrimination among similar species. The best performances were obtained with pelagic fish. The classifier thus appeared to be set up with the appropriate machine learning techniques. The app can be a useful tool to support consumers in verifying the authenticity of seafood they purchase. Its power and its applicability can be more and more improved and widened just enlarging the database, regarding number of pictures and number of species.

O4.2

PORTABLE AUTHENTICATION TECHNOLOGIES - THEIR PLACE IN DETECTING SPIRIT DRINKS FRAUD

Goodall, I.^{1,*}, Harrison, S.¹, Eccles, R.¹

1-The Scotch Whisky Research Institute, Edinburgh, United Kingdom

*corresponding author e-mail: ian.goodall@swri.co.uk

Keywords: spirit drinks, portable authentication, screening technologies

This presentation highlights the successful outcomes of the FoodIntegrity project's work on portable authentication techniques for spirit drinks. It also considers the current perception within the spirit drinks industry of these technologies, noting opportunities for improvements in their design and utilisation, as well as potential barriers that could restrict development or uptake by stakeholders.

The FoodIntegrity project's work on spirit drinks authenticity and safety has tackled various objectives fewer than two themes - improving analytical methods for the detection of non-authentic/unsafe products and developing knowledge and expertise amongst those involved in spirit drinks authentication.

Principal areas of investigation into improving analytical methods have included:

- The evaluation of numerous rapid, portable authentication technologies such as UV-Vis, NIR and Raman spectroscopies, pH and conductivity analysis pens, and handheld density meters.
- The identification of a range of key markers for illegally added flavourings in counterfeit spirit drinks.
- The examination of the authentication potential provided by traditionally laboratory-based analyses in formats that are gradually becoming more portable, such as low-field NMR, GC-MS and direct injection MS.
- Key elements in developing knowledge and expertise have tackled:
 - The development and promotion of a spirit drinks authentication network of contacts.
 - The creation of a website within the FoodIntegrity project, dedicated to spirit drinks authentication, complete with training resources, project outcomes and additional information tools.

The deliverables from these objectives have been disseminated by several routes. However, to ensure that appropriate feedback could be obtained on the project outcomes, a Spirit Drinks Authentication Workshop was held at the Scotch Whisky Research Institute on the 20th June 2018, with attendees from industry, academia, research institutes, government enforcement agencies and technology providers. The report from this workshop clearly highlighted the intense interest in the advantages that portable techniques could offer the authentication of spirit drinks. There was considerable discussion about how technologies could be employed and developed, and firm views expressed on the characteristics an effective portable authentication solution should possess. The outcomes and learnings from the FoodIntegrity research in this area, as well as the opinions of the attendees at the recent workshop, are used as the basis for this presentation.

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

O4.3

PHASMAFOOD: A NEW GENERATION OPTICAL FOOD SENSING DEVICE AND AN OPEN SOFTWARE ARCHITECTURE DELIVERING FOOD-TECH INNOVATION

Weesepoel, Y.^{1,*}, Müller-Maatsch, J.¹, Ebbinge, L.¹, Koutalieris, G.², Alewijn, M.¹

1-RIKILT–Wageningen University & Research, Wageningen, the Netherlands

2-INTRASOFT International SA, Athens, Greece

*corresponding author e-mail: yannick.weesepoel@wur.nl

Keywords: maximum 4 significant keywords for indexing

PhasmaFOOD is an EU collaborative R&D project funded by the Horizon 2020 Programme. It aims at delivering a miniaturized multi-sensor optical sensing device for the detection of food safety threats such as food spoilage, food fraud and aflatoxins. The system is based on heterogeneous fluorescence, visible and near infrared spectroscopy technologies and is supported by a software architecture that delivers fast characterisation of foods, encompassing an extendable framework for the deployment of smart chemometric algorithms, data fusion strategies and reference laboratory measurements. The built-in algorithms address data mining and data analysis methods from non-destructive, non-invasive instruments and are independent of the food type and food-tech application. This project aims to address three food safety and authenticity use cases by utilizing low-cost and miniaturized spectroscopic equipment in combination with multivariate statistics in order to screen a sample within seconds.

In the first phase of the project, a large number of pilot applications is explored by using a conceptual modular scanner, using fluorescence, visible, near infrared sensors as well as a standard cell-phone camera. This was performed in conjunction with the design and construction of the PhasmaFOOD sensor prototype. In this session we focus on first results obtained by the sensor array, in particular pilots on adulterated skimmed milk powders. This pilot show-casts the advantage of using multiple optical and so-called ‘one-class model’ multivariate statistics. This type of statistics is trained to detect anything that is “not normal”, and hence this can be used for authentication, regardless of the type of adulterant or whether the adulterant has been used in the underlying database.

Next to assessing food fraud, the PhasmaFOOD also considers aflatoxin detection and food spoilage of fresh produce like meat, fish, vegetables and fruits. This application may have significant impact in the supply chain of agricultural products such as corn and grains. The framework proposed by PhasmaFOOD can lead to new forms of food-tech applications and growth, especially in the rural regions of Europe where the technological and broadband penetration is still low, while the economic growth coming from food value-chains is rapidly growing. As by September 2018, the PhasmaFOOD prototype scanner, including mobile and web applications has been delivered and is now being evaluated on performance.

PhasmaFOOD has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 732541. www.phasmafood.eu.

O4.4

ON-SITE ANALYSIS OF INDIVIDUAL PORK CARCASSES FOR THE PREDICTIONS OF QUALITY PARAMETERS IN IBERIAN HAM

Pérez-Marín, D.C.1,*, Garrido-Varo, A.1, Riccioli, C.1, de Pedro, E.1, Fearn, T.2, Piotrowskiy, C.3, Doña, R.3, Blanco-Valero, A.4

1-Faculty of Agriculture & Forestry Engineering (ETSIAM), Universidad de Cordoba, Campus de Rabanales, Carretera de Madrid km. 396, 14071 Cordoba, Spain.

2-Dep. of Statistical Science, University College London, Gower Street, London WC1E 6BT, UK

3-AUNIR. The Dovecote, Pury Hill (Business Park), Nr Alderton, Towcester, UK

4 *Ibérico de Bellota*, SA (IBESA). Polígono Cárnico, s/n, 14440 Villanueva de Cordoba, Spain

*corresponding author e-mail: pa1gavaa@uco.es

Keywords: NIRS portable devices; in situ analysis, Iberian pig carcasses classification, fatty acid profile

Acorn Iberian ham (*Jamón Ibérico de Bellota*) is one of the most expensive luxury food products produced in Europe, with a highly appreciated smell and flavor, and a first-rate consumer acceptance. Its recognized high-sensorial quality and health properties are mainly due to the traditional outdoor feeding system (*Montanera*) of Iberian pigs (IP), which provides high standards of animal welfare. Nowadays, the use of “special compound feeds”, to simulate the fat composition of the acorns through the inclusion of sources of oleic acid – among others- leads to high oleic acid in the adipose tissue of the animals, like those found in pigs fed outdoors. The traditional rearing system with acorns, which is natural and seems simple, it is in practice highly sophisticated, leading to many opportunities for mislabeling and fraud and causing much confusion in the market, where prices for a cured leg of Iberian ham range from hundreds to thousands of euros. Currently, the official quality-control systems used for determining the feeding regime of IP are just based on on-farm inspection, 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. Both methods are costly and time-consuming, and only provide information on batches of animals rather than individual pigs, an important consideration where the product, as in this case, reaches high market prices.

Previous research undertaken by the report’s authors have demonstrated the ability of NIRS at-line not only for the prediction of the fatty acid profile, but also for classification of carcasses according the feeding regime or in other words, according the price [1], [2]. The main goal of this study is to evaluate, fine-tune, validate and implement a portable miniaturized NIRS spectrometer (MicroNIR Onsite Lite, Viavi Solutions Inc.) for on-site quantitative (fatty acid profile) and qualitative (Premium, Non-premium) of individual Iberian pork carcasses at the slaughterhouse. The data set comprised 495 samples from 45 different producers. The samples were measured in two years: 66 were measured in 2016 and the remaining 429 in 2017. Of the 495 samples, 265 were premium grade (acorn and grass fed) and 230 were non-premium grade (compound feeds).

Quantitative models were developed using PLS, Bayesian approach and LOCAL regression methods and were evaluated through the standard errors of cross validation (SECV) or standard errors of prediction (SEP) ranging from 0.83 to 0.84 for palmitic acid (C16:0), 0.94 to 0.99 for stearic acid (C18:0), 1.47 to 1.56 for oleic acid (C18:1) and 0.53 to 0.58 for linoleic

acid (C18:2). In general terms, the results obtained with the MicroNIR portable instrument are quite a bit worse than the obtained previously using more expensive monochromator instruments at-line[3].

For classification purposes, we used the spectral data to make a direct classification as Premium or Non-premium classes, without going via a quantitative prediction of the fatty acids. Accepting from the outset that there will be samples for which the classification is uncertain, it seems important to be able to quantify that uncertainty. For this reason, the initial focus is on approaches whose output has the form of probabilities of class membership. There are many different algorithms that can be used to build such classification rules, too many to try them all on any one application. One major consideration in choosing an algorithm is that it is desirable that it should give probabilities for each category, not just a yes/no assignment. The appropriate action in the case of a report on a supposed premium carcass that "This is non-premium category with probability 0.55" is likely to be different from that following a report that "This sample is non-premium with probability 0.99". The focus has thus been on methods that return probabilities, and on the examination of those probabilities to see how well-calibrated they appear to be. Linear discriminant analysis, Quadratic discriminant analysis, a nonparametric approach and other classifiers (Logistic regression, Support Vector Machine, Ensemble subspace discriminant and Ensemble bagged trees) were evaluated.

The three Bayesian methods evaluated provided acceptable results in terms of classification success (up to 98% of sample correctly classified in validation with 230 calibration samples with probabilities of 0.9 or over), though the probabilities from QDA are clearly too extreme. Logistic regression gives similar results and will be investigated further to see if it can be tuned more successfully and examine the quality of the probabilities it produces. The work reported here clearly demonstrates the feasibility of using the MicroNIR device for on-site classification of carcasses. However, more samples would be desirable since, although 45 producers are represented, many of these only contribute a small number of samples, whilst some contribute 40 or 50. It would have been desirable to validate by splitting the samples according to producer. Given the above, in 2018 a total amount of 199 pig carcasses belonging to 12 different producers were analysed. These are currently being used to validate the models and will be added to the current dataset.

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O5.1

DATA SHARING AS A SOLUTION TO IDENTIFY FOOD ISSUES EARLY

Lucas Luijckx, N.B.^{1,*}, Brewster, C.¹, van de Brug, F.¹, Minnens, F.², Verbeke, W.²

1-TNO Innovation for Life, Zeist/Soesterberg, the Netherlands

2-Ghent University, Ghent, Belgium

*corresponding author e-mail: Niels.lucasluijckx@tno.nl

Keywords: data sharing, trust, early identification, data analysis

Issues and incidents around food products have always arisen and will always arise again. Stakeholders in the food production network work together from different perspectives and responsibilities to prevent or mitigate these issues and -preferably- to be prepared for any emerging issues. Different stakeholders will use different tools and resources to arrange their risk management processes. Also, they have differing access to data that support these processes. Besides, there is such an overwhelming amount of (big) data that it is almost impossible for individual stakeholders to find the relevant information, check for completeness or gaps and interprets that information.

Under the EU project Food Integrity a feasibility study was performed on data sharing in the food production network with the aim to identify food integrity issues, at the earliest possible moment. This package consisted of two major pillars of work: a stakeholder survey in three rounds and a detailed analysis of existing systems and methods on data sharing and analysis. Two main questions were the basis of this project: (1) Are stakeholder willing to share information within the network and under which conditions, and, (2) what would a sharing system look like in view of current technologies?

We present a global framework or architecture for a data sharing system with an outlook on the type of data sources, including a view on stakeholder involvement. Also, what information and scenario analyses could evolve from it. A position on the governance of such a system and the conditions under which it could operate will be discussed. This latter part of discussion will be largely based on the outcomes of the stakeholder survey that reported the opportunities and strengths of information sharing among stakeholders, but also the potential hurdles.

O5.2

FINDING THE NEEDLE IN THE HAYSTACK: PREVENTING FOOD FRAUD BY COMPARING PRODUCTION VOLUMES WITH TRADE VOLUMES

Herrmann, G.A.^{1,*}

1-Organic Services, Tutzing, Germany

*corresponding author e-mail: g.herrmann@organic-services.com

Keywords: food fraud prevention, integrity management, cloud-based solution, supply chain integrity

Dealing with the issue of food fraud can feel like looking for the needle in the haystack. There are hardly any witnesses as consumers often become victims of the crime unknowingly, and even food companies that buy food products are often unaware of the exact origin and history of the product that they purchase. It is therefore not surprising that when food fraud scandals rocked the food industry (such as the horsemeat scandal), the European competent authorities, law enforcement, and policymakers found themselves staring at the haystack, not knowing where to start. The European Union's decision to turn to science, asking them to help find the needle – was the right reaction at a pivotal moment, and what led to the inception of the FoodIntegrity project.

The FoodIntegrity project chose a focus on analytical science and originally consisted of 13 work packages. After a gap analysis was conducted, 9 additional work packages were added to the project. Based on the gap analysis, a new topic issue was added to the agenda of the FoodIntegrity project: the issue of supply chain integrity and information-sharing between supply chain actors. Therefore, Organic Services joined the FoodIntegrity project with its work package 16: Improving Supply Chain Integrity through Data Sharing.

Now that our studies as well as the FoodIntegrity project are coming to an end, we look back at our learnings: based on our research, we conclude that comparing production volumes of food commodities with trade volumes of food commodities by establishing mass balances is an effective food fraud prevention and detection measure. Based on 4 case studies and interviews with 20 food supply chain actors and food industry experts, we can describe the many different techniques used to commit food fraud as resulting in the sale of a less valuable product disguised as a more valuable product. That means that the volume of the more valuable product is artificially increased along the food supply chain. When combined with certification data, a mass balance approach detects when traded volumes are not plausible and therefore prevents food fraud.

System-wide solutions based on registration or certification of all system participants can be envisioned. Based on our feasibility study, we identified three prerequisites for successful Check X system applications:

- All required supply chain actors within the defined system must participate,
- All production and trade must be recorded/ (estimated), and
- All relevant product qualities must be recorded.

With these prerequisites, the mass balance approach requires much less data than for example a batch traceability approach, is less cumbersome and prone to gaps than other approaches such as internal audits and/ or the building of close business relationships and can prevent fraud from happening rather than detect it once it has happened.

Finally, we can conclude that this is where our contribution to the FoodIntegrity lies, and what the contribution of our integrity management solution Check X aspires to be. If we return to the metaphor of searching the needle in the haystack, then analytical science and specifically product analysis – by focusing in on understanding the outcomes of fraudulent practices – is a way of understanding the needle. This allows companies to avoid the purchase and trade of such tainted food products. And it allows law enforcement to trace the steps from the needle to the one who committed the fraud. However, if you don't know the character of the needle, the choice of the adequate tool for identifying the needle can be cumbersome, expensive and may fail. Our integrity management solution Check X proposes a different approach: Instead of following the assumption that food fraud is taking place and detecting fraudulent practices as they manifest in the product, food fraud defense programs should use their knowledge to prevent food fraud by comparing produced volumes to traded volumes and preventing food fraud before it has occurred. What we propose is to define the properties of hay, and the size of the hay stack, thus excluding anything that does not fit these definitions, and complement this approach with targeted analytical tools.

O5.3

A SYSTEMATIC REVIEW INTO EUROPEAN CONSUMER PERCEPTIONS OF FOOD AND AUTHENTICITY

Kendall, H.^{1,*}, Clark, B.¹, Rhymer, C.¹, Kuznesof, S.¹, Brereton, P.¹, Frewer, L.J.¹

1-School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU.

2-School of Biological Sciences, Queens University Belfast, Belfast, 97 Lisburn Road Medical Biology Centre, Belfast, BT9 7BL.

*corresponding author e-mail: helen.kendall@newcastle.ac.uk

A number of recent incidents of food fraud detected across European food supply chains have challenged public confidence in the authenticity of food and reduced consumer trust in the integrity of food system actors and the governance of supply chains. Given the increased prominence of food fraud instances across Europe, it is important to understand consumer attitudes towards food fraud and the relationship between consumers and aspects of food integrity, such as authenticity, trust and risk-benefit perception. A systematic review of the literature of European consumer attitudes was undertaken to synthesise interdisciplinary evidence available in this area, in order to support existing and future research and identify gaps in knowledge for research prioritisation. Three databases were searched, Scopus and ISC Web of Knowledge and Google Scholar, for empirical studies published in the past 20 years.

The results were screened in a two-stage process, yielding 16, published from 2012 onwards. Given the diversity of data generating methodologies adopted by the studies (including willingness-to-pay assessments, in-depth interviews, focus groups, social media analysis, online deliberations and surveys), thematic analysis was undertaken. Results reveal consumer concern regarding food fraud, with response varying across European countries. Consumer attitudes towards traceability systems were shown to be positive, with positive attitudes and beliefs and willingness to pay for traceable products that could guarantee the authenticity and safety of food. Preliminary analysis suggests similar drivers of consumer concern to those identified by perceptual work conducted with Chinese consumers within the EU Commission funded Food Integrity project, however a more formal empirical analysis is needed.

From a policy perspective, the findings support the identification of consumer's concerns regarding the potential risks associated with food fraud, which will enhance consumer communications intended to abate consumer worries in this regard. From an industry perspective it provides important insights to companies operating or wishing to operate within Europe regarding consumer attitudes and expectations with regards to food authenticity and quality and supports product differentiation within this market.

O5.4

TRACING MEDITERRANEAN HIGH VALUE FOOD PRODUCTS: THE REALMED APPROACH

Alegria, C.^{1,*}, Giovanetti, M.¹, Araus Ortega, J.L.², Ogrinc, N.³, Podgornik, M.⁴, Camin, F.⁵, Taous, F.⁶, Lauteri, M.⁷, Reis, P.⁸, Atti, N.⁹, Máguas, C.¹

1-cE3c–Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal;

2-Universitat de Barcelona (UB), Barcelona, Spain

3-Jožef Stefan Institute (JSI), Ljubljana, Slovenia

4-Science and Research Centre Koper, Institute for Oliveculture, Koper, Slovenia

5-Fondazione Edmund Mach (FEM), San Michele All'Adige, Italy

6-Centre National De L'énergie, Des Sciences Et Techniques Nucleaires (CNESTEN), Rabat, Morocco

7-Istituto di Biologia Agroambientale e Forestale (IBAF), Porano, Italy

8-Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

9-Institut National de Recherche Agronomique de Tunisie (INRAT), Tunis, Tunisie

*corresponding author e-mail: csmaleqria@gmail.com

Keywords: Mediterranean products authenticity, Iberian black pig, acorns, isoscapes

REALMed is an international project that promotes the authenticity and valorisation of Mediterranean (Med) traditional products. Its aims are to provide reliable technological tools for authentication and quality assessment; to make these tools largely accessible to stakeholders and consumers; and to promote traditional knowledge and the sustainable development of local economies. REALMed offers the unique opportunity for a transdisciplinary approach, addressing authenticity of traditional value products by applying innovative research techniques managed by a permanent team of experts. REALMed products under testing are: the Iberian black pig (Portugal and Spain), Argan Oil (Morocco), Truffles (Italy and Slovenia) and Mountain Lamb and Kid (Tunisia). All these products are highly recognized by own intrinsic and extrinsic quality traits.

Most are especially valuable due to existing links to their origin. The Med area is globally recognised for boosting healthy dietary lifestyle driving consumers to demand proof of authenticity and, to achieve this, there is the need to develop tools able to reach the market and policy efforts to confirm the authenticity of Med products. Clear geographical references can increase food products competitiveness and even broaden the range of consumers based on products reputation. Nonetheless, geographical references are insufficient to guarantee the origin of the food product, but when linked to the uniqueness of the productive system environment, the products geographical origin can be confirmed and traced. Among methodological approaches for determining the geographical origin, isotopes are one of the best tools for fingerprinting. Stable isotope ratio analysis (SIRA) is a well-established and acknowledged method for authenticity testing, being able to differentiate substances from its unique isotopic signature, reflecting the biochemical and physiological processes, climate, geography, geology, agricultural practices, and processing factors. Isoscapes are a way to visualize the complex information deriving from isotopic ratios analysis and can be considered as a template for data analysis and visualization, and as a common platform and language for establishing a dialogue among specialists and the public.

The innovation of REALMed approach is clearly associated with a mechanistic framework (from local to regional scales) based on GIS and statistical models, that will enable analysis of the relationships between isotope ratios and the fractionation processes associated with local climate data, as well as plant and animal physiology, geology and pedology: Isoscapes. In this sense, this project seeks the integration of isotope analysis and climatic-geologic-geographic data and the application of computational mathematics to reach origin authenticity of Med products. Indeed, REALMed is invested to deliver a faster, cheaper and user-friendly way to authenticate Med products, through isoscapes of carbon, oxygen and nitrogen to answer scientific or stakeholders and producers' questions regarding the provenance of Med products via their isotopic signatures. In the Iberian Peninsula, target product is the renowned Iberian black pig and acorns are an integrant part of its feeding system, responsible for the meat and derived products prime-quality (fatty acid and aromatic composition). Grazing is restricted to the "montanheira" (pannage) period, corresponding to the beginning of the annual pasture production cycle and acorn production (October to February).

Due to the particularity of this feeding system, acorns can be used as tracers of geographic origin for the Iberian black pig through respective isotopic signatures. Thus, for the Iberian black pig stable isotope profile definition, a massive acorn sample collection has already been carried out: 82 sites, 110 and 46 acorn samples of *Quercus rotundifolia* and *Q. suber*, respectively (each sample represents an individual tree). Pork fat sample collection as also started, with samples being collected from independent pig herds from different producers (each sample represents 30% of a pig herd). Multivariate analysis and geospatial data analysis were applied to illustrate the result of the correlation between acorn isotopic composition and environmental variables, as well as the correspondent values of pork fat tissues. The results indicate that is of crucial importance for the future Iberian black pig meat/products geographic origin strategy, to define the relation between acorn isotopic signature and pig fat production during the "montanheira" period.

Acknowledgments

REALMed is funded by ARIMNet2 -2014-2017, an ERA-NET coordinated by INRAFrance and funded under the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration, under grant agreement no. 618127.

O6.1

A STANDARDIZED DEFINITION OF TERMS RELATING TO FOOD AUTHENTICITY AND FOOD FRAUD

Olsen, P.^{1,*}

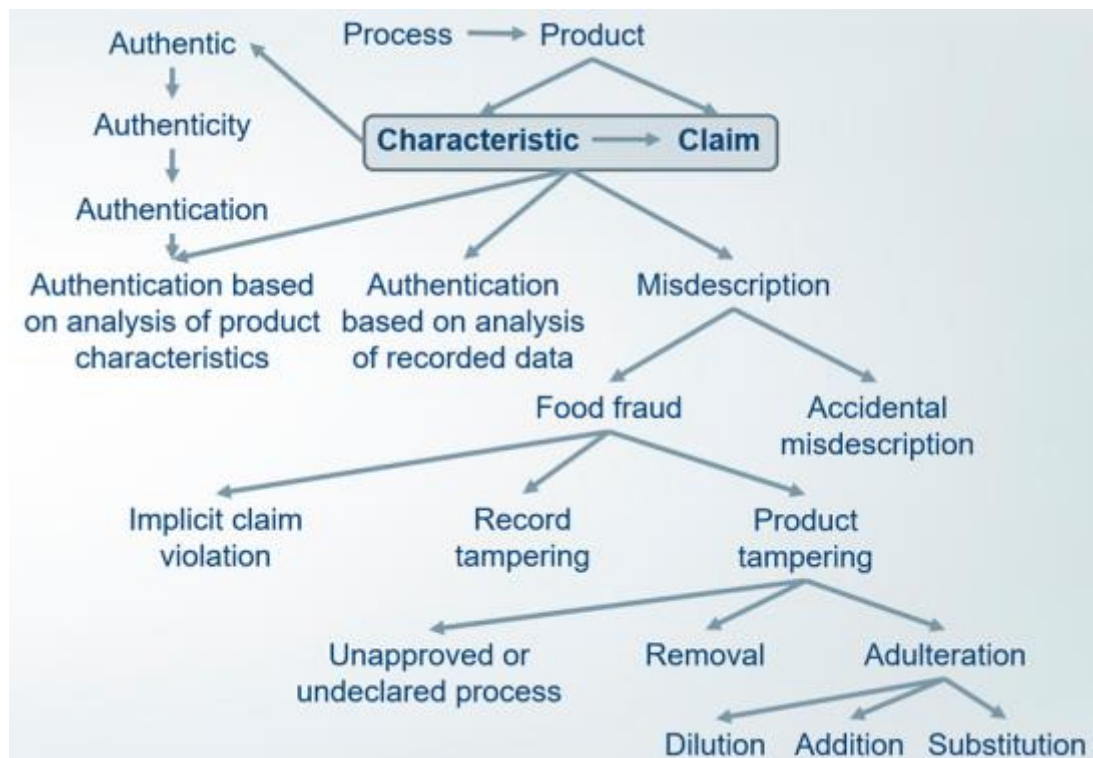
1-Nofima, Tromsøe, Norway

*corresponding author e-mail: petter.olsen@nofima.no

Keywords: authenticity, food fraud, standard

Food authenticity and food fraud are multi-disciplinary research fields without well-established definitions for the related terms and concepts. For the research in this area to progress, it is important to arrive at a common understanding of what various terms and concepts mean. Various general standards exist, and various specialized standardization efforts are underway.

This presentation focuses on the consensus-based European standard "CEN WS/86 - Authenticity in the feed and food chain – General principles and basic requirements" that was delivered by the H2020 Authent-Net project, and it highlights the discussions and decisions that were made during the standardization process, and what the final results and recommendations were.



A key distinction in the standard is between actual product 'characteristic' and related product 'claim'. It is when there is a mismatch between these two that we get 'misdescription' and 'food fraud', and this mismatch underlies the definitions of the various types of food fraud, and the other related terms.

O6.2 GUIDANCE DOCUMENT TOWARDS FOOD AUTHENTICITY, AN INITIATIVE OF ILSI EUROPE.

Pöpping, B.^{1,*}, Buck, N.², Bánáti, D.³, Brereton, P.⁴, Calancha, M.⁵, Gadanho, M.⁶, Gendel, S.⁷, Kelly, S.⁸, Lesueur, C.⁹, Menzel, M.¹⁰, Morling, A.¹¹, Saner, S.¹², Spink, J.¹³, Wiggers, V.-D.¹⁴

1-Scientific Advisor and Chair of the Expert Group at ILSI Europe Authenticity of Food Task Force, Frankfurt, Germany

2-General Mills and Chair of the ILSI Europe Authenticity of Food Task Force, Nyon, Switzerland

3-ILSI Europe, Brussels, Belgium

4-Queen's University, Belfast, UK

5-Arla Foods, Leeds, UK

6-SGS, Lisbon, Portugal

7-United States Pharmacopeia (USP), College Park, Maryland, United States

8-International Atomic Energy Agency (IAEA), Vienna, Austria

9-Danone, Paris, France

10-Südzucker Group, Obrigheim, Germany

11-Food Standards Agency (FSA), London, UK

12-Mérieux NutriSciences, Istanbul, Turkey

13-Michigan State University, Michigan, USA

14-Cargill, Wormer, The Netherlands

*corresponding author e-mail: bert.popping@focos-food.com

Keywords: food authenticity, gap analysis, novel approaches, tools

The International Life Sciences Institute (ILSI) brings together scientists from industry, academia and the public sector to deliver science of the highest quality and integrity in the areas of food safety, nutrition, consumer behaviour and sustainability. ILSI has established a Task Force on Food Authenticity with members from governmental and inter-governmental organisations, the food industry, and academia. The immediate purpose of this group is to review the field of food authenticity and provide both a concise overview and identify best practices. In a first phase of the work an Expert Group is studying umbrella topics that are independent of the type of ingredient, these topics include:

- Definition and terminologies related to food authenticity
- Sources of data on existing and emerging food authenticity incidents
- Identification and description of existing and planned governmental or official projects
- Identification and consolidation of existing approaches to managing food authenticity that are used by food manufacturers

The first phase of work will be followed by a second phase wherein specific types of food authenticity will be reviewed, such as those related to geographic origin, and issues related to specific categories of food commodity and ingredients. The purpose of the second phase will be to identify known vulnerabilities and mitigation methods for those specific foods. In both phases of the work, it is not intended to create new tools, rather to provide a consolidated understanding of food authenticity and identify best practices. However, in a final phase it is intended to review the information collected, for the purpose of identifying gaps and potentially proposing solutions to close these. In this presentation we will discuss the plan and progress of the ILSI Expert Group.

O6.3

HOW CAN FOOD SAFETY STANDARDS HELP IMPROVE FOOD FRAUD MITIGATION?

Séchet, B.^{1,*}

1-IFS Management, Paris, France

*corresponding author e-mail: sechet@ifs-certification.com

Keywords: food fraud, mitigation tools, food safety standards, risk assessment tool

As one of the GFSI benchmarked standards, IFS has been working on implementing food fraud mitigation plan into its requirements after the “horse gate” scandal. IFS recognized that one of the main issues is related to the lack of knowledge of the different actors within the supply chain. Therefore, a Product Fraud Mitigation Guideline has been developed to encourage stakeholders to gain more knowledge about the existing risks related to their products and suppliers. In addition, IFS will offer to all its members, a risk assessment tool, to help in the identification of the major risks within a given product sector or geography. This tool is linked with the major international food safety alert databases. After highlighting the international context, the presentation will give an overview of the pragmatical approach recommended by the IFS and the tools that are proposed.

O6.4

FOOD FRAUD AND FOOD AUTHENTICITY: NEW DEFINITIONS AND A NEW APPROACH

Carter, J.

1-IFAAO

*corresponding author e-mail: joanne@truefoodies.com

The efforts to address food fraud have been largely, if not entirely, unsuccessful. When food fraud became an issue again after melamine in milk in China, and horsemeat in lasagna in Europe, the food industry assigned responsibility for food fraud to food safety professionals. While food fraud can sometimes present food safety problems, looking at food fraud from a strictly food safety perspective has resulted in traditional food safety approaches being used to address a problem which is economic and criminal.

It is important to take a fresh look at food fraud and the newer concept of food authenticity. A new kind of standard is necessary to address these issues and governments and organizations are struggling to find new approaches. Notwithstanding this fact, Codex Alimentarius and other organizations have begun to look at food integrity, fraud and authenticity through slightly different lens.

The first step in developing a new approach is to establish definitions. Definitions set the tone for the approach to food fraud and authenticity. IFAAO will present the historical definitions of food fraud and comment on their benefits and their weaknesses. IFAAO will also discuss the global approaches to food fraud and talk about a new solution which requires collaboration from multiple stakeholders.

O7.1

THE FUTURE OF NGS (NEXT GENERATION SEQUENCING) ANALYSIS IN TESTING FOOD AUTHENTICITY

Haynes, E.^{1,*}, Pardo, M.A.², Jiminez, E.², Helyar, S.³

1-Fera, York, UK

2-AZTI, Derio Bizkaia, Spain

3-Queen's University Belfast, Belfast, UK

*corresponding author e-mail: edward.haynes@fera.co.uk

Keywords: Next Generation Sequencing, authenticity.

The authenticity of foodstuffs is an important issue for consumers, regulators, producers and processors, as fraudulent practices can negatively affect consumer confidence and safety, as well as the operating models of legitimate businesses. This review provides an overview of the current applications of Next Generation Sequencing related to food authenticity, highlighted through a number of case studies. Additional, specific areas of interest included the range of NGS platforms available, databases which can be interrogated, bioinformatic tools (both open source and commercially operated), validation of NGS, and limitations and appropriate uses of the technologies. We also make suggestions as to how NGS may be applied in the future at a range of levels, including control laboratories, producers and retailers.

07.2

NGS-BASED METABARCODING FOR THE ANALYSIS OF PLANT FOOD PRODUCTS

Logacheva, M.D.^{2,*}, Krinitsyna, A.A.^{1,2}, Omelchenko, D.², Speranskaya, A.S.^{1,2,3}, Fedotova, A.V.¹, Khafizov, K.^{2,3}, Ayginin, A.A.^{2,3}, Kasianov, A.S.^{2,4}

1-Lomonosov Moscow State University, Moscow, Russia

2-Skolkovo Institute of Science and Technology, Moscow, Russia

3-Central Research Institute of Epidemiology, Moscow, Russia.

4-Vavilov Institute of General Genetics, Moscow, Russia.

*corresponding author e-mail: maria.log@gmail.com

Keywords: metabarcoding, herbal teas, spices, honey

Plants play an essential role in human nutrition being the key components of many food products. They are especially popular as a component of spices and health products. Consumers tend to perceive plant products (especially from wild growing plants) as “healthy” and “natural”. However, this is not always the case; the lack of quality control can lead to serious health problems. Incongruence between the labeled and real composition of food products of the plant origin is an issue. Most often, it happens due to the adulteration for economic benefit or errors during collection and processing of raw plant materials. Thus, it is important to have a reliable instrument for precise analysis of food composition to ensure food safety and quality. A very promising approach for the analysis of such products is DNA metabarcoding due to its high resolution and sensitivity.

However, its application to food analysis requires several methodology optimizations in DNA extraction, amplification and library preparation. We tested and optimized protocols for DNA extraction from a wide range of plant-containing food (herbal teas and coffee substitutes, spices, honey, candies) available on Russia market, assessed DNA quantity and quality and performed metabarcoding analysis using nuclear marker ITS1. We found widespread deviations from the declared content. In particular, many herbal teas lack declared components; the presence of extraneous components was also registered. The most frequently detected undeclared plants are common field weeds (*Elymus*, *Convolvulus*, *Calystegia*) that could be mixed with product component during collection of raw plant material from fields or from the wild and several edible cultivated plants (*Triticum*, *Secale*, *Brassica*, *Coriandrum*) that could be mixed with product during transportation, storage and/or packaging. In a set of herbal teas that were claimed to contain fireweed (*Epilobium angustifolium*, a tea substitute popular in Russia) we found instead *Lythrum salicifolia*, a plant with similar appearance but different biological activity.

For honey the most widespread deviation from the declared content is the presence of sunflower. In Russia sunflower honey is one the cheapest honey varieties thus this is the most likely economically motivated adulteration. The widespread adulteration and/or contamination of plant food products call for the wider application of metabarcoding for their analysis and for the reconsideration of customers' attitude toward these products. The study is supported by the Ministry of education and science of Russia; project # 14.609 21.0101, unique identifier RFMEFI60917X0101.

O7.3

A NOVEL METABARCODING APPROACH TO COMPLEX FOODS: USING THE OXFORD NANOPORE'S MINION TO FIGHT FRAUD

Helyar, S.^{1,*}, Dicks, K.¹, Gavin King, P.¹, Haughey, S.¹, Elliott, C.¹

1-Institute of Global Food Security, Queen's University Belfast, Belfast, UK

*corresponding author e-mail: helyar@qub.ac.uk

Keywords: NGS, herbs, method comparison, complex food matrix.

This study used the Oxford Nanopore Technologies MinION, a novel, rapid NGS platform to determine the authenticity of herb samples and to quantify the levels of adulteration. While the error rates for the platform are high, and less well understood than for more established platforms such as Illumina, these issues can be addressed. The oregano samples we tested here had been previously tested with a LC-MS/MS method, allowing us to compare the results.

A three locus, tiered approach was utilised for the sequencing, based on the combination of easily amplifiable and alignable *rbcL* region (which does not always discriminate between closely related species), combined with *matK* (which does not amplify all species due poor conservation in the universal primer-binding site), and the highly variable ITS2 region. These three fragments also range in size from ~500bp to ~1500pb, so balancing use of the MinION advantage for long sequencing reads, with the potentially degraded DNA present in the herb samples. These 3 loci are employed together for the identification of all plant species present in the herb samples. Known reference samples (both fresh and processed) were sequenced along with commercial samples.

To date, researchers have been utilizing conventional methods including a range of chemical and genetic techniques to target food authenticity. However, these methods are generally either costly, time consuming, or both, delaying effective and timely control actions. The emergence of new tools for real-time diagnostics, such as the Oxford Nanopore MinION, have recently proven useful for the rapid detection of Ebola and Zika viruses, even in low resourced laboratories. Here we demonstrate that these tools can now be applied to food authenticity.

O7.4 MINIATURIZED DEVICE FOR ISOTHERMAL DNA AMPLIFICATION

Carvalho, J.^{1,*}, Garrido-Maestu, A.¹, Azinheiro, S.¹, Ipatov, A.¹, Prado, M.¹

1- International Iberian Nanotechnology Laboratory, Department of Life Science, Nano4Food Unit, Food Quality & Safety Research Group, Braga, Portugal

**corresponding author e-mail: joana.carvalho@inl.int*

Keywords: Isothermal DNA amplification, miniaturized device

In the last years, the interest for DNA-based analysis in the food sector has grown exponentially. Many studies have been published using these techniques for different applications, such as foodborne pathogen detection, identification of genetically modified organisms (GMOs), detection of allergenic ingredients and food authenticity and traceability applications. DNA is a very interesting target molecule for food authenticity studies due to its high stability and durability when compared to other molecules, such as proteins. In addition, it is present in every cell of plants and animals, which are the major constituents of food, and the DNA sequence is independent from geographical, climatic or agronomical factors [1]. However, these techniques usually require large specialized equipment and trained personnel, being still very expensive and time-consuming, therefore limiting their application in the food industry.

The miniaturization of well-established DNA analysis methods brings several advantages compared to the conventional approaches, providing efficient analysis tools to the food industry and food control authorities. Miniaturized DNA analysis devices require smaller volumes, allowing the use of less quantity of reagents, reducing the costs of analysis and improving the performance of the system by allowing faster and more sensitive analysis [2]. Moreover, these technologies are more suitable for automatization, decreasing the risk of contamination during the analysis process. Another advantage is their portability, allowing in situ detection.

One of the main steps of DNA analysis is DNA amplification, being polymerase chain reaction (PCR) the most widely adopted method. However, in the last years, the development of alternative isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), has been increasing [3, 4]. These methods present several advantages over PCR, such as the ability to be performed at constant temperature, the higher tolerance to the presence of inhibitors, the reduced reaction time and, in the case of LAMP, the possibility of naked-eye detection [5, 6]. Moreover, the equipment and trained personnel requirements are greatly reduced with these approaches. These advantages also make isothermal DNA amplification techniques more suitable to be integrated in a miniaturized device.

At the International Iberian Nanotechnology Laboratory (INL), our research group is working on the development of tailored, miniaturized and automatized devices to perform the steps of DNA analysis. Regarding DNA amplification, we have been working on the development of isothermal amplification methods for different applications and we are currently developing and optimizing a miniaturized device to perform this type of amplification. This device includes a high-performance closed-loop Peltier module for temperature control connected to a precision platinum sensor for temperature monitoring. Preliminary results have shown the potential of this device to perform isothermal DNA amplification.

Acknowledgments

This work was supported by the project NANOeaters (0181_NANOEATERS_1_e), under the EP - INTERREG V A España Portugal (POCTEP), and by the project Nanotechnology Based Functional Solutions (NORTE-01-0145-FEDER-000019) supported by Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). It was also supported by the partnership agreement project between the Confederación Hidrográfica del Guadalquivir and the International Iberian Nanotechnology Laboratory for the development of a system of early detection of the zebra mussel through analysis of environmental DNA.

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O7.5

AUTHENTICATION OF SHRIMP PRODUCTS IN THE UK

Naaum, A. M.^{1,*}, Helyar, S.¹, Elliott, C.¹

1-Institute for Global Food Security, Queen's University Belfast

*corresponding author e-mail: naauma@gmail.com

Keywords: shrimp, DNA barcoding, food authenticity

Food fraud is a well-documented global issue in the seafood industry. DNA methods are often employed to not only identify baseline issues, particularly in cases of species substitution, but also to prosecute offenders and help monitor supply chains. There have been some studies published to date of shrimp authenticity using various approaches; however shrimp is heavily underrepresented in studies of seafood fraud compared to fish. This is of particular concern in shrimp, as fraud may further be linked to support of modern day slavery, which has been identified as an issue for this commodity. In these cases, geographical origin is also of critical importance. Market products from the UK will be tested using DNA barcoding to determine the incidence of species mislabelling in shrimp products commonly available for consumer purchase. This data will be used to develop a portable test for real-time PCR identification of specific target species using a novel, lab-free, portable system. In addition, novel spectroscopic methods are used to determine the geographical origin of shrimp products tested, providing a further level of authentication. This work will help establish a baseline for mislabelling of species and/or geographical origin in shrimp products purchased in the UK, presented here for the first time. The available authentication methods will be compared to identify best practices for shrimp authenticity testing, with recommendations on their potential for use within the supply chain to help assure authenticity and increase transparency for shrimp products.

O8.1

USING THE FOOD AUTHENTICITY KNOWLEDGE BASE: CASE STUDIES FOR FOOD OPERATORS AND REGULATORS

Maquet, A.^{1,**}, Morin, J.-F.^{2,*},

1-European Commission, DG Joint Research Centre (JRC), Geel, Belgium

2-Eurofins, Nantes, France

*corresponding authors e-mail: JeanFrancoisMorin@eurofins.com

**alain.maquet@ec.europa.eu

Keywords: knowledge base, food regulator, industry, vulnerability assessment, mitigation

The latest Global Food Safety Initiative (GFSI) Guidance Document includes recommendations on how industry should counter food fraud. Main food safety management schemes such as the British Retail Consortium Standard (BRC) and the International Featured Standard Food (IFS Food) have now included specific requirements for mitigating food fraud in their certification schemes.

The Food Authenticity Knowledge Base, an on-line database developed by FoodIntegrity and hosted by the Joint Research Centre within the EC Knowledge Centre for Food Fraud and Quality, helps food business operators and regulators tackling food fraud and complying with the EU regulation and available guidance documents. It provides information on various food fraud practices and frequently used adulterants together with recommended analytical strategies.

Several examples, including different food matrices, illustrate the Knowledge base added-value and how it can be used by stakeholders. Initiated either by an external alert (e.g. JRC Food Fraud Reporter) or by a suspicion, a search in the Knowledge base helps stakeholders to identify existing testing solutions for ensuring authenticity and safety of a given food product or ingredient.

As part of a vulnerability assessment or during the design of a mitigation plan, the Food Authenticity Knowledge Base will help documenting when, where and how the integrity of food products entering or leaving a factory is verified.

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www.foodintegrity.eu

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<https://ec.europa.eu/jrc/en/food-fraud-and-quality/monthly-summary-articles>

O8.2

THE FOOD AUTHENTICITY RESEARCH NETWORK (FARNHUB) FOR SHARING AND ACCESSING INFORMATION ON FOOD AUTHENTICITY ACTIVITIES

Vermeulen, P.*¹, Berg Sorbahl, P.², Tomaniova, M.³, Olsen, P.², Baeten, V.¹

1-Food and Feed Quality Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

2-NOFIMA, Tromsø, Norway

3-Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic

*corresponding author e-mail: p.vermeulen@cra.wallonie.be

Keywords: food, authenticity, fraud, web platform

One of the objectives of the Authent-Net project is to establish a dynamic and sustainable European information platform, named the "Food Authenticity Research Network Hub (FARNHub). This platform is a web-based portal where users can get an overview of currently available resources related to food authenticity for each country or for each food sector. This includes papers and documents (scientific or other), ongoing projects, online databases, an overview of funding bodies with contact points, news stories and regulations on food authenticity. Analytical methods are addressed by the Food Integrity project (WP2) through the [Food Integrity Knowledge Base](#)

The FARNHub application is now available online on <http://farnhub.authent.cra.wallonie.be/> for search and view content. By giving open access to this web tool, all possible users who have an interest in food authenticity (funding bodies, industries, regulatory authorities, research organisations and other stakeholders) can benefit from the hub and its content.

A map available on http://www.authent-net.eu/AN_FARNH_click_map.html gives statistics on the number of publications, projects, news, and includes links to the 14 National status reports and 4 commodity status reports developed by groups of experts from the resources stored in the FARNHub tool.

Any update, adding can be suggested and provided to email farnhub@cra.wallonie.be. A network of national representatives involved in the Authent-Net project has been created to approve new entries and update the database.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 696371 (Authent-Net). The authors thank all the AuthentNet partners and in particular the countries representatives which can be contacted through farnhub@cra.wallonie.be.

O8.3

THE FOOD AUTHENTICITY NETWORK: THE ONE-STOP-SHOP THAT CAN HELP PROTECT THE INTEGRITY OF YOUR FOOD

Elahi, S.^{1,*}, Ellison, S.¹, Woolfe, M.²

1-LGC Limited, Teddington, UK

2-Food Authenticity Consultant to LGC Limited, Thames Ditton, UK

*corresponding author e-mail: Selvarani.Elahi@lqcgrou.com

Keywords: Food authenticity, fraud, mitigation

The Food Authenticity Network (www.foodauthenticity.uk/) is a free open access 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 government funded initiative that was born out of the 2013 horsemeat issue and brings together all those with an interest 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.

Membership is free and it's very quick to join so if you're not a member then please visit www.foodauthenticity.uk and sign-up today for the latest information on food authenticity. The Network is now 3 years old and has nearly 900 people from 41 countries registered as members of the website and >1,260 followers of the Twitter account (www.twitter.com/fauthenticity). The Network also has a Google page rank score of number 1 for a search on the term 'food authenticity'. In addition to the focus on food authenticity testing best practice, links to the major global food fraud mitigation guides have been placed on the Network so that supply chain integrity (mitigation) and testing is covered. The Network is a one-stop-shop for all things related to food authenticity testing and food fraud mitigation as shown by Figure 1.



Figure 1: Key Features of the Food Authenticity Network

Our vision for the Network is to grow it internationally so that it becomes a truly global network aimed at fighting food fraud in today's global food supply chain. To aid this, the Network will transition to being industry led from January 2019 at which point it will cease to be solely UK government funded. This talk will highlight the key features of the Network and outline our plans to create a global self-sustaining network aimed at fighting food fraud in a unified and cost efficient manner that can also help capacity building in countries that cannot afford to have dedicated food authenticity programmes of their own.

Acknowledgments

Department of Environment, Food and Rural Affairs
Food Standards Agency
Food Standards Scotland
Department of Business, Energy and Industrial Strategy.

O8.4 FOOD INTEGRITY “INDUSTRIAL INTEGRATION TOOLS”

Suman, M.^{1,*}, Lambertini, F.¹

1-Advanced Laboratory Research, Barilla G. & R. F.Ili SpA, via Mantova 166, 43122, Parma, Italy

*corresponding author e-mail: michele.suman@barilla.com

Keywords: industrial integration, food authenticity, practical tools, guidelines

Major risks related to food frauds are: (i) For the consumer: Immediate or long-term health issues, Unintentional consumption of substances contrary to particular ethical / religious beliefs, Financial loss (purchase of overpriced, adulterated or damaged goods); (ii) For food business stakeholders: Decrease of consumers' confidence in the whole sector, Damaged reputation, Financial loss (purchase of overpriced, adulterated or damaged products or raw materials). In this presentation, it will be given a deeper look into FoodIntegrity EU Project (FI) Industrial Integration work, delivering concrete tools and information on how food industry can protect the business from fraud issues, hence strengthening the European food supply chain and assuring the circulation of authentic and reliable products.

Among practical instruments that help food industries into assuring the integrity of their products, FoodIntegrity provided online resources such as FI Knowledge Base & FI Network (simplifying, the first one to find the right analytical strategies to use; the second to suggest which organizations/authorities in different countries have the right expertise for performing them). Whether there is the need to defend the quality of food products against frauds by legal means, stakeholders can rely on FI scientific opinions that can be used as a reliable point of reference. A selection/evaluation of relevant chemical/molecular-biology markers from the industrial perspective, which can be connected to specific quality/authenticity aspects of both raw materials/ingredients and corresponding finished products has been created.

Testing and validation of: (i) rapid screening high-throughput technologies (whether they are targeted or non-targeted) & multivariate approaches, (ii) profiling/fingerprinting/ targeted or non-targeted fundamental methods have been executed. A complete set of infographics concerning foods, risks, analyses and specific case studies is also available online, together with a so-called FI App available for Android/ios smartphone platforms and designed to transfer useful tips to fight the daily battle against food frauds, as a useful starting point to comprehend the complexity of the matter and inspire the desire to learn more.

Finally, we have also laid a set of industrial guidelines, which describe the procedures that any company can adopt to prevent and counteract frauds through the whole supply chain.

Besides providing stakeholders and operators with materials accessible for immediate use, FI want to achieve long-term benefits for them and for everyone in the field: that is why a strong effort in dissemination activities and training to researchers and industrial operators has been put in place, permitting the access to the latest updates on the themes of interest from a cross-disciplinary point of view which brings together analytical chemistry, quality management, marketing, consumer science and economic science. Protecting the authenticity is more than a legal obligation. It is about offering a guarantee of safety, quality, health and taste. It is about preserving the trade, tradition and food culture of a company, giving to the correspondent food products more economic and ethical value and therefore strengthening the trust that consumers place in food industry itself.

Setting guidelines and toolboxes

✓ **Infographics**

✓ **App (Android, ios)**

App **FOOD INTEGRITY** → **Case**

Oil & fats

Type of risk
Addition of foreign/refined/deodorized/... oil to EVO.
Addition of pigments in order to improve the color.
Mislabeling.

Type of analyses
GC-MS; LC-MS; IRMS
HPLC-DAD; LC-MS
IRMS

Chemical Markers
GC-MS; LC-MS; IRMS
HPLC-DAD; LC-MS
IRMS

To bring together available data on industrially exploited analytical tool for detection of food fraud directly in your mobile device.

Looking for Solutions

□ **Industry Perspective - Matrix**

RAW MATERIAL	TYPE OF RISK (fraud, adulteration, contamination)	TYPE OF ANALYSIS	COMMENTS
<p>Milk and Derivatives</p>	Melamine addition to apparently increase the protein content in powdered milk	LC-MS Analysis, FTIR,...	High request of milk powders ww; at each stage of the food chain operators can do frauds to increase productions or to earn more money
	Adulteration with milk of not declared geographical origin	Stable isotopes analysis	
	Type of animal, if declared (cow, sheep, buffalo, etc...). Adulteration with milk of different animal species (e.g. sheep's milk instead of cow milk, etc...)	ELISA; LC-MS; qPCR (DNA probes), Metabolomic	
	Addition of non/milk fat&oil into dairy products	Fatty acids and tryglicerides composition&profiles (e.g. by GC-MS, DART-HRMS, MALDI,...)	
	Addition of water	Crisoscopic point; Vapor Pressure Osmometry; Conductivity	

□ **Industry Markers for Quality & Authenticity**

RAW MATERIAL/ INGREDIENT/PRODUCT	TYPE OF RISK (fraud, sophistication, contamination)	TYPE OF ANALYSIS USUALLY ADOPTED	CHEMICAL MARKERS
<p>Milk and Derivatives</p>	Substitution of natural mozzarella cheese with imitation	HPLC	Lysinoalanine
	Apparent increase of the protein content/dry matter	LC-MS	Melamine
	Substitution of PDO cheese with others	HPLC; GC; LC-MS; IRMS, ICPMS	Free aminoacids / peptides profiles. Stable isotope ratios ² H/ ¹ H, ¹² C/ ¹³ C, ¹⁵ N/ ¹⁴ N, ³⁴ S/ ³² S; Trace element profile
	Addition of other "non-milk" fats/oils	GC-MS; DART-HRMS	Fatty Acids/Triglycerides profiles
	Addition of pigments (e.g. beta-carotene,...) in order to increase color	HPLC-DAD	Carotenoids profile

O8.5 FOOD FRAUD EARLY WARNING SYSTEM

Donarski, J.^{1,*}, Rainford, J.¹

1-Fera Science Ltd, Sand Hutton, York, YO41 5LZ, United Kingdom

*corresponding author e-mail: JamesDonarski@Fera.co.uk

Keywords: Early Warning System, big data, economic analysis

Food Fraud in general is typically motivated by economic gains. Therefore, the occurrence of fraud is expected to be associated to prevailing economic conditions, and dynamics of international markets (e.g. changes in supply and demand due to natural disaster or crop failure, etc.) may present lucrative opportunities for fraudsters to commit fraud. As part of the FoodIntegrity project, we developed an Early Warning System (EWS), which monitors global signals of over 5000 foodstuff commodities utilising various statistical methods together with network analysis and unsupervised machine learning to identify ongoing disruptions as signs of a lucrative opportunity, and provides early insights allowing food businesses and regulatory bodies to proactively mitigate risks. This presentation will provide an overview of the system and will include a retrospective analysis of the horsemeat incident in January 2013 to demonstrate the utility of the system.

The improved availability of “big-data” in relation to food trade and the increased sophistication of learning algorithms enables opportunities to improve our understanding of conditions leading to economically motivated adulteration in foodstuffs. As supply chains become increasingly global such automated tools have the potential to become a key component in strategic response to fraud risk within the industry.

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

O8.6

DEVELOPMENT OF A FOOD FRAUD MEDIA MONITORING SYSTEM

Marvin, H.^{1,*}, Bouzembrak, Y.²

1-RIKILT Wageningen University and Research

2-RIKILT, Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands

*corresponding author e-mail: Hans.marvin@wur.nl

Keywords: food fraud, early warning, media

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. Within Work Pack 8 of the EU FP 7 project FoodIntegrity tools and approaches have been developed to help direct and prioritise industry monitoring and regulatory enforcement activities against food fraud.

As part of such early warning system, we developed a food fraud tool (MedISys-FF) that collects processes and presents food fraud reports published in the media worldwide automatically. MedISys-FF is part of the European Media Monitor (EMM) and is updated every 10 minutes 24/7.

Food fraud reports were collected with MedISys-FF for 16 months (September 2014 to December 2015) and bench marked against food fraud reports published in Rapid Alert System for Food and Feed (RASFF), Economically Motivated Adulteration Database (EMA) and HorizonScan.

The results showed that MedISys-FF collects food fraud publications with high relevance > 75% and the top 4 most reported fraudulent commodities in the media in the period tested were i) meat, ii) seafood, iii) milk and iv) alcohol. These top stories align with those found in RASFF and EMA but differences in frequency are apparent. It is concluded that the newly developed MedISys-FF can be a useful information source to help risk managers to combat food fraud.

O8.7 FOOD AUTHENTICITY RISK ASSESSMENT: 30 YEARS OF EXPERIENCE ENHANCED THROUGH DATA MINING

Jamin, E.^{1,*}

1-Eurofins Analytics France, Nantes, France

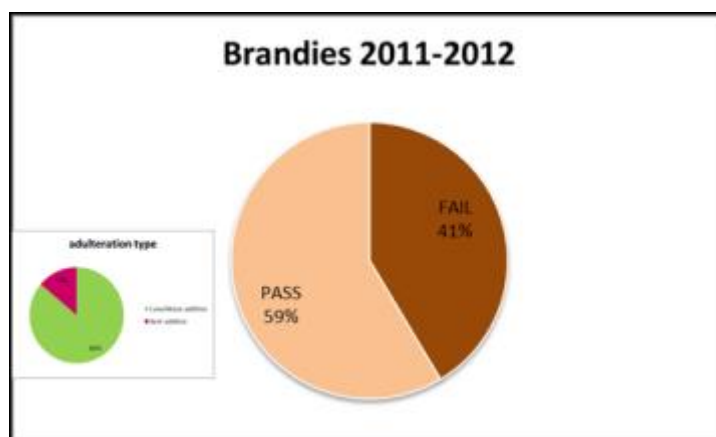
*corresponding author e-mail: ericjamin@eurofins.com

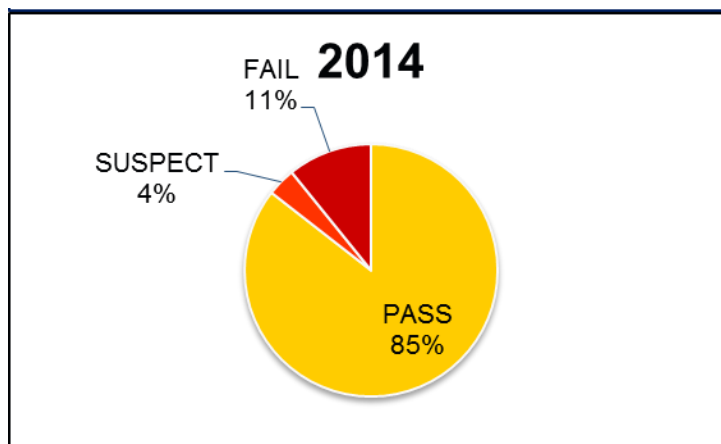
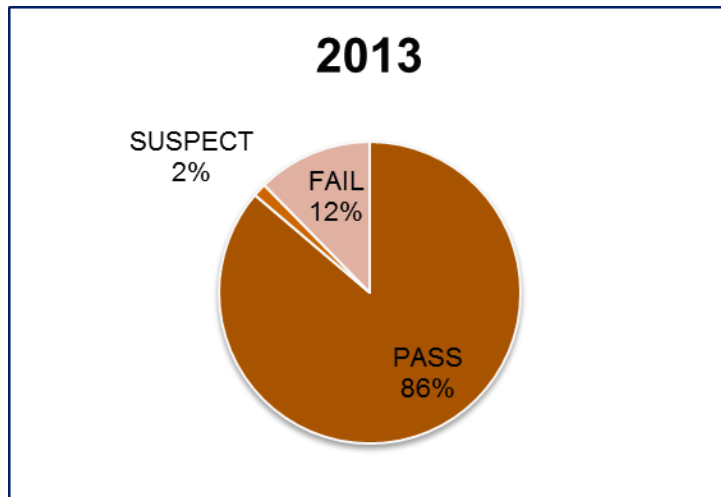
Keywords: Food authenticity, databases, market results, innovation

Sound authenticity testing is always based on reference data established by analysis of samples of known provenance. Therefore two prerequisites are necessary for a tailored food fraud risk management plan: first actual data with good quality in terms of representativeness of the samples taken as reference, secondly a good data analysis method with suitable statistical tools and a long-term reproducibility.

With 30 years of experience in the field, our authenticity competence centre has produced a huge amount of results from worldwide supplies. Market observations over a long period often show some recurrence, because the simplest way to extend food with adulterants usually remains valid. However the appearance of new products, new market conditions and new methods to authenticate them, often produces rises and fall of non-compliance rates over time. Therefore when looking at authenticity risk of commodities, is it important to consider the context in the life cycle and economical context of food fraud.

These considerations will be illustrated by examples from real life, and some recent trends will be highlighted. For example the graphs below display the changes of non-compliance rates observed on grape alcohols analysed by the SNIF-NMR method in our laboratory. During the years 2011-2, we observed a rise of non-compliance samples, which appeared to be linked with a market change: due to the lack of available eau-de-vies from Western Europe at that time, brandy makers were using vinic alcohols from third countries outside Europe. Authenticity testing revealed mislabelling practices in those countries, what led buyers take measures to avoid them. Over the next two years, the rate of non-compliance decreased significantly, but reached a plateau still justifying some regular surveillance.





O9.1

IS IT ORGANIC? WHAT DO EXISTING ANALYTICAL TECHNIQUES HAVE TO OFFER AND HOW CLOSE ARE WE TO IMPLEMENTING THEM?

Kelly, S.^{1,*}

1-Food and Environmental Protection Laboratory, Joint FAO/IAEA Division of Nuclear Applications in Food and Agriculture, Department of Nuclear Sciences and Applications., International Atomic Energy Agency, Vienna International Centre.

*corresponding author e-mail: S.Kelly@iaea.org

Increasing consumer demand for organic products has meant rapid expansion worldwide of the organic retail sector. Whilst the founders of the organic farming movement placed considerable value on close links between producers and consumers, the demand for organic produce has widened this gap. The organic sector is becoming increasingly dominated by corporate players that may not share some of the less tangible benefits of the organic philosophy. Notwithstanding the pros and cons of this evolution of the organic retail sector, the globalisation of organic markets must inevitably place an increased burden on certification/inspection bodies and traceability systems on which the authenticity of the organic produce depends. Thirteen years ago, Siderer et al. (2005) proposed the introduction of supplementary analyses and tests of organic merchandise in order to verify labelling claims and in the intervening years much research has been reported in the scientific literature; but how much closer are we to implementing these 'supplementary analyses'?

In this presentation I aim to give an overview of a range of analytical techniques that have been advocated as having the potential to discriminate between different facets of organic and conventional cultivation to verify labelling claims. These include; established and validated techniques such as pesticide and veterinary drug residue analysis, stable nitrogen and oxygen isotope analysis to differentiate between crops grown with the application of synthetic (inorganic) nitrogen or manure based fertiliser; trace metal analysis to establish markers for mineral supplementation or the possible effects of arbuscular mycorrhizal fungi association in organic soils; metabolite profiling, fluorescence microscopy, phosphorus nuclear magnetic resonance spectroscopy and other potential techniques. Some information about the routine application analytical surveillance will also be reported.

O9.2

ENHANCED FRAUD PREVENTION THROUGH COMBINING SUPPLY CHAIN AND SATELLITE INFORMATION – REDUCING VULNERABILITIES IN ORGANIC CERTIFICATION

Sutter, A.^{1,*}

1-Organic Services, Tutzing, Germany

*corresponding author e-mail: a.sutter@organic-services.com

Keywords: food fraud prevention, integrity management, cloud-based solution, supply chain integrity, vulnerability, organic certification

Our presentation „ Enhanced fraud prevention through combining supply chain and satellite information – reducing vulnerabilities in organic certification” will present the hypothesis that effective fraud prevention in organic supply chains relies on a mass balance approach based on the combination of two types of data: certification data and transaction data. As the mass balance calculation is based on field size and yield to determine the plausibly produced organic production volume of an organic producer, the integration of satellite data that proves the existence of the certified acreage and identifies production data can enhance the efficiency of such an integrity management system (as well as the efficiency of inspection and certification).

This hypothesis will be field tested against applications in Italy and Kazakhstan, where organic integrity systems with the objective to prevent fraud have been established. The study involves a range of stakeholders in Kazakhstan including the Ministry of Agriculture, the Kazakh Accreditation Body, and the National Centre for Certification, private certifiers, the Agrarian University, traders and farmers. The pilot project is funded by the FAO, Italy with Naturland Association, and Germany as partner organization. The two service providers TALAP, Kazakhstan and Organic Services, Germany are responsible for implementing the project. The Italian FederBio Integrity database was established in 2016 and included all Italian growers, a high percentage of traders and processors with data from the Italian Accreditation Body on certified operations.

The study builds on a previous study conducted in the framework of the EU-funded FoodIntegrity project, which led to the formulation of the above-mentioned hypothesis. The FoodIntegrity study had concluded – following more than 25 qualitative interviews with supply chain actors and industry experts of four different food industries – that Check Organic is able to prevent and/ or detect fraud in food supply chains. The study also identified prerequisites for the successful application of the mass balance checks of Check Organic. One prerequisite is that a relevant part of production and trade of the concerned product and within the defined system (either national or company based) must be recorded. Where this data is not readily available through other sources, the integration of GIS/ satellite systems with Check Organic was identified as a possible means to ensure the presence of this prerequisite. The conclusions drawn in the FoodIntegrity study have then helped to shape a new project: Organic Services partners with a GIS system provider, to develop and implement advanced GIS/ satellite tools with the objective of enhancing fraud prevention and reducing vulnerabilities in organic certification. The GIS system will be integrated with the Supply Chain Monitor (list, graph, and dashboard) and Supplier Volume Monitor (mass balance) of Check Organic.

The presentation for this conference will present the additional tools that are under consideration to be used in combination with Check Organic to reduce the vulnerability of organic certification through inspector visits' independent proof of 'crops grown', 'establishing crop rotation on fields', 'aerial photography submitted by farmers', 'list of fields and verification of fields through polygon data', 'identification of wild collection areas' and else.

O9.3

AUTHENTICATION OF ORGANIC VEGETABLES FROM REAL FARMS USING STABLE ISOTOPE RATIOS: A LEGISLATIVE INITIATIVE FROM THE ANDALUSIAN GOVERNMENT. INDIRECT IMPACT ON NEW FERTILIZERS LEGISLATION.

Moreno-Rojas, J.M.^{1,*}, Montenegro, J.C.¹, Cuevas, F.J.¹

1-Department of Food Science and Health. Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Avenida Menéndez-Pidal, s/n, 14004, Córdoba, Spain.

*corresponding author e-mail: josem.moreno.rojas@juntadeandalucia.es

Keywords: stable isotopes, organic production, traceability, legislation.

Almería (SE Spain) has become the main supplier in Europe in several crops such as tomato, cucumber, zucchini and pepper in terms of production and exports. Organic producers claim to the administration to do continuous efforts to increase the controls and to avoid fraud. At present, the main problem of the organic sector is the absence of an analytical technique to verify the origin of the products.

A new organic production regulation will come into force in 2021 (EU regulation n° 2018/848) this fact urges for finding robust analytical techniques used in real farms under a theoretical background to validate the necessary sanctions to the producers that commit fraud. Numerous attempts have been done with different techniques to study the authentication of the organic production. The S-IRMS technique used in this broad study of the organic horticultural Andalusian production have two great strengths: the previous results in literature (Bateman et al. 2007a) that should be revised and the robustness of the samples used regarding genetics (crop varieties), years of production and the diverse organic production systems under study. Moreover, the isotopes signatures used in this field should be complemented with other information such as fertilizers and soils that are included in our study.

This broad study of the main organic horticultural product verification in real farms (Almería, SE Spain) using stable isotopes have been launched by IFAPA at request of the regional ministry of agriculture and food of the Andalusian government.

A total of 686 samples have been collected during 2016, 2017 and 2018. Tomatoes, zucchinis, peppers and cucumbers were produced in greenhouses under (real) commercial agronomic conditions in thirty-seven farms. The different farms used conventional and (certified) organic methodologies of cultivation with no indications given by our research group. The ratio of the stable isotopes of nitrogen ($\delta^{15}\text{N}$) of the fruits, soil, and fertilizers were analyzed. The entire harvest was monitored throughout the cycle of production. The sampling was performed periodically (every 2-3 weeks) and the samples were analyzed separately. The analyses and relations under a new holistic perspective considering soil, fertilizers and fruits improve the verification of a unique food-based value of the farms opening a new framework in the organic food sector and the organic-food chain verification.

Additionally, the important screening of isotopic data of fertilizers done during this study has led to a broad isotopic databank of fertilizers (for organic and conventional cultivation) updating the previous literature (Bateman et al. 2007b). Those results have highlighted the

lack of information of an important number of fertilizers as hydrolysed protein derivatives (new types) and chelated nutrients authorized in organic production due to a complicated fit of both types in the current legislation (pending to be updated). This fact could lead to a support for the elaboration of the new fertilizers legislation.

A) Organic (blue) and conventional (red) peppers samples ($\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$); B) organic (blue) and conventional (red) tomato samples ($\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$)

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Regulation (EU) 2018/848 of the European parliament and of the council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007.

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Acknowledgements

This work has been funded by the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) and the European Rural Development Fund (ERDF) through the Project 'Viabilidad de la relación de isótopos estables de nitrógeno ($^{15}\text{N}/^{14}\text{N}$) como metodología para la caracterización de la producción ecológica frente a la convencional (PP.PEI.IDF201601.1)'. JCM is granted by a research contract funded with previously mentioned funds.

O9.4

BIO-FRAUD-SCAN – CONCEPT FOR A METHOD TO CHECK AUTHENTICITY OF ORGANIC FOODS VIA PESTICIDE METABOLITES

Hofsommer, M.^{1,*}

1-GfL Gesellschaft für Lebensmittel-Forschung mbH, Berlin, Germany

*corresponding author e-mail: info@gfl-berlin.com

Keywords: organic, fraud, pesticides, metabolites

Turnover with organic food is still continuously increasing. It can happen easily that supply cannot keep up with demand. This fact together with higher prices and margins make organic food susceptible to fraud. To verify the authenticity of organic products several analytic strategies are under discussion. The rules of organic legislation (EG 834/2007) respectively EU 2018/848 makes restrictions to the use of fertilizer and plant protection products. Both could consequently be starting points for an analysis method, both with pro's and con's. In state of the art laboratories nowadays more than 500 pesticides are easily detectable by the use of LC- and GC-MS/MS down to 10 ppb. However pesticides do not remain on plants as such but are being metabolized so the active principle (analytical target) is degraded. This frequently occurs to a point where the pesticide is no longer detectable, making the analysis useless for the authentication of the organic status.

In our concept we approach the issue by looking at pesticide metabolites which are not covered by MRL legislation. Just like any organism plants have developed strategies how to deal with xenobiotics. After a phase I and phase II reaction the resulting products are not eliminated from the plant but incorporated into compartments of the vacuole. This makes them promising analytical targets to verify the use of plant protection products, respectively the organic status.

The concept will outline an approach how to discover potential targets and detecting these highly polar molecules with capillary electrophoresis (CESI) and/or ion chromatography coupled to tandem mass spec.

The project "Bio-Fraud-Scan" is financially supported by the European Regional Development Fund.

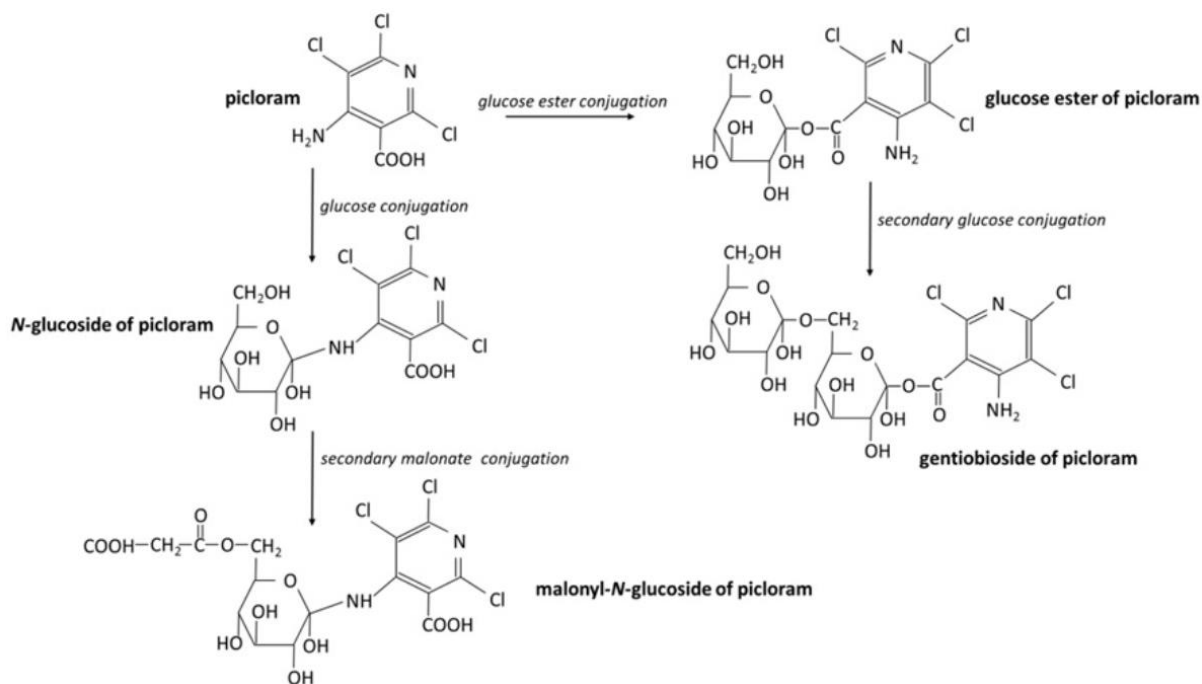


Figure 1: Conjugation and secondary conjugation of picloram in leafy spurge (*Euphorbia esula* L.) as proposed by Frear et al. (1989)

Frear, D. S., E. R. Mansager, and H. R. Swanson. 1989. Picloram metabolism in leafy spurge: isolation and identification of glucose and gentiobiose conjugates. *J. Agric. Food Chem.* 37:1408–1412.

O11.1

KEY SUCCESS FACTORS FOR AN INFORMATION SHARING SYSTEM TO PREVENT AND DETECT FOOD INTEGRITY ISSUES: INSIGHTS FROM A STAKEHOLDER CONSULTATION

Minnens, F.^{1,*}, Sioen, I.², Lucas Luijckx, N.B.³, Verbeke, W.¹

1-Department of Agricultural Economics, Ghent University, Ghent, Belgium

2-Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium

3-TNO, Zeist, the Netherlands

*corresponding author e-mail: Fien.minnens@ugent.be

Keywords: food integrity, transparency, information sharing, food supply chain, stakeholders

One of the biggest challenges facing the food industry is assuring food integrity (FI). Dealing with complex FI-issues requires a multi-dimensional approach. Preventive actions and early reactive responses are key for the food supply chain. Information sharing could facilitate the identification and prevention of FI-issues. This study investigates attitudes towards a food integrity information sharing system (FISS) among stakeholders in the European food supply chain. Insights into stakeholders' interest to participate and their conditions for joining a FISS are assessed.

The stakeholder's consultation consisted of three rounds. During the first round, a total of 119 food industry stakeholders (46% SMEs) – covering all major food sectors susceptible to FI-issues – participated in an online quantitative survey between November 2017 and February 2018. The second round, an online qualitative feedback survey in which the findings were presented, received feedback from 61 stakeholders from industry, authorities and research. In May 2018, 37 stakeholders discussed the results in further detail during an interactive workshop.

Three distinct groups of industry stakeholders were identified based on reported frequency of occurrence of and likelihood of detecting FI-issues. Food industry stakeholders strongly support the concept of a FISS with an attitude score of 4.49 (S.D. =0.57) on a 5-point scale; and their willingness to participate is high (81%). Consensus exists regarding the advantages a FISS can yield towards prevention and detection. A food safety authority (74%) or a newly established organisation (84%) was believed to be the most suitable third parties to organise a FISS. Reactions diverged concerning the required level of transparency, the type of data stakeholders might be willing to share in a FISS and the role authorities can have within a FISS. Four key success factors for a FISS are defined: a new organisation should manage a FISS, data and information confidentiality need to be guaranteed, food safety authorities need to be involved, all actors in the food supply chain need to join.

O11.2

TOWARD AN NMR-BASED MONITORING OF COFFEE ORIGIN? AN INDUSTRIAL CASE-STUDY

Recht, R.^{1,*}, Febvay, L.^{1,2}, Daoudé-Werner, D.¹, Hamon, E.¹, Viraswami, V.³, This, H.²

1-Aerial, Illkirch, France.

2-UMR 1145, AgroParisTech, Inra, Université Paris-Saclay, Massy, France.

3-Les Cafés Sati, Strasbourg, France.

*corresponding author e-mail: r.recht@aerial-crt.com

Keywords: NMR fingerprinting, chemometrics, coffee authenticity, roasting impact.

The market value of a coffee is greatly influenced by its origin and production mode. There is therefore a strong stake for roaster companies to be able to (i) ensure the geographical provenance of their raw materials and (ii) justify the origin and technical path of their finished products. Studies published in the literature have demonstrated the feasibility of discriminating green and roasted coffees of different origins by Nuclear Magnetic Resonance (NMR) and chemometrics. Although proofs of concept have been provided at the scale of the continent or of specific countries, they are often not representative of the reference portfolio of an industrial, taking into account the diversity and natural variability of the raw materials as well as the impact of roasting process.

The objective of this project is to assess the capabilities of NMR coupled with chemometrics to discriminate the coffee references (pure origin) of a roaster company. 124 green coffee samples from 8 countries were collected and analysed by high resolution ¹H NMR. The molecular fingerprints obtained were compared by chemometric analysis. The impact of real industrial roasting kinetics was also investigated on one of these references. The results obtained allowed to establish a database specific to the manufacturer's reference catalogue. It also enabled to link key parameters of the roasting process to evolutions in the metabolic fingerprint of the products.

This study highlights the challenges to establish databases for the authentication of raw materials and processed products. It continues the efforts undertaken in the systematic control of the authenticity of foodstuffs by NMR.

O11.3

MRM-MS OF MARKER PEPTIDES DISTRIBUTION AS A TOOL FOR AUTHENTICATION OF SINGLE-CUT MEAT PRODUCTS

Kłosowska-Chomiczewska, I.E.^{1,*}, Nalazek-Rudnicka, K.², Wasik, A.², Macierzanka, A.¹

1-Department of Colloid and Lipid Science,

2-Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

*corresponding author e-mail: ilochomi@pg.edu.pl

Keywords: meat authentication, MRM-MS, meat cuts, marker peptides

In meat products, a chopped meat can be easily substituted with a meat of another species or from a different than declared part of animal, usually of a lower price. One of the most known meat scandal was discovered in 2013 when horse meat was found in beef burgers available from a number of the UK supermarkets. Number of methods was developed to protect customers against meat adulteration. These methods typically allow for distinguishing animal species. Some of them can also serve for distinguishing *i.e.* sex of the animal, breed, feed intake, and even wild to farmed meat ([Ballin, 2010](#)). However, there is no analytical method for authentication of different types of meat cuts, despite that a lot of products is made of single types of cuts. The variations in myoglobin (Mb) content in different pork muscles ([Beecher, Cassens, Hoekstra, & Briskey, 1965](#)) indicates however, that protein/peptide approach can be used not only to differentiate animal species but also the types of meat cuts used in food manufacturing.

Therefore, the aim of our research was to examine the distribution of selected proteins in raw and processed meat cuts in the context of their authentication. For that purpose we have adopted the HPLC-MS/MS MRM mode method proposed by Watson *et al.* ([Watson, Gunning, Rigby, Philo, & Kemsley, 2015](#)) for authentication of raw meat samples. We have optimized a number of steps in the method in order to obtain high sensitivity and good repeatability required for reliable analysis of processed meat products. The repeatability of the method was significantly improved (coefficient of variation <10%) and the sensitivity was increased up to sevenfold as compared to initial method.

In our marker peptide approach Mb and myosin (My) were used as parent proteins to differentiate types of pork, beef, and chicken meat cuts. For each type of meat cut, samples were obtained from three individual animals and analyzed separately in order to assess the level of individual variability. The content of Mb in raw pork depended on the type of meat cut, being the highest in knuckle and shoulder, and the lowest in ham and loin. Moreover, the differences between meat cuts were more pronounced than the level of individual variability. Neither the distribution of Mb peptides in beef nor My peptides in chicken varied significantly enough to be used for processed meat cuts authentication.

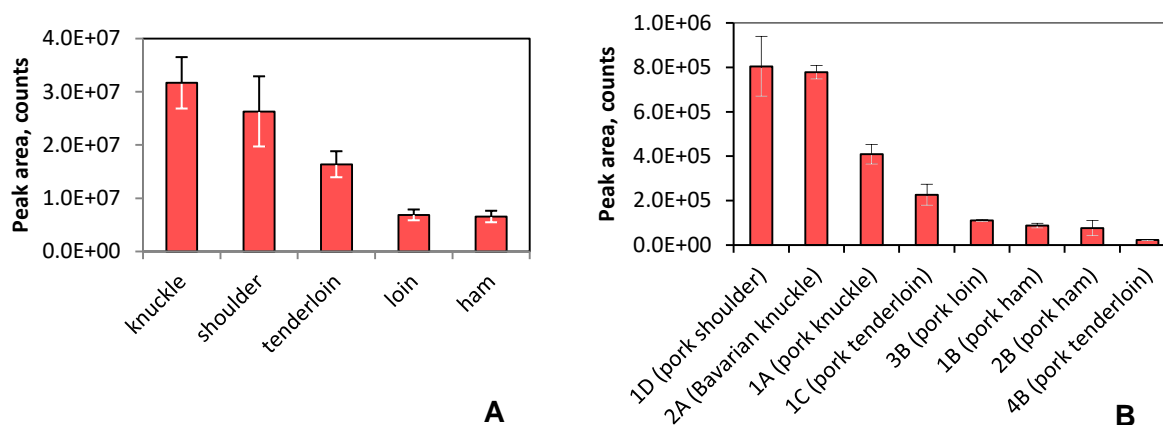


Fig. 1. Distribution of Mb originating peptide marker GHPETLEK in (A) raw pork meat cuts, (B) processed meat products made of different types of single pork cuts. Data are presented as means \pm SD ($n = 3$).

The Mb distribution pattern in commercially available processed pork meat products made of single types of meat cuts followed the pattern observed for raw pork cuts (Figure 1), independently of the complexity of the food processing used at a manufacturing stage. This indicates, that peptide approach combined with HPLC-MS/MS in MRM mode allows for authentication of commercially available meat products made of different types of meat cuts.

The distribution of Mb and My in different meat cuts was explored in the context of meat authentication for the first time. Our results lay foundations for development of new, reliable method for protecting consumers and producers against meat fraud.

Acknowledgments

The work was funded from the EU Project FOODINTEGRITY (grant agreement FP7-KBBE-2013-7-613688) through a research grant to Gdańsk University of Technology. The work was also supported by the Polish Ministry of Science and Higher Education (grant 3891/7.PR/2018/2).

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O11.4

NEW RAPID GC-MS METHOD VERSUS CONVENTIONAL PYCNOMETRY: WHAT IS A REAL ALCOHOLIC STRENGTH OF THESE SPIRITS AND LIQUEURS?

Stupák, M.^{1,*}, Pulkrabova, J.¹, Kocourek, V.¹, Hajslova, J.¹

1-University of Chemistry and Technology Prague, Technická 5, 166 28, Prague, Czech Republic

*corresponding author e-mail: michal.stupak@vscht.cz

Keywords: alcoholic strength, GC-MS, pycnometry, spirits

With regards to a high excise duty to which alcoholic beverages, specifically spirit drinks, are subjected in many countries, incorrect declaration of alcoholic strength is one of fraud categories encountered in control of some products at the market. In the Regulation (EC) No. 2870/2000 laying down the Community reference methods for the analysis of spirit drinks, there are listed three 'classic', well established methods applicable for the 'official' measurement of the alcoholic strength: (i) pycnometry, (ii) electronic densimetry, and (iii) densimetry employing hydrostatic balance. However, in the case of density measurement using pycnometry, the process of distillation i.e. separation of the ethanol (and other volatile compounds) from the extractive matter, i.e. substances which do not distill, is rather time consuming, moreover, a relatively large volume of sample, at least 100 ml, is required. It should be also noted, that in addition to alcohol, other volatiles contained in controlled sample are transferred into distillate (Regulation (EC) No. 2870/2000), what may in some extent, pose a problem.

The main aim of this study was to develop and apply a rapid and sufficiently accurate method for determination of alcoholic strength in various distilled spirits, wines and some other beverages with higher amounts of dissolved solids and/or volatile compounds, e.g. liqueur. It is believed that the use of gas chromatography coupled to mass spectrometry (GC-MS) enables not only control whether the criteria stated in 'official methods' for alcoholic strength determination are met, but, in addition, also allows to overcome all the limitations/drawbacks of other methods published until now. Six types of spirit drinks (rum, grappa, pear brandy, vodka, egg liqueur) and four wines (merlot, cuvee, Moravian muscat and port) were used for the method validation. All these samples were also tested by pycnometry for the content of ethanol and it showed a good compliance with our GC-MS method. Moreover, the trueness of data generated by the new GC-MS method was proven through participation in proficiency tests by FAPAS®, round 1368 and 1377 (testing materials - whiskies) the z-scores 1.2 and -1.4, respectively, were acceptable. Subsequently, two liqueur samples (citrus and bitter) were tested for the alcoholic strength by pycnometry and our new GC-MS method (see Table I). Those samples were also analyzed for the volatile compounds other than ethanol.

Table 1: Comparison of alcoholic strength in two liqueur samples obtained by pycnometry, GC–MS method and declared value.

Alcoholic strength (% v/v)	Citrus liqueur	Bitter liqueur
Declared	40.0	35.0
Pycnometry	39.9	35.0
GC-MS	36.7	33.3

The content of ethanol obtained by GC–MS method was significantly lower due to high content of volatiles originating from essential oil, which was added during manufacturing process and the content is approximately 3% (v/v) in the final product. As documented in Fig. 1A, the citrus liqueur was very rich in volatile compounds and high amount of volatiles was also found in distillate used for the pycnometry measurement (see Fig. 1B). On this account, in the two samples, the alcoholic strength was by 3.2% (v/v) and 1.7% (v/v) higher than the results by GC–MS, respectively.

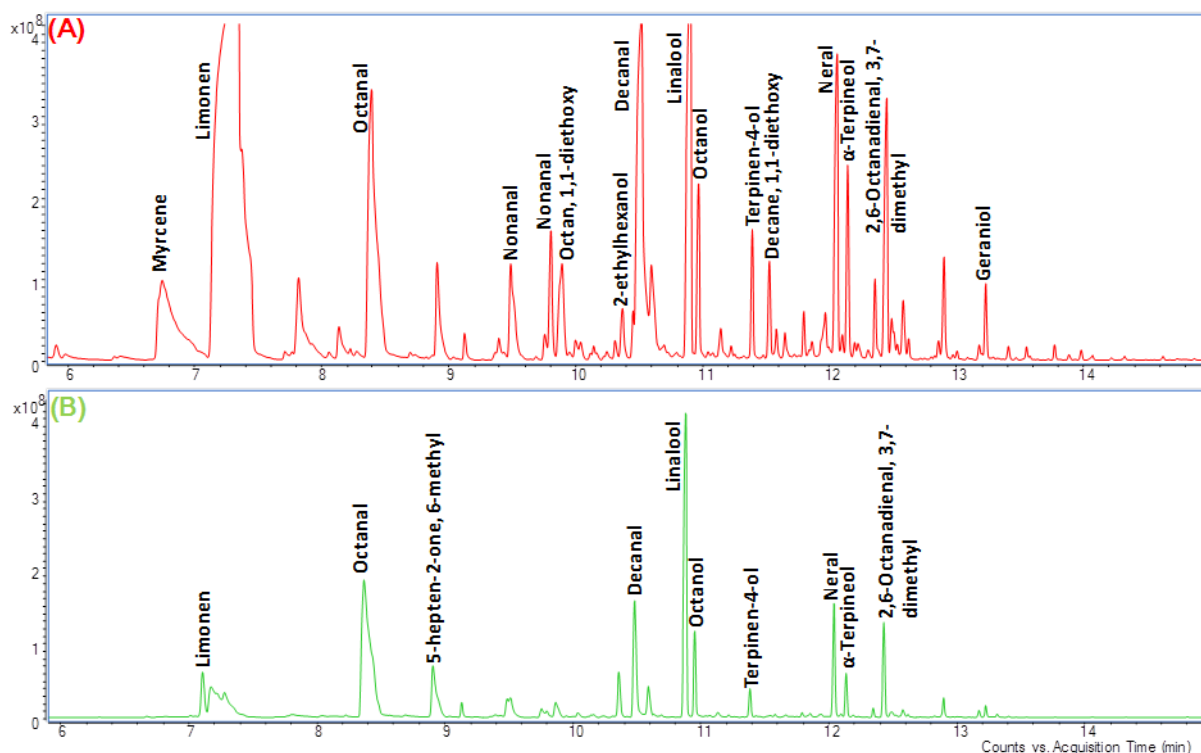


Figure 1: Analysis of A-citrus liqueur sample and B-its distillate used for pycnometry measurement (total ion chromatogram-TIC).

O11.5

IS IT ORGANIC? COMPOUND-SPECIFIC STABLE ISOTOPE RATIO ANALYSIS FOR AUTHENTICITY TESTING OF ORGANICALLY GROWN VEGETABLES

Novak, V.^{1,*}, Adler, J.¹, Husted, S.¹, Holst Laursen, K.¹

1-Plant and Soil Science Section and Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

*corresponding author e-mail: vlno@plen.ku.dk

Keywords: compound-specific, organic agriculture, plants, stable isotopes

High-value plant products such as organically grown vegetables are at risk of adulteration as they are sold at premium prices compared to their conventional counterparts. Synthetic fertilizers are prohibited in organic plant production that mostly relies on nutrient supply from animal or green manures or compost. Analytical methods that can verify how plants were fertilized are therefore needed to prevent and reveal fraud in the organic sector. This has spurred the development of stable isotope-based methods that can document the fertilization history of plants. Among these methods, nitrogen isotope analysis of bulk tissue ($\delta^{15}\text{N}$) is one of the most widely used. However, several studies have shown that $\delta^{15}\text{N}$ is biased by the large variation in fertilization strategies used in organic and conventional agriculture and thus has limited applicability for organic authentication (Laursen *et al.*, 2013).

Recent pilot studies have demonstrated that oxygen isotope analysis of plant-derived nitrate ($\delta^{18}\text{O}_{\text{NO}_3}$) can discriminate crops that have been grown with synthetic or organic fertilizers. However, the analytical procedure for $\delta^{18}\text{O}$ analysis of nitrate is costly and labour intensive (Laursen *et al.*, 2013; Mihailova *et al.*, 2014). The aim of the current study was therefore to develop a novel and fast method, based on oxygen isotope analysis of sulphate ($\delta^{18}\text{O}_{\text{SO}_4^{2-}}$), for discrimination between organic and conventional potatoes. Potatoes, cabbage and carrots were field grown with synthetic fertilizers (NPK), animal slurry, or legume based green manures for two years. Potatoes were grown at three different locations in Denmark, while cabbage and carrots at one location in Denmark. Freeze-dried and homogenized vegetables were used directly for bulk analysis of carbon, nitrogen and oxygen ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) by isotope ratio mass spectrometry (IRMS). Oxygen isotope analysis of plant-derived nitrate was based on bacterial denitrification, which is a 2-week procedure (Laursen *et al.* 2013). Oxygen isotope analysis of plant-derived sulphate was based on water extraction, filtering, precipitation as BaSO_4 , drying and IRMS analysis (Novak *et al.* 2018, in prep.).

Bulk $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of vegetables did not reveal differences between fertilization strategies. However, bulk $\delta^{15}\text{N}$ values were higher for slurry fertilized vegetables compared to systems based on legumes or NPK. The oxygen stable isotope ratio of sulphate showed statistically higher values in vegetables fertilized with NPK compared to both of the organic fertilization strategies with the exception of animal manure fertilized carrot. The nitrate stable isotope analysis of oxygen showed that NPK fertilized potatoes was statistically higher than organically fertilized (Figure 1). For carrot and cabbage the oxygen isotope analysis of nitrate did not reveal any statistical difference. The lower $\delta^{18}\text{O}$ values in nitrate and sulphate from organic systems are suggested to be caused by assimilation of soil water with low $\delta^{18}\text{O}$ values during nitrogen and sulphur mineralization.

It is concluded that oxygen isotope analysis of plant-derived sulphate ($\delta^{18}\text{O}_{\text{SO}_4^{2-}}$) can reveal how plants have been fertilized. Sample preparation related to the sulphate-based isotope analysis is much less labour and equipment intensive compared to nitrate isotope analysis. It thus represents a promising new method for authenticity testing of organically grown vegetables.

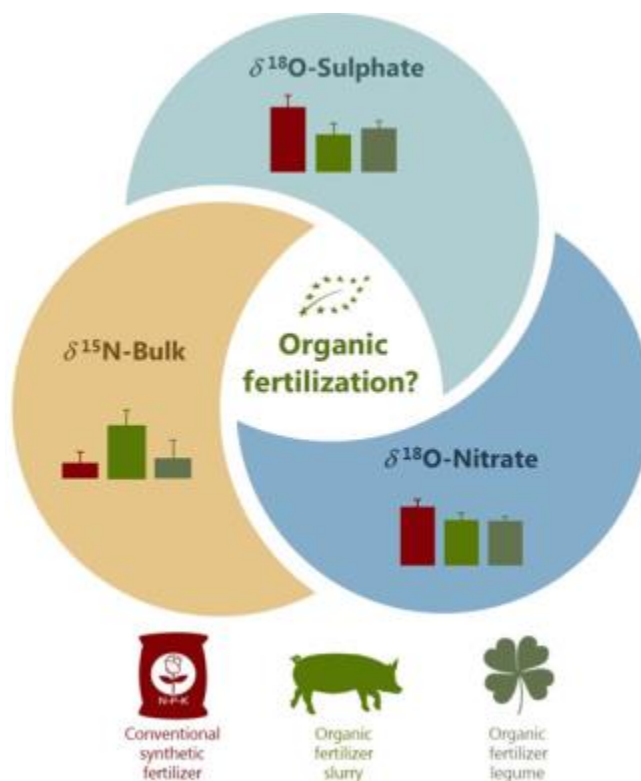


Figure 1:

Graphical overview of stable isotope methods used for analysis of field-grown potatoes. Results from stable isotope analysis of nitrogen in potato bulk tissue, and compound-specific oxygen isotope analysis of potato-derived sulphate or nitrate are shown. Potatoes were fertilized with conventional synthetic fertilizer, or two organic fertilizers: pig slurry or legume-based green manure. Bars in graphs show means + SD (n=12).

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O11.6

USING AMBIENT MASS SPECTROMETRY AND CHEMOMETRICS TO RAPIDLY DETERMINE POULTRY PRODUCTION SYSTEM AUTHENTICITY

Birse, N.¹, Chevallier, O.², Pringle, S.³, Stead, S.³, Elliott, C.¹

1-Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, UK BT9 5AG

2-Mass Spectrometry Core Technology Unit, School of Biological Sciences, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, UK BT9 5AG

3-Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, UK SK9 4AX

Keywords: poultry, ambient, mass spectrometry, chemometrics

Food safety, security and the authenticity of food products has become an increasingly important focus for consumers in the aftermath of several major food scandals over the last decade. The deliberate adulteration of milk products with melamine was followed by the adulteration of beef products with horsemeat and then the contamination of eggs with the insecticide fipronil. The negligent or deliberate mislabeling of poultry in certain UK slaughterhouses and cutting plants is the most recent event to receive significant press coverage. Consumers have tried to alleviate their concerns about adulteration and contamination by purchasing higher priced products which are described as being more sustainable, having higher welfare standards, offering health benefits or being from an organic production system. The food industry has been happy to encourage this, as higher priced products typically offer greater profitability.

The industry has added additional tiers to existing production systems and developed new products whilst investing in new branding and certification schemes to attract customers and drive sales. The additional tiers added to production systems and new products being produced have created additional risk for suppliers and for the consumer. Supply chains and production lines have become so complex; it is now increasingly difficult to be certain that the labelling on products is accurate and that the correct product is found with the correct labelling. The industry lacks fast, cheap and reliable analytical platforms which can confirm whether or not the product in a packet is what it claims to be.

We will present the findings of our latest work which makes use of the rapid evaporative ionisation mass spectrometry (REIMS) ambient mass spectrometry system with iKnife electrosurgical sample system in combination with chemometric modelling to develop a platform which can quickly and cheaply determine the authenticity of the product and correctness of labelling in chicken products. REIMS has been chosen as the system has previously shown significant promise in the detection of adulterated meat products by identification of tissue species. We will discuss the work that has been done to build on these foundations to deliver a platform capable of identifying not only different species, but different breeds and production systems within a species, in this case, chicken.

O11.7

A NOVEL MULTI-PLATFORM HIGH RESOLUTION MASS SPECTROMETRY NON-TARGETED APPROACH FACING EXTRA VIRGIN OLIVE OIL ADULTERATION

Cavanna, D.^{1,2,*}, Hurkova, K.³, Serani, A.⁴, Dall'Asta, C.², Hajslova, J.³, Suman, M.¹

1-Advanced Laboratory Research, Barilla G.& R. F.lli SpA, via Mantova 166, 43122, Parma, Italy

2-Department of Food and Drug, University of Parma, Parco Area delle Scienze 95/A, 43124 Parma, Italy

3-University of Chemistry and Technology, Department of Food Analysis and Nutrition, Technicka 3, 166 28 Prague 6, Czech Republic

4-COTECA Srl Consulenze Tecniche agroindustriali, 56121, Pisa, Italy

*corresponding author e-mail: daniele.cavanna@barilla.com

Keywords: non-targeted mass spectrometry, extra virgin olive oil, food frauds, LC-HRMS

High resolution mass spectrometry was used in the past for the detection of frauds on extra virgin olive oil products [1] [2] [3]. However, at the moment, scientific studies are not largely focused on the detection of soft refinements of Olive Oils, even if the dilution of EVOO with these samples is probably one of the most common frauds applied on the market [4]. This inter-laboratory work would like to identify some chemical markers responsible of this process. Refined oils (deodorized and deacidified) were created on a laboratory scale starting from low quality olive oils and were analyzed together with a set of pure EVOO samples and with mixtures of adulterated and pure EVOO at different percentages.

Sample preparation was executed according to previous works [5] [6]. UHPLC- HRMS analyses were performed with a SCIEX® triple TOF 6600 analyzer and with a Thermo® Q-Exactive Orbitrap analyzer. Instrumental conditions were the same for all the instruments involved in the project. After the analyses, raw data were processed with PeakView® (for triple TOF data) and Compound Discoverer® (for Orbitrap data); chemometric data analyses were performed with Markerview® (Sciex) and SIMCA® software (Umetrics). Different unsupervised and supervised models were created and the scores plots clearly highlighted a separation between the pure Extra Virgin Olive Oil samples and the soft refined samples.

The features responsible of these clusterizations were selected and an attempt of compound identification was performed. A group of molecules related to the adulteration processes were identified: they were at least upregulated in the refined oils or even only detected in these fraudulent samples and in their mixtures. The markers selected applying the same experimental design with different instruments in different laboratories were compared. This research represents a robust untargeted metabolomic approach able to identify specific compounds that can be considered markers of soft refinement processes in Extra Virgin Olive Oils. The detection of these molecules (performed with easier “target” methods) will be a proof of fraudulent issues in commercial samples [7].

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O12.1 IS INDUSTRY THE MAIN VICTIM OF FOOD FRAUD?

Brereton, P.^{1,*}

1-Institute for Global Food Security, Queen's University Belfast, UK

*corresponding author e-mail: paul.brereton@qub.ac.uk

Keywords: food fraud, general food law

It has been commonly acknowledged that the first and main victims of food frauds are consumers. They are certainly the high profile victims and obviously need to be protected, but are they the most affected? What about the honest producer whose livelihood is being threatened by unfair competition from malevolent actors in the food chain? Who protects supports or even acknowledges their plight? Have we produced a system that, due to this lack of protection, actually encourages food fraud as the only means of survival?

The General Food Law Regulation (178/2002) ensures a high level of protection of human life and consumers' interests in relation to food, while ensuring the effective functioning of the internal market [1]. With food fraud rife, prosecutions low, penalties minimal, reduced government surveillance, is effective functioning of the internal market really taking place?

Food fraud can take place anywhere in the supply chain, with most frauds being business to business. By focusing on the consumer, in terms of food fraud, are we merely trying addressing some of the symptoms rather than address the cause? The rationale of industry the primary victim of food fraud will be explored together with implications for regulators and enforcement bodies of a possible change in approach.

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O13.1

CHALLENGES IN MOVING FROM TARGETED TO NON-TARGETED MASS SPECTROMETRIC METHODS FOR FOOD FRAUD ANALYSIS

Suman, M.^{1,*}, Cavanna, D.¹, Righetti, L.², Elliott, C.³

1-Advanced Laboratory Research, Barilla G.& R. F.lli SpA, via Mantova 166, 43122, Parma, Italy

2-Department of Food & Drug, University of Parma, Parco Area Scienze 95/A, 43124, Parma, Italy

3-Faculty of Medicine, Health & Life Sciences, Queen's University Belfast, BT9 6NG, Belfast, Ireland

*corresponding author e-mail: michele.suman@barilla.com

Keywords: Non-targeted mass spectrometry, metabolomics, food authenticity, scientific opinion, harmonization.

Detecting and measuring food fraud is a challenging analytical task since a very wide range of food ingredients and types may be adulterated by numerous potential adulterants, many of which are yet unknown. To date most of the methods applied for the control of food fraud are targeted methods, which are focused on the detection of one or a few classes of known compounds. There is an increasing availability of solutions and applications based on high-resolution mass spectrometry (HRMS), allowing parallel non-targeted approaches, novel compound identification and retrospective data analysis. For these types of methods, sample handling must be minimal to allow the inclusion of as many as possible chemical categories.

However, data-handling of such methods is much more demanding, together with the potential requirement to integrate multiplatform data as well as conducting data fusion. To allow the processing of massive amounts of information based on the separation techniques and mass spectrometry approaches employed, effective software tools capable of rapid data mining procedures must be employed and metabolomics based approaches does appear to be the correct way forward. To verify the relevance of modelling results, appropriate model validation is essential for non-targeted approaches, confirming the significance of the chemical markers identified. The present work is devoted to review and assess the current state of the art with regards non-targeted mass spectrometry in food fraud detection within many food matrices and to propose a harmonized workflow for all such applications [1].

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O13.2

TO TARGET OR NOT TO TARGET? DEFINITIONS AND NOMENCLATURE FOR TARGETED VERSUS NON-TARGETED ANALYTICAL FOOD AUTHENTICATION

Holst Laursen, K.^{1,*}, Zederkopff Ballin, N.²

1-University of Copenhagen, Faculty of Science, Department of Plant and Environmental Sciences, Plant and Soil Science Section & Copenhagen Plant Science Centre, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark.

2-Danish Veterinary and Food Administration, Soendervang 4, 4000 Ringsted, Denmark

*corresponding author e-mail: holst@plen.ku.dk

Keywords: analytical chemistry, food authentication, non-targeted, targeted

Analytical methods that can offer fast, cost-effective and reliable food authenticity testing at several points in the food production and retail chain are urgently requested. Targeted methods such as stable isotope ratio analysis [1] still have much to offer but it is increasingly acknowledged that food is a complex matrix and should thus be treated and analyzed by techniques that can embrace this complexity. The use of non-targeted analytical methods in food authentication has therefore rapidly increased during the past decade. Examples are authentication of fish, olive oil and spices by spectrometry and spectroscopy based techniques [2-4].

The increasing use of non-targeted analyses across several scientific disciplines has brought together a mixture of analytical traditions and terminologies and terms such as profiling, signature, fingerprinting, analytical marker etc. are inconsistently used. Consequently, the scientific literature on food authentication often includes different approaches and a variety of definitions and nomenclature for both targeted and non-targeted analysis.

While non-targeted fingerprinting methods are still taking the initial steps into the food authenticity community much more work is required to validate and harmonize these methods and the associated data. An essential prerequisite is a common understanding of the analytical principles of targeted versus non-targeted food authentications. At the conference, novel definitions and nomenclature of targeted and non-targeted authentication methods will be presented as a first step towards harmonization. Biological, chemical, and microscopy-based examples of targeted and non-targeted approaches will be presented while discussing the associated possibilities and limitations for analytical food authentication.

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O13.3

INVESTIGATIONS ON THE COMPARABILITY OF FINGERPRINTING DATA – LEARN TO WALK BEFORE YOU RUN

Lörchner, C.^{1,*}, Fauhl-Hassek, C.¹, Drescher, S.², Esslinger, S.¹

1-German Federal Institute for Risk Assessment, Berlin, Germany

2-Technische Universität Berlin, Berlin, Germany

*corresponding author e-mail: carolin.loerchner@bfr.bund.de

Keywords: non-targeted analysis, authentication of food, statistical data analysis

Sudan red in chili powder, melamine in baby food, and denaturants in spirits - these are just a few examples of harmful food adulterants occurred in recent years. Not least because of the extensive media presence, the awareness of consumers regarding the authenticity of food is increasing. Besides document-based approaches the authenticity of a product is examined by complex and time-consuming laboratory tests. Non-targeted analysis offers a great potential to identify e. g. deviations from the expected product composition. These analytical techniques are based on the acquisition of a so-called chemical fingerprint of the respective food for instance by spectroscopic methods such as Fourier Transform Infrared Spectroscopy (FT-IR). The generated fingerprint data are used for statistical data analysis (univariate or multivariate) to investigate different authenticity issues.

A particular challenge with regard to analytical authentication by these fingerprint methods is the comparability and sharing of spectral data obtained in different laboratories. In the simplest case this already includes the application of different devices of the same manufacturer and the same type. Interestingly, this under-investigated aspect is an essential step towards the joint usage of spectral databases and another module to be developed in the field of standardization of fingerprint techniques, to ensure a uniform procedure in the laboratories.

With the presented study, various approaches for the optimization of comparability of FT-IR fingerprint spectra acquired by different devices of a variety of edible oils will be presented. For this, the use of a specific correction factor and the optimization by data pre-processing like Piecewise Direct Standardization (PDS) will be illustrated and respective classification results will be compared and discussed.

Acknowledgement: The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

O13.4

DEVELOPMENT OF UNBIASED AND MULTI USERS CLASSIFICATION TOOLS BASED ON NON-TARGETED ANALYSIS: THE CRUCIAL ROLE OF THE STATISTICAL EQUIVALENCE OF THE “SCALED NMR SPECTRA” IN THE VALIDATION PROCESS

Gallo, V.^{1,2,*}, Garino, C.³, Arlorio, M.³, Ragone, R.², Todisco, S.², Rizzuti, A.², Latronico, M.^{1,2}, Mastrorilli, P.^{1,2}, Pontrelli, S.², Intini, N.^{2,4}, Triggiani, M.¹, Locatelli, M.³

1-Politecnico di Bari, Bari, Italy

2-Innovative Solutions S.r.l., Noci (BA), Italy

3-Università del Piemonte Orientale, Novara, Italy

4-ARPA Puglia, Bari, Italy

*corresponding author e-mail: vito.gallo@poliba.it

Keywords: non-targeted analysis, NMR, database sharing, food classifiers

In the fields of food quality assessment and food authentication, protection of consumers and producers requires improved methods to face increasing fraudulent activities. Currently, the panorama of the official tests is dominated by the targeted methods. Limits of targeted methods are known along with the potentiality of the non-targeted approach. The scientific challenges in moving from targeted to non-targeted analysis for food fraud testing have been discussed in the framework of the Food Integrity project (INTELLITrace-WP18) and some actions have been proposed, including the use of non-targeted NMR analyses.

In this presentation, the application of NMR based non-targeted methods will be presented and discussed. In particular, the way to develop unbiased and multi-users classification tools based on non-targeted analysis will be shown taking into account the unique feature of the NMR spectra. Indeed, based on theory, NMR spectra can be subdivided into intervals that once scaled to a reference signal, result independent on instrumental features (hardware configuration, manufacturer, age, magnetic field, probehead, etc.). In other words, by suitable scaling procedures, NMR can generate statistically equivalent spectra and this paves the way to the creation of systems characterized by spectral database sharing, data input by many spectrometers, multi-users apps connected to the same database, as shown in the following scheme.

Experimental evidence of the statistical equivalence of the scaled NMR spectra¹ will be also presented to support theory. Moreover, a practical application for identification of the origin of durum wheat will be shown emphasizing the possibility to recognize a wheat sample analysed by any NMR spectrometer by enquiring a classification model trained with NMR spectra recorded with a single NMR spectrometer. Finally, the validation procedure followed to demonstrate the reliability of the whole classification tool will be detailed.

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O13.5

A GUIDANCE FOR THE ANALYTICAL VALIDATION OF UNTARGETED METHODS: RESULTS, CRITICISMS AND PERSPECTIVES FROM THE FOOD INTEGRITY PROJECT

Arlorio, M.^{1,*}, Garino, C.¹, Locatelli, M.¹, Portinale, L.¹, Leonardi, G.¹, Monaci, L.², de Dominicis, E.³, Godula, M.⁴, Mafra, I.⁵, Gallo, V.⁶

1-Università degli Studi del Piemonte Orientale "A. Avogadro", Vercelli, Italy

2-Istituto di Scienze delle Produzioni Alimentari-Consiglio Nazionale delle Ricerche, Bari, Italy

3-Mérieux NutriSciences Italia, Resana, Italy

4-Thermo Fisher GmbH, Dreieich, Germany

5-REQUIMTE-LAQV, Universidade do Porto, Porto, Portugal

6-Innovative Solutions srl, Bari, Italy

*corresponding author e-mail: marco.arlorio@uniupo.it

Keywords: untargeted methods, validation, guidelines,

The concepts of food integrity, food traceability and food authenticity must be considered three priorities in the areas of food, health and consumer protection. Integrity and authenticity of food can be assessed by exploiting targeted or untargeted analytical methods, using many analytical approaches. Several Boards (both academic and not) have developed standards and guidance for targeted methods, but at the moment they do not cover the untargeted methods. This gap was highlighted by the Food Integrity EU Project and addressed within the WP18 by the "INTELLItrace" Consortium, who engaged in their harmonization and standardization.

As pre-requisite, the harmonization of untargeted analytical approaches goes through a robust validation procedure. The application of mathematical prediction tools on large data sets ("big data") allows interpreting them and thus provides a more accurate description of the population. The criteria for the validation of these untargeted methods (often relying on very different analytical techniques) are hard to identify; as result of the work of WP18, some criticisms were identified and a general guidance for their validation was drafted.

The identification of the best procedures for sampling, of the most suitable analytical protocols, of the correct pre-processing steps, of the most performing statistical classification models and of the acceptability criteria for the validation were deeply analysed and discussed also with other Partners of FI (taking into account both the literature previously published and the Guidelines already proposed and worldwide disseminated [1-3]). All these findings will be reported and discussed in this oral communication, as principal outcome of the research.

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Acknowledgments

This work has been supported by the European project FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688)

O14.1

WILL USE OF BLOCKCHAIN TECHNOLOGY ENSURE FOOD TRACEABILITY AND AUTHENTICITY?

Olsen, P.^{1,*}

1-Nofima, Tromsøe, Norway

*corresponding author e-mail: petter.olsen@nofima.no

Keywords: authenticity, food fraud, standard

Blockchain technology is taking the business world by storm; it is described as a disrupting technology, and by some considered to be the biggest digital revolution since the internet itself. The most well-known application of blockchain technology is the Bitcoin digital currency, but it is also being used to facilitate smart contracts, crowd-funding, electronic voting, and more. There are obvious applications of blockchain technology in product supply chains where it can be used to support persistent, incorruptible records of statements and transactions.

This presentation briefly describes what blockchain technology is, and what functionality it offers. The basic components of a traceability system for food products are outlined (Olsen & Borit, 2018), and the applicability of blockchain technology is analyzed for each component. The overall conclusion is that while blockchain technology can solve, or at least remedy, some specific problems related to food traceability and authenticity, the technology is currently being oversold, and there is significant hype around this particular application of blockchain.

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O14.2

BLOCKCHAIN / DLT AND PRODUCT AUTHENTICITY IN THE AGRI-FOOD SECTOR - SUSTAINABLE, ARTISANAL AND OTHER SPECIALIST CATEGORIES

Delaney, F. ¹

1- *Origin Chain, Dublin, Ireland*

*corresponding author e-mail: info@originchain.eu

Keywords: sustainability, trust, digitalisation, blockchain

Organic packaged food sales in Ireland grew by 4% to €118 million in 2016 and are expected to continue to see strong demand given rising consumer disposable incomes (Euromonitor International, Organic Packaged Foods in Ireland, 2018).

Within Europe, interest in and demand for organic food production continues to grow in both local and export markets. In the 10 years prior to 2016, the organic food market in the EU more than doubled to \$35 billion (USDA FAS GAIN Report: E18034).

Ireland makes one of the smallest contributions to the EU organic farming area, with just 1.6 % of land (73,000 hectares) certified in 2015. This represents an increase of 53 percent over 2010. In 2016, there were 1,787 certified organic farmers representing 2% of total farmers, below the European average of 5-6%. More than 500 of these registered as organic in 2015 when there was a funding program in place (USDA FAS GAIN Report: E18034).

Irish Organic Farmers and Growers Association (IOFGA) describe a reluctance to become organic certified, despite a belief that farming practice is close to organic, because of lack of program funding (discontinued after 2015) and the overhead of additional process and paperwork. (Irish Independent, Farm Ireland interview, 2016)

In Ireland, growers and producers of local and 'sustainable-though-uncertified' produce, benefit from consumer-interest marketing labels including 'artisanal', 'heritage', 'local' and 'eco-friendly'.

While demand for high-quality Irish organic and artisanal prepared food produce continues to grow it is suggested that Irish produce must deliver on provenance, quality, health, ethics and sustainability to meet rising demand for organic and sustainable food. (USDA FAS GAIN Report: E18034).

These demands can be met with new technologies that reliably and publicly record provenance and compliance data across supply chains and deliver traceability insights on real world entities utilising unique and smart digital identities. UDI's help us to track certification, location, logistic and sensor-type data from source to destination facilitating product traceability and also recall under-pinning proof-of-origin and verification of standards.



Figure 1. Visualising the value chain - Origin Chain's new agri-trust initiative creates a new provenance verification channel between producers and consumers in the global digital marketplace.

O14.3

LABORATORY 4.0 AND BLOCKCHAIN: HYPES, BUZZWORDS - CONFUSION OR ADDED VALUE? VIEWS OF AN INSTRUMENT SUPPLIER

Jansen, C.^{1,*}

1-Mettler-Toledo, GmbH Schweiz

*corresponding author e-mail: christoph.jansen@mt.com

Keywords: Blockchain, IIOT, Big Data, Smart Lab

IIOT, Lab 4.0, Blockchain, Big Data, Cloud etc. Buzzwords currently lead many discussions of strategic planners in companies of productions and laboratories. The technical background is often very complicated and needs translation of the relevant aspects for the decision makers. Nobody wants to miss an opportunity. But what are the risks? Cybercrime is a big threat, a lot bigger than anticipated and every day we hear about new threats. Blockchain promises to become a solution but we already read that it has the attention of hackers: the more difficult, the more appealing. Blockchain, such as Bitcoins, also bears other pitfalls: in order to create security, the effort for encrypting is huge. The power consumption for computing and cooling is so big that has to be done in special centers, which are also attractive targets for hackers. Blockchain technology as it is offered today has one bigger disadvantage: it is slow. Real time data recording via Blockchain today is not possible.

Big Data

Data are valuable and the more you have, the more it feels like sitting on a treasure trove. With proper algorithms you could extract anomalies from big data collections, context you cannot see from single data sets. However, even anomalies often don't give you answers. With topics, such as the popular example "predictive policing", opportunity and threat are very close neighbors. Without a doubt DNA sequencing is a good example for useful application of big data. However, I'm still skeptical about consultants for exaggerated data collection. Again, the collection of big data though blockchain today would fail due to the fact that this technology is rather slow.

Data Integrity through IIOT

Industrial Internet of Things (IIoT) in production and laboratories is the umbrella term for many buzzwords.

One of the fundamental IIOT ideas is making objects (things) intelligent enough to share information within a workflow. This is to reduce manual interaction of personnel for transcribing all kind of information. Seamless data flows between components increase data integrity and data quality. With integer documentation we also contribute to better food integrity. Manual transcription is known to be very error prone and makes intentional fraud also much easier.

Who can make it happen?

All these topics remain simple theory if there is no company ready to implement certain aspects in their product portfolio. Someone needs to see a business benefit.

While for some aspects, such as Blockchain, we, the instrument suppliers, currently don't see a useful balance of effort/risks and benefit, there are some fields where we really see high

customer value for our workflows. This is everything that reduces manual transcription and grants easy gap free documentation.

The trend for analytical instruments is improving direct communications between components.

For example, an exchangeable burette contains the chemical name and its titer (the actual concentration), pH electrodes memorize when they were last calibrated and the calibration parameters. This makes them "plug and play" devices, reduces errors and provides seamless documentation. Pipettes can have an asset management system that tells the user which pipette can be found in which lab and provides information about the calibration status. There are many more examples in lab equipment for this kind of seamless dataflow. The technology behind all those features is RFID, wireless, contact free, short range communication. The memory of the tags is powered by induction.

For the current Blockchain technology we don't see the value added application in these workflows. At the end of the lab workflow, the final analytical result is transferred into a lab informatics system or directly into the ERP system, e.g. for product release or quality documentation. This data flow is ideally performed with software that is capable of controlling the instrument and collecting all necessary meta data. Raw data storage and audit trail recording are also important aspects of data integrity, to comply with regulatory requirements.

Having the same software for a bundle of instruments allows dataflow between the instruments and reduces the implementation and maintenance effort. Open standards projects are in the development but also struggle for a number of reasons. There are off the shelf software solutions available that connect and control many instruments and fulfill requirements of data integrity.

Again, business cases drive instrument suppliers to invest into working solutions. If we'll see that for blockchain, we will work on the implementation for our workflows.

O15.1 EIT FOOD STRATEGIC AGENDA FOR INNOVATION

Groba, M.^{1,*}

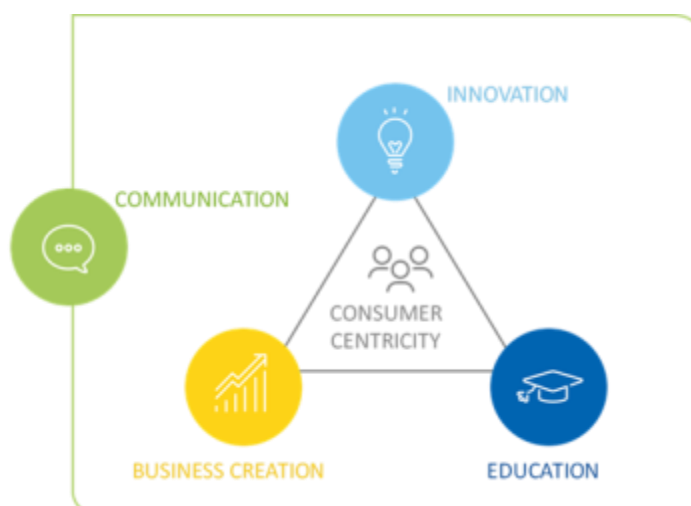
1-EIT Food, Leuven, Belgium

*corresponding author e-mail: Mercedes.groba@eitfoo.eu

EIT Food is one of the Knowledge and Innovation Communities (KIC) of the European Institute of Innovation and Technology ([EIT](#)).

The consortium has broad coverage in EU member states and associated countries and is a unique partnership of over 50 leading companies, universities, and research institutes representing the entire food system. This includes leading international industry such as Nestlé, PepsiCo, Siemens, John Deere, Colruyt Group, Döhler, DSM, Sodexo, Bosch, industry sector is complemented by a number of leading universities and research institutes e.g. KU Leuven, TU Munich, ETH Zurich, universities of Reading, Cambridge, Turin, and Warsaw, This is accompanied by the RisingFoodStars association, which members are excellent European agro-food tech start-ups.

EIT Food's mission is to catalyse the transformation of the food system by building, managing and empowering a sustainable and trusted multi-stakeholder community with a central role for the consumer. EIT Food is putting Europe at the centre of a global transformation in how food is innovated, produced and valued by society. With a strong, trusted and growing partnership and an engaged multi-stakeholder community, we are creating a new kind of food system that is innovative, resilient and sustainable. Our approach puts the needs, concerns and ideas of consumers at the core of what we do to drive a more resource-efficient, secure, trusted and transparent food system. We achieve this by going beyond the integration of the Knowledge Triangle of Education, Business Creation and Innovation by including Communication as an equal, fourth functional area to implement our Strategic Agenda until 2024.



EIT Food partners have defined the following Strategic Objectives:

1. Overcome low consumer trust; EIT Food supports European citizens in the transition towards a smart food system that is inclusive and reassuring.

2. Create consumer valued food for healthier nutrition; EIT Food enables individuals to make informed and affordable personal nutrition choices.
3. Build a consumer-centric connected food system; EIT Food develops a digital food supply network with consumers and industry as equal partners.
4. Enhance sustainability through resource stewardship; EIT Food develops solutions to transform the traditional 'produce-use-dispose' model into a circular bio-economy.
5. Educate to engage, innovate and advance; EIT Food provides 'food system' skills for more than 10,000 students, entrepreneurs and professionals through advanced training programmes.
6. Catalyse food entrepreneurship and innovation; EIT Food fosters innovation at all stages of business creation.

This will be achieved by:

- Developing new talents: EIT Food education programmes will attract and engage new talent thanks to innovative course content which is designed to overcome the 'silos' of knowledge and skills in specific areas of the food system. EIT Food will introduce new learning methods, entrepreneurial tools, and business practices that empower students, professionals and executives at all career stages to become entrepreneurial champions in Europe's food sector.
- Supporting business creation and acceleration: EIT Food will boost the competitiveness of the EU food sector and ensure that Europe remains the number one global exporter of food and drink. EIT Food will proactively support entrepreneurs in transforming their ideas into businesses through the entire start-up cycle and in clearly defining their market. It will generate future entrepreneurial champions in the food sector who will fulfil their ambition to improve nutrition, achieve food security and promote resource-efficient food systems.
- Create consumer-valued food for healthier nutrition: EIT food will develop innovative tools and technologies that support personalised diet profiles combined with the ability to self-assess the impact of customised diets through non-invasive home diagnostics, mobile devices and individual online coaching. This will help to narrow the current gap between people's intentions and actual behaviour towards healthier foods and lead to an improvement of people's health across Europe.

O15.2

AUTHENTNET – BUILDING A FOOD AUTHENTICITY NETWORK

Foster, L.^{1,*}, Aguilera, V.²

1-Department for the Environment, Food and Rural Affairs, 2 Marsham St, London SW1P 4DF, UK

*corresponding author e-mail: lucy.foster@defra.gsi.gov.uk¹;
victor.aguilera@defra.gsi.gov.uk²

Keywords: analytical tools, fraud, collaboration, knowledge transfer

Europe leads the way in producing high quality food. A thriving economy depends on systems that are in place to add value and ensure the integrity of the food chain. Following the horsemeat incident, a Food Authenticity Funders Network was established under a H2020 Coordination and Support Action project 'Authent-net' to better align food authenticity and food fraud research activities across EU countries. This network addressed a gap in providing mechanisms to improve coordination and knowledge exchange around food integrity research between member States.

A key task of the Funder Network was to develop a high-level Strategic Research Agenda (SRA) which set out areas of common interest on food authenticity and food fraud. This document built on work carried out under the Authent-net project to undertake mapping of funder activity on food authenticity research at a national level, and review gap analysis work by member States on priorities. Activities were also undertaken to identify, refine and align transnational priorities for research to inform policy making to tackle food fraud and identify opportunities for collaboration.

This SRA sets out areas of common interest in food authenticity research and food fraud related activity identified by the Food Authenticity Funders Network. Priority areas for collaboration address research to develop analytical tools to detect food fraud, their uptake and use, and approaches to food fraud prediction and prevention. These shared priorities will provide a framework, around which to facilitate cooperation across Member States through research collaboration, data sharing and knowledge exchange. Enabling collaboration will contribute to more effective research outputs at EU level, and raise awareness of the tools available to address food.

Acknowledgments

With thanks to all the Authentnet funding partners for their continued engagement.



POSTER
ABSTRACTS

P3.1

FAST AND GLOBAL AUTHENTICITY SCREENING OF SPICES USING $^1\text{H-NMR}$ PROFILING: EXAMPLE OF BLACK PEPPER

Portaluri, V.^{1*}, Thomas, F.^{1*}, Jamin, E.¹, Lafeuille, J.-L.²

1–Eurofins Analytics France, NANTES, France

2–McCormick & Company, Inc., CARPENTRAS, France

*corresponding author e-mail: freddythomas@eurofins.com,

Keywords: NMR profiling, authenticity, spices, pepper.

According to a recent study from French customs, about half of the commercialized spices are considered as being adulterated, and even more among ground spices. Black pepper samples, representative of the whole world production (Vietnam, India, Brazil, Indonesia ...), were collected over one year and analyzed according to an analytical pathway including organic solvent extraction and untargeted ^1H NMR spectroscopy. An interpretation tool was developed in order to determine the authenticity of black pepper from our database, whose spectra are displayed on figure 1. Common adulterants such as maltodextrine or starch were detected from 10% addition experiments. Principal Component Analysis (PCA) was used to analyse spectral data in an untargeted unsupervised way, and a geographical discrimination was found between Asia and America (fig. 2). That same fast and untargeted approach can be applied to many other spices.

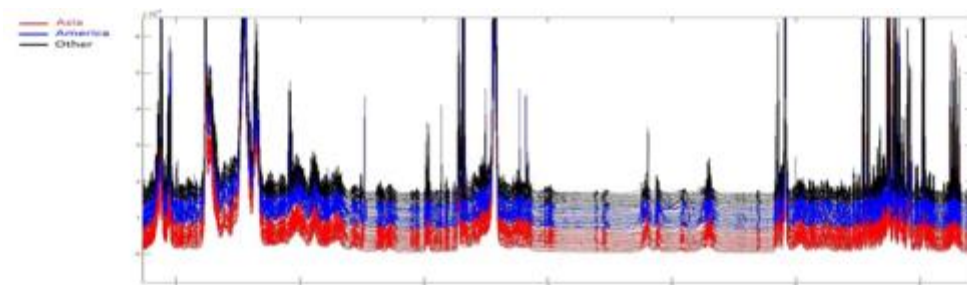


Figure 1: Black pepper $^1\text{H-NMR}$ spectra database. Pepper origin: Blue = Asia, Red = America, black = other.

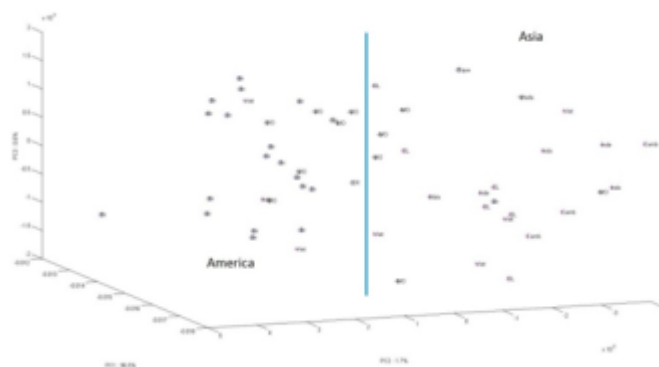


Figure 2: Database projection on PC1 (96.7%), PC2 (1.7%) and PC3 (0.6%).

P3.2

UNTARGETED PROTEOMIC BASED ANALYSIS USING A TRIPLE TOF MS FOR DISCRIMINATING WILD TYPE FROM FARMED SALMONS

Monaci, L.^{1,*}, Brockmeyer, J.^{2,**}, Florino, G.M.¹, Fresch, M.², Brümmer, I.², Losito, I.^{1,3}

1-Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR), Via Amendola 122/O, 70126 Bari, Italy

2-Institut of Biochemistry and Technical Biochemistry, Department of Food Chemistry, University Stuttgart, Allmandring 5b, 70569 Stuttgart, Germany

3-Department of Chemistry and SMART Inter-department Research Center, University of Bari "Aldo Moro", Via E. Orabona 4, 70126 Bari, Italy

*corresponding author e-mail: linda.monaci@ispa.cnr.it **jens.brockmeyer@lc.uni-stuttgart.de

Keywords: salmon authenticity, untargeted analysis, LC-high Resolution Mass Spectrometry, multivariate statistical analysis.

Salmon is one of the most valuable and beneficial fish sold worldwide, also thanks to the enormous benefits on human health related to its consumption. The increasing demand of this product on the market has led, on the one hand, to the depletion of the wild type species in the oceans, on the other hand, to the intensification of farming practices in aquaculture systems. In the last years, the increase of food fraud and adulterant substitutions with serious consequences on the consumers and the economic systems, has solicited the development of new analytical and fast methods able to detect illegal manipulation or food mislabeling.

Non targeted methods have recently emerged as an alternative and rapid strategy to confirm food authenticity. Despite the classical targeted methods mainly aimed at detecting specific indicators, untargeted analysis represents a novel tool able to collect a multitude of data, not correlated to known parameters that are further processed via advanced statistical tools. In the present communication, an untargeted workflow based on High Resolution Mass Spectrometry analysis was applied to the protein fraction of farmed and wild type salmon extracts (belonging to *Salmo salar* species) aiming at assessing its capability to discriminate between both salmon groups. The protein mixture extracted by using an urea based buffer, was further enzymatically cleaved by using trypsin and then submitted to a cleanup step before LC-HRMS analysis. MS/MS experiments were performed on a triple TOF-MS in DIA analysis by applying the SWATH mode and data evaluation was further accomplished by using chemometric software. A preliminary analysis (PCA) was carried out on all peptides detected after data processing and the peak area values were used to highlight any eventual discrimination between both salmon groups.

The resulting score plot referred to the PCA using all peptides already demonstrated a clear separation between wild type and farmed salmons. In addition, an evaluation on the extracted ion chromatograms (XICs) relative to the most abundant peptides in wild or farmed salmons showed that some peptides displayed a different abundance, in terms of intensities, in farmed or wild salmons, suggesting the possibility to identify a list of candidate peptide markers capable of discriminating farmed from wild salmons. In conclusion, the application of untargeted LC-HRMS/MS approach, integrated by multivariate statistical analysis, appears to be a promising tool for the discrimination of farmed and wild type salmons.

Acknowledgment:

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

P3.3

UNTARGETED HIGH RESOLUTION MASS SPECTROMETRY FOR DISCRIMINATING AUTHENTIC SAFFRON

Monaci, L.^{1,*}, de Angelis, E.¹, Pilolli, R.¹, Godula, M.², Hollosi, L.², Garino, C.³, Arlorio, M.³.

1-Institute of Sciences of Food Production, National Research Council of Italy, Bari, Italy,

2- Thermo Fisher Scientific, Germany

3-Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Novara, Italy

*corresponding author e-mail: linda.monaci@ispa.cnr.it

Keywords: saffron, adulteration, DART, High Resolution Mass Spectrometry

Saffron is a valuable and highly appreciated spice derived from the dried red stigmas of the flowers of the cultivated plant *Crocus sativus* L. It is commonly used as a coloring and flavouring agent in food preparation. In addition, it is a good source of flavonoids, proteins, sugars, vitamins, amino acids, mineral matter, gums, and other chemical compounds that make it a health promoting spice. To produce saffron spice, harvested stigmas are submitted to a mild food processing peculiar of the region of production thus it could be considered a traditional product, which aroma and chemical composition strictly depend on the geographic location of production. Due to its high costs of production, saffron is one of the most expensive spices commercialized across the world, and often susceptible of adulteration.

Different plant-derived adulterants have been discovered, and the most frequently involve cut and/or dyed *Carthamus tinctorius* L. petals and *Curcuma longa* L. powdered rhizomes (Kanti et al., 2011). Several analytical methods have been reported in literature for the detection of plant adulterants in saffron samples based on different techniques (e.g. Maggi et al., 2011, Zalacain et al., 2005, Tarantilis et al., 2004, Zougagh et al., 2005) although official methods (ISO 3632-1; ISO 3632-2) exist. Very recently, high-performance liquid chromatography coupled to high resolution mass spectrometry (HRMS) was successfully proposed for saffron authentication/traceability according to the geographical origin based on untargeted metabolic fingerprinting (Rubert et al., 2016).

In this work, we investigated two HRMS based approaches one using ESI ionization coupled to LC separation and the other based on DART ionization before HRMS detection to assess saffron authenticity by an untargeted metabolic approach. Pure saffron samples and saffron spiked with different amounts of *Carthamus* and *Curcuma* adulterants were extracted with a mix of ethanol and water to extract the majority of metabolites and final MS fingerprints obtained were used for authenticity assessment and/or adulteration detection. The produced spectra were then processed via the commercial software Compound Discoverer v.2.1 SP1 (Thermo Fisher Scientific). The detection and grouping of the unknown compounds by setting a mass accuracy ≤ 5 ppm was further accomplished, together with a preliminary statistical analysis of the integrated peak areas.

As grouping factor "Type of adulterant" (*Carthamus* and *Curcuma*) was set and data were further pretreated by aligning the extracted chromatograms on the respective retention time. In order to filter the compound list to the species most suitable in discriminating pure saffron from the adulterated one, Volcano plots combining the statistical significance of the identified compounds and magnitude of change in the extracted peak areas, were investigated. Edited compounds list constrained by means of p-value thresholds was then subjected to statistical evaluation by PCA. The present communication will provide a comparison of results by using

both approaches demonstrating that HRMS in positive polarity combined with a proper statistical treatment might discriminate between authentic and adulterated saffron.

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Acknowledgement:

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

P3.4

SCREENING OF AGAVE SYRUP FOR ADULTERATION USING NMR-PROFILING

Dübecke, A.^{2,*}, Wiezorek, T.¹, van der Meulen, J.¹, Bartz, S.¹, Bachmann, S.¹, Beckh, G.¹

1-Quality Services International, Bremen, Germany

2-Tentamus Center for Food Fraud (TCF²), Bremen/Berlin, Germany

*corresponding author e-mail: arne.duebecke@tentamus.com

Keywords: agave, fraud, NMR, profiling

Alternative sweeteners are gaining more and more importance. Among these, agave syrup is the most popular especially for organic markets and vegan nutrition. Due to its low glycemic index it is well appreciated for diet purposes. Agave syrup is a risk product in terms of economically motivated adulteration based on high production costs due to a long growing period of the agave plant before harvesting (approx. 8-14 years). The “success” of an adulterated product is based on a sophisticated adulteration method using hardly detectable sugars or syrups. With every improved or new method to determine adulteration the “food fraud business” develops more pure sugar syrups, which cannot be detected with “simple” methods. For instance, you can easily buy syrup without a detectable trace of oligosaccharides – so the analysis of an oligosaccharide profile or the detection of polyglucanes will always be negative and gives a false negative result.

At the moment, several techniques for agave syrup authenticity testing are available from different private laboratories. Also, Mexico as main producer has recently created a governmentally approved paper (“NOM-003-SAGARPA-2016”) as an official guideline for the characterization of pure agave syrup. We developed a screening method by means of NMR-profiling. As other profiling methods, also this one relies on a database of authentic samples as well as experimentally adulterated samples. 485 samples were analyzed with NMR and other methods. Furthermore, we conducted a study on market samples from Mexico and compared the results from ¹³C-IRMS analyses with those from NMR-profiling. Finally, results were evaluated in context of the standard NOM-003-SAGARPA-2016.

P3.5 THE ISOTOPIC APPROACH TO THE AUTHENTICITY OF CYPRIOT INNOVATIVE SPIRIT “CERATONIA”

Kokkinofta, R.^{1,*}, Ioannou, E.¹, Economidou, N.¹, Tzioni, E.¹, Savvidou, C.¹, Louka, C.¹.

1-State General Laboratory, P.O.Box 28648, Nicosia, Cyprus

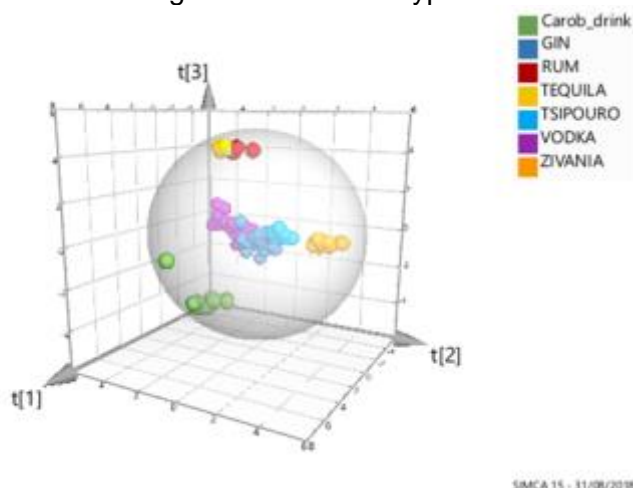
*corresponding author e-mail: sqslsnif@cytanet.com.cy

“Ceratonía” is an innovative alcoholic beverage produced in Cyprus from carobs. Given the proven commercial value of this product, it is necessary to establish “Ceratonía” as a traditional spirit with unique characteristics.

In the framework of “Black Gold” project funded by the University of Cyprus, the composition and specific characteristics of “Ceratonía” were studied and compared to similar products from other regions, in order to differentiate it and certify its origin. In the present work, the combined information from isotopic ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ by IRMS and D/H by SNIF-NMR spectroscopy appears to create a unique isotopic fingerprinting of “Ceratonía”.

In a total of 157 alcoholic beverages, PCA and PLS-DA differentiated the carob drinks in a separate group, with only “outlier” a carob liqueur. This group is similar to the group of Cypriot traditional zivánias, close to tsipouro and apart from vodka and gin. The rum and tequila drinks are presented in a group, without the possibility of being distinguished by the applied method. The chemometric analysis of the results for comparison “Ceratonía” from others highlighted its uniqueness.

It is believed that this differentiation in stable isotopes of “Ceratonía” is related to the unique geological and climatic conditions existing in the island of Cyprus.



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P3.6 NUTRITIONAL MARKERS OF CAROBS: AN APPROACH TO ORIGIN CLASSIFICATION

Kokkinofta, R.^{1,*}, Giannopoulos, S.¹, Stylianou, M.A.², Agapiou, A.²

1-State General Laboratory, P.O.Box 28648, Nicosia, Cyprus

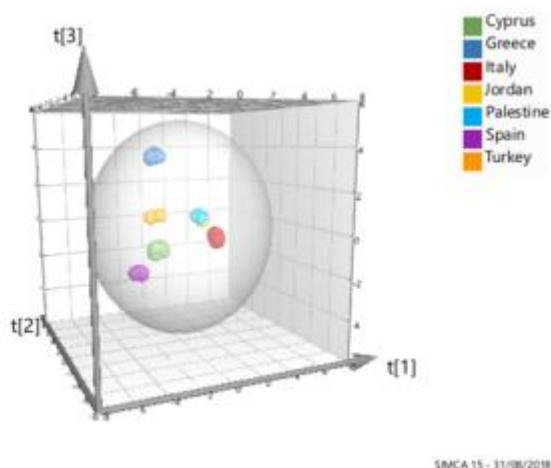
2-University of Cyprus, Department of Chemistry, P.O. Box 20537, 1678, Nicosia, Cyprus

*corresponding author e-mail: agapiou.agapios@ucy.ac.cy / sqslsnif@cytanet.com.cy

Carobs can be characterized as functional foods with low fat content, high content in dietary fibers and source of minerals. In the present study, which is a part of a wider investigation on carobs, 76 carob samples from 7 different Mediterranean countries (Cyprus, Greece, Spain, Turkey, Jordan and Palestine) were analysed for their nutritional composition, in order to identify markers for their authenticity. Moisture, ash, fat, proteins, sugars (fructose, glucose, and sucrose), dietary fibers and minerals (Ca, K, Mg, Na, P, Cu, Fe, Mn, Zn) were determined following official methods. Due to the large number of data produced, chemometric techniques were employed to analyze these and draw conclusions. The samples of different geographical origin were discriminated with >90% success in total. The carobs from Cyprus, Italy and Spain were correctly classified without error. The main discriminators found to be the dietary fibers, the carbohydrates and Fe, Zn and Mn, which emphasize their specific nutritional value and add value to the product.

The results appear to suggest that the above approach is a powerful tool for the successful discrimination of carobs origin.

This work carried out in the framework of the "Black Gold" project, financially supported by the University of Cyprus.



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P3.7**TRACEABILITY OF ANIMAL RENNET THROUGH $\delta^{15}\text{N}$ ANALYSIS OF CHYMO SIN**

Bontempo, L.^{1,*}, Camin, F.¹, Ziller, L.¹, Franceschi, P.², Molteni, A.³, Corbella, R.⁴, Verga, I.³

1-Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach 1, 38010 San Michele all'Adige, Italy

2-Unit of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach 1, 38010 San Michele all'Adige, Italy

3-Cagliificio Clerici S.p.A. and Sacco S.r.l., Via Manzoni 29, I-22071 Cadorago (CO), Italy

4-Caglio Bellucci S.r.l., Via Vito Bering 57, 41123 Modena (MO), Italy

*corresponding author e-mail: luana.bontempo@fmach.it

Keywords: rennet, stable isotope ratio of nitrogen, authenticity

Chymosin is a protease that curdles the milk casein. Animal rennet is the first discovered source of chymosin and its use is mandatory for the production of PDO cheeses such as Parmigiano Reggiano and Grana Padano. Among the available alternatives, the fermentation-produced chymosin is the most competitive because it has similar activity of animal rennet, with a much lower price. Analytical tools are necessary in order to distinguish the two types of chymosin and verify the compulsory use of animal rennet in the production of PDO cheeses.

In this work, a method to analyse the stable isotope ratio of nitrogen ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$) in chymosin after extraction has been developed. The $\delta^{15}\text{N}$ values of animal rennet range from 5.7‰ to 8‰, whereas the $\delta^{15}\text{N}$ values of the fermentation-produced chymosin are significantly lower, ranging from -5.3‰ to 2.2‰. A threshold value of 5.7‰ has been defined for the identification authentic animal rennet. The method can be used to detect the authenticity of animal rennet on the market.

P3.8**AUTHENTICITY TESTING OF COCONUT WATERS,
VANILLA FLAVOURS AND FRESH APPLES BY THE USE
OF ADVANCED FORENSIC ANALYTICAL TOOLS.
DEVELOPMENTS AND FUTURE TRENDS****Psomiadis, D.^{1,*}, Horváth, B.¹, Zisi, N.¹, Koger, C.¹, Bodiselitsch, B.¹***1-Imprint Analytics GmbH, Werner von Siemens Str. 1, 7343 Neutal, Austria**corresponding author e-mail: psomiadis@imprint-analytics.at**Keywords: food authenticity, vanilla, coconut waters, origin testing**

Food fraud, by definition, is a human-induced practice which lies beneath the surface of food supply chain and food manufacture. Modern fingerprinting techniques are increasingly utilized in order to scrutinize food matrices and products as a matter of source, provenance, origin and authenticity. However, food authenticity testing involves a complicated and still undefined area of analytical verification tools. We are still far from harmonization and technical synergies, which would enable multidisciplinary solutions, automatized mechanisms and digitalization. Market trends always create fertile ground for food fraud and financial gains. As a practical example, we present three market scans on certain popular product types: coconut waters (detection of undeclared added sugar), vanilla-flavored products (detection of different vanillin types) and fresh fruits (geographical origin of apples).

Indicatively, 38% of the tested coconut waters have been found to include undeclared added sugar, 26% of the declared as authentic vanilla-flavored products have been identified to include non-vanilla flavors and 12% of the investigated apples did not comply with the fingerprint of their declared provenance. Using advanced forensic analytical tools, the detection of adulterated products is enhanced and the detection limits are improved. This serves the real market conditions in respect with the food fraud practices. The authenticity testing study includes also the evaluation of advanced methods, beyond the standards which are established and commonly used. By this, the improved detection and verification of claims is justified, aiming to open a discussion on good practice and fit-for-purpose methods. Looking to the future trends, the conclusions from the previous points are elaborated to identify potential developments that may serve the food sector.

P3.9

EFFECT OF WHEY BASED FILM COATINGS ON VARIOUS PROPERTIES OF KASHAR CHEESE

Jalil, H.M.^{1,*}

1-Food Engineering and Science, Raparin University, Slemani, Iraq.

*corresponding author e-mail: hawbashmhedin@gmail.com

In this study, the effects of whey protein based films on various properties of kashar cheese were examined. In the study, edible film solutions based on whey protein isolate, whey protein isolate + transglutaminase enzyme and whey protein isolate + chitosan were produced and Kashar cheese samples were coated with these films by dipping method and stored at +4 °C for 60 days. Chemical, microbiological and textural analyzes were carried out on samples at 0, 30 and 60 days of storage. As a result of the study, the highest dry matter and total nitrogen values were obtained from uncoated control samples. This is an indication that the coatings limit water vapor permeability. The highest acidity and pH values obtained from the samples as storage results were 3.33% and 5.86%, respectively, in the control group samples.

Both acidity and pH rise in these groups, is a consequence of the buffering of pH changes of hydrolysis products which are as a result of proteolysis occurring in the sample. Nitrogen changes and lipolysis values, which are indicative of the degree of hydrolysis of proteins and triglycerides in kashar cheese, were generally higher in the control group. This result is due to limiting the micro organism reproduction by limiting the gas passage of the coatings. Hardness and chewiness values of the textural properties of the samples were significantly reduced in uncoated control samples compared to the coated samples due to maturation. The chitosan film coatings used in the study limited the development of mold yeast until the 30th day but after that did not yield successful results in this respect.

P3.10

CHEMICAL PROFILING OF WHISKIES USING ORBITRAP GC-MS

Hajslova, J.¹, Stupák, M.¹, Pulkrabova, J.¹, Cole, J.², Silcock, P.², de Dobbeleer, I.³

1-Institute of Chemical Technology, Prague, Czech Republic,

2-Thermo Fisher Scientific, Runcorn, UK

3-Thermo Fisher Scientific, Breda, the Netherlands.

Whisky is a premium distilled spirit beverage produced using long-established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, has high commercial value, and can be economically important in the regions of the world where it is produced and consumed. As such, it is essential that whisky producers are able to obtain an accurate and comprehensive chemical profile that is characteristic of their individual product. This work aims to demonstrate the application of a complete untargeted chemometric workflow using the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS to detect and identify chemical components in whisky. This proof-of-concept study also shows the process of identifying chemical differences in whiskies of different origins.

The results of this proof-of-concept study show that the Q Exactive GC system is an ideal analytical tool for comprehensive chemical profiling of complex matrices, offering high performance full scan analysis. Software tools enable fast and accurate differential analysis to be performed to isolate unique features of samples. Routine mass resolution of 60,000 FWHM and consistent sub-ppm mass accuracy ensures selective and confident compound detection and identification.

P3.11

DETERMINATION OF MEAT AUTHENTICITY USING A COMPREHENSIVE TARGETED PROTEOMIC STRATEGY AND HIGH-RESOLUTION MASS SPECTROMETRY

Ruiz Orduna, A. ¹, Husby, E. ¹, de Dobbeleer, I. ², Gosh, D. ³

1-Département de Biomédecine Vétérinaire, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QB, Canada,

2-Thermo Fisher Scientific, Breda, The Netherlands.

3-Thermo Fisher Scientific, San Jose, US

Introduction

Due to the internationalization of food production and distribution, there has been a significant increase of food fraud in recent years. Food fraud can have serious health implications and occurs when food manufacturers implement unethical practices, such as making false label claims as well as using additives and fillers within their products to increase profitability. This has been a serious concern, and in 2013, horse and pig DNAs were detected in beef products sold by several retailers (DG Health and Consumers, European Commission). In an effort to control this within the food industry, certification of meat authenticity must be delineated for all regulatory agencies.

The application of proteomics in the meat science field is focused on improving meat quality while increasing meat production and revenue. To ensure that food safety regulations are being met, food-testing laboratories require more advanced analytical strategies to test for adulteration and to expose many of these unethical, albeit profit-generating, tactics. Since mass spectrometry (MS) is considered a gold standard in protein research, it is also used as a method for detecting marker proteins that support animal tissue identification. In this application, meat adulteration was tested using a well-defined proteogenomic annotation and carefully selected surrogate tryptic peptides. This novel method is a new technique for determining meat authenticity and composition using a state-of-the-art high-resolution Orbitrap™ MS.

P3.12

A FAST ION CHROMATOGRAPHY METHOD FOR ANALYSING SUGARS IN INSTANT COFFEE

Dewsbury, P.^{1,*}, Patil, S.¹, Rohrer, J.¹

1-Thermo Fisher Scientific, Sunnyvale, USA

*corresponding author e-mail: paul.dewsbury@thermofisher.com

Keywords: coffee, sugars, adulteration, ion chromatography

Carbohydrates are an important constituent of the coffee beans, forming about 50% of the green coffee bean. These carbohydrates undergo complex changes during the roasting process and can affect the final taste and aroma properties of the coffee. Carbohydrate content is used for detecting coffee adulteration

A fast method for profiling sugars in instant coffee using High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) and a High Concentration Carbohydrate Analysis kit is demonstrated where instant coffee sugars are ionized in a strong base, separated by High-Performance Anion Exchange (HPAE) chromatography, and detected by Pulsed Amperometric Detection (PAD). Analysis is facilitated by the Thermo Scientific™ Dionex™ Integriion™ HPIC™ system.

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.

P3.13

PROFILING OF SUGARS IN HONEY BY ION CHROMATOGRAPHY

Dewsbury, P.^{1,*}, Aggrawal, M.¹, Hu, J.¹, Rohrer, J.¹

1-Thermo Fisher Scientific, Sunnyvale, USA

*corresponding author e-mail: paul.dewsbury@thermofisher.com

Keywords: honey, sugars, adulteration, fingerprint

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

P3.14

EA-IRMS: TRACING THE GEOGRAPHICAL ORIGIN OF ROASTED AND GREEN COFFEE USING ISOTOPE FINGERPRINTS

Brodie, C.¹, Kracht, O.¹, Hilkert, A.¹

1-Thermo Fisher Scientific, Bremen, Germany.

Keywords: coffee, hydrogen, oxygen, isotopes

Coffee is one of the most popular beverages worldwide, sourced from different geographical regions and exported through a commercial chain that usually involves several intermediates. To ensure that coffee beans come from labelled locations, laboratories need an analytical solution, enabling to discriminate geographical origin, with a special emphasis on the country of origin.

Roasted and green coffee beans have a fingerprint, a unique chemical signature that allows them to be identified: isotope fingerprints of carbon, nitrogen, sulfur, hydrogen and oxygen have been reliably used for origin, authenticity and product label claim verification.

In this poster, we report isotope measurements from green and roasted coffee beans measured using the Thermo Scientific™ EA IsoLink™ IRMS System. These data illustrate how isotope fingerprints can determine the origin of coffee beans. Consequently, it is evident that isotope fingerprint approach helps support legislation on food integrity and labelling (EC Reg. No. 1169/2011) and product geographical indication/origin (EC Reg. No. 510/2006) and therefore, protects consumers and brands.

P3.15

AUTHENTICITY CONTROL OF BEVERAGES AND WATER WITH GASBENCH II SYSTEM USING HYDROGEN AND OXYGEN ISOTOPE FINGERPRINTS

Radke, J.¹, Brodie, C.¹, de Castro, M.¹, Bonanomi, M.¹, Kracht, O.¹, Hilker, A.¹

1-Thermo Fisher Scientific, Bremen, Germany.

Keywords: wine, hydrogen, oxygen, isotopes

In this presentation the application of stable isotope fingerprints in beverage and water in food 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 in beverages and the technology used is also provided. It can easily be adapted to water in food (e.g. meat).

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 with the isotope equilibration technique (Method OIV-MA-AS2-12, *EU regulation no. 822/97*). This 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 standardized technique in food and beverage fraud prevention and food safety. For wine in the EU isotope signals are stored in the EU-WineDatabase or other national databases, e.g. $>1400 \delta O^{18}$ of water in European wines, national databases).

P3.16

ISOTOPE FINGERPRINTS: ORIGIN OF TEQUILA WITH GC COUPLED WITH ISOTOPE RATIO MS

Tuthorn, M.^{1,*}, Juchelka, D.¹, Brodie, C.¹, Griep-Raming, J.¹, Hilker, A.¹

1-Thermo Fisher Scientific, Bremen, Germany

*corresponding author e-mail: mario.tuthorn@thermofisher.com

Keywords: isotopes, IRMS, chromatography, tequila

The blue agave (*Agave tequilana* Weber var. Azul) is a native plant of the Jalisco region in Mexico and is an important economic product that, by law, is the only one allowed to be used in the production of tequila. Globally, tequila is a popular alcoholic beverage, which has led to increasing demand and thus production, with a subsequent increase in export value to the Mexican economy. This provides for an opportunity of economically motivated fraud either by adulteration and mislabeling of original tequila or production of fake tequila.

Gas chromatography/isotope ratio mass spectrometry provides a powerful tool for determining carbon, oxygen and hydrogen isotope fingerprints in beverages and food. Thermo Scientific™ TRACE™ 1310 GC coupled with Thermo Scientific™ GC IsoLink II™, Thermo Scientific™ ConFlo IV™ Universal Interface and a Thermo Scientific™ DELTA V™ isotope ratio mass spectrometer offers a solution for identifying the purity and adulteration of products.

Biosynthesis of organic molecules in *A. tequilana* requires water that comes principally from rainfall. Therefore, oxygen isotope fingerprint of the *A. tequilana* plant, and local sugars used in mixed tequilas, is primarily given by the rainfall water in those regions and can provide a geographical tool for origin. Here we report carbon and oxygen isotope fingerprints from commercial tequila, sugar cane and the *A. tequilana* plant. Coupled $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of ethanol allow differing the original branded mixed tequila from *A. tequilana* and sources of sugar (corn and cane). This indicates that mixed tequila can be clearly differentiated from pure tequila, which derives 100% from *A. tequilana*. In addition, it also shows the difference between *A. tequilana*, original mixed tequila and sugar sources, meaning that adulterated and mislabeled tequila can be differentiated from original tequila and original source ingredients.

P3.17

ISOTOPE FINGERPRINTS: AUTHENTICATION OF HONEY BY LC COUPLED WITH ISOTOPE RATIO MS

Tuthorn, M.^{1,*}, Juchelka, D.¹, Brodie, C.¹, Griep-Raming, J.¹, Hilbert, A.¹

1-Thermo Fisher Scientific, Bremen, Germany

*corresponding author e-mail: mario.tuthorn@thermofisher.com

Keywords: isotopes, IRMS, HPLC, honey

Honey is considered as a value-added food of natural origin, yet it is simply structured regarding its composition, which makes it prone to economically motivated adulteration by addition of sugars of other sources. Testing for adulteration can be done using various methods, including melissopalynological pattern analysis, sensory analysis, amino acid profile analysis, and others. The limit of detection of these methods is generally in the double digit percent range, depending on the type of sugars added. The introduction of bulk ¹³C isotope analysis by White and Doner in 1978 was a major step towards establishing better methods sensitivity. But carefully selected mixtures of sugars can mimic both, the bulk ¹³C composition and the sugar profile of the natural product.

Compound specific isotope analysis can refine the authenticity fingerprints of honey. The methodology based on the chromatographic separation of the carbohydrates and carbohydrate fractions and the subsequent isotopic analysis by coupling LC with isotope ratio mass spectrometry (IRMS). Carbohydrates eluting from the ion exchange column are chemically oxidized to carbon dioxide within the aqueous mobile phase using the Thermo Scientific™ LC IsoLink™ interface. Dissolved CO₂ is removed from the liquid phase and entrained into a stream of helium. The individual CO₂ peaks in helium - which correspond one to one with the peaks of the individual compounds - are subsequently dried and then admitted to the IRMS system. The individual ¹³C/¹²C isotope ratios are determined by Thermo Scientific™ DELTA V™ isotope ratio mass spectrometer for the following carbohydrates and carbohydrate fractions: i) glucose, ii) fructose, iii) disaccharides, iv) trisaccharides, and v) oligosaccharides.

The $\delta^{13}\text{C}$ of bulk honey is determined by analyzing pure honey samples in a Thermo Scientific™ EA IsoLink™ IRMS system coupled to a Delta V mass spectrometer. Samples are prepared by encapsulation in tin foil and introduced into the elemental analyzer without additional treatment. The protein fraction is prepared by Na₂WO₄ precipitation from aqueous sample solution, dried and analyzed using the configuration described above. The resulting maximum difference between the following values is being calculated: a) bulk $\delta^{13}\text{C}$, b) protein $\delta^{13}\text{C}$, and c) the individual carbohydrates and fractions (i –iv, above). A large difference in the values (on the order of 1‰ or greater) might indicate adulteration and requires further investigation. This work describes a multi-parametric methodology, looking at both, bulk and compound specific $\delta^{13}\text{C}$, deducing isotope fingerprints and identifying adulteration.

P3.18

FAST, ACCURATE AND PRECISE AUTOMATED GRAVIMETRIC SAMPLE PREPARATION APPLIED TO NMR-BASED AUTHENTICITY CONTROL OF HONEY AND OLIVE OIL

Schnell, N.^{1,*}, Weinelt, P.¹, Hofmann, G.², Schuetz, B.², Steck, A.²

1-Mettler Toledo AG, Im Langacher 44, 8606 Greifensee, Switzerland

2-Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany

*corresponding author e-mail: nutsima.schnell@mt.com

Keywords: honey and olive oil authenticity, food fraud, gravimetric sample preparation, NMR spectroscopy

Honey and olive oil are at very high risk of food fraud. All techniques developed to profile food quality and authenticity is confronted with a big challenge: to detect subtle deviations, caused by adulteration, besides natural variances in complex matrices. One of the most important prerequisites is reproducibility - both in respect of sample preparation and measurements.

Bruker BioSpin GmbH has developed, market-released and ISO-17025-accredited several food profiling methods (for fruit-juice, wine and honey), based on ¹H-NMR spectroscopy combined with multivariate statistical chemometrics. Further matrices are under development, including olive oil profiling. The supreme reproducibility of ¹H-NMR allows worldwide lab-to-lab spectra comparison and collective database buildup.

METTLER TOLEDO, on the other hand, has made significant progress in rapid and accurate sample preparation by developing its novel Quantos Automated Liquid Dosing Solution for gravimetric sample preparation (GSP). It guarantees easy-to-use, economic and highly reproducible mixing of liquid samples and solvents, minimizing variations otherwise brought in if liquid dosing is performed manually with pipettes. In addition, dedicated METTLER TOLEDO software solutions (e.g. LabX) allows sample tracking, supports barcode readers, data import/export to EXCEL or data integration with information system.

For optimum ¹H-NMR spectra reproducibility, also the sample preparation step (i.e. the solvent-sample ratio) has to be kept as constant as possible from sample to sample. When prepared manually, this is tedious and time-consuming for the viscous honey samples, and even more trouble-prone for olive oil dissolved in the toxic and highly volatile deuterated chloroform.

The Quantos Liquid Dosing System was successfully tested for the honey and olive oil sample preparation, using application-tailored dosing methods developed by METTLER TOLEDO in cooperation with Bruker BioSpin GmbH, and yield a very promising 50% reduction of sample preparation time both for honey and olive oil with unmatched reproducibility (see figure).

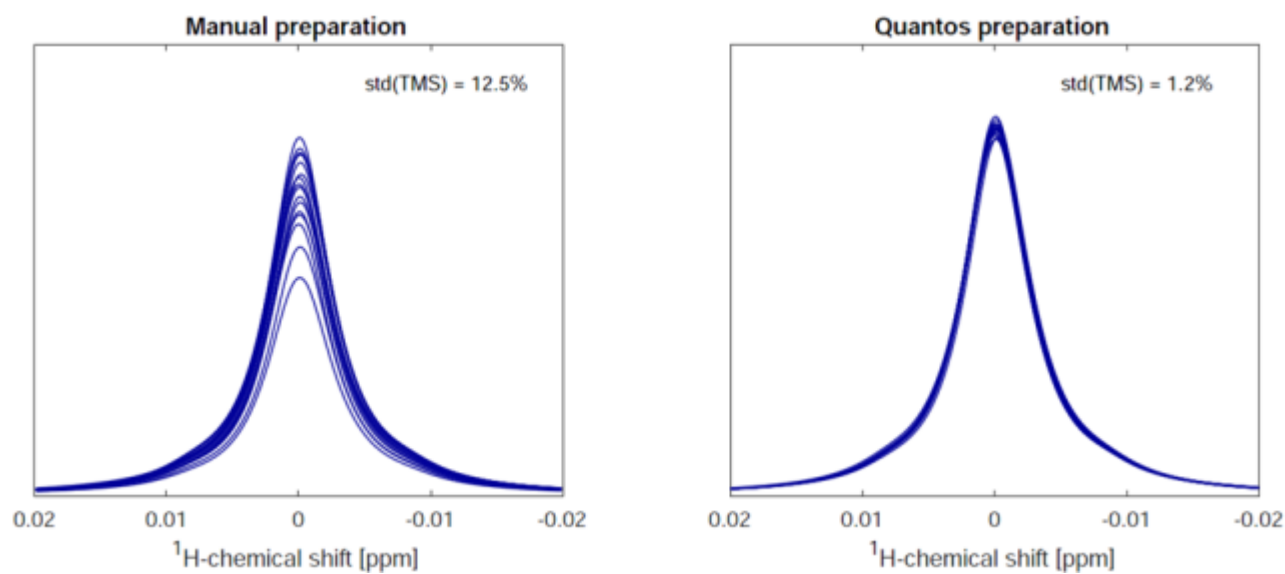


Figure: Comparison of manual and Quantos-based preparation of olive oils, demonstrated by the signal of tetramethylsilane (TMS, ^1H -NMR chemical shift reference). Being extremely volatile, TMS is a very good indicator for precise, fast and reproducible sample preparation.

The first results of the honey and edible oil sample preparation, performed with the Quantos Liquid Dosing Solution, are presented.

P3.19

VALIDATION AND QUALITY ASSURANCE OF FINGERPRINTING METHODS FOR AUTHENTICATION OF FOOD – A REVIEW UPDATE 2015-2017

Raeke, J.^{1,*}, Riedl, J.¹, Esslinger, S.¹, Fauhl-Hassek, C.¹

¹-German Federal Institute for Risk Assessment, Berlin, Germany

*corresponding author e-mail: julia.raeke@bfr.bund.de

Keywords: standardisation, model validation, quality control, system challenge

Non-targeted analytical methods have recently gained in importance for the authentication of food [1, 2]. In these so-called fingerprinting approaches spectroscopic or spectrometric data form the basis for a comprehensive characterisation of the investigated matrix and enable, for example, the differentiation of samples according to their botanical or geographical origin as well as the identification of impurities and adulterations. The complex data matrices which are obtained are evaluated using multivariate statistical methods. Although many studies have already demonstrated the feasibility of fingerprinting approaches, there remain still some challenges before these methods can be used in routine analysis and food surveillance [1, 3]. Important aspects are for example suitable and reliable validation strategies and the implementation of quality assurance measures. Different recommendations and strategies have already been proposed in various publications [1, 3, and 4].

In the overview presented here, the current status of application and implementation of such concepts in the scientific literature was examined. For this purpose, publications published between January 2015 and October 2017 were analysed. The systematic search using specific keywords employing the internet literature database “Scopus” yielded 89 results. The studies covered various food matrices and analytical methods, including near and mid infrared (NIR and MIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) (Figure 1).

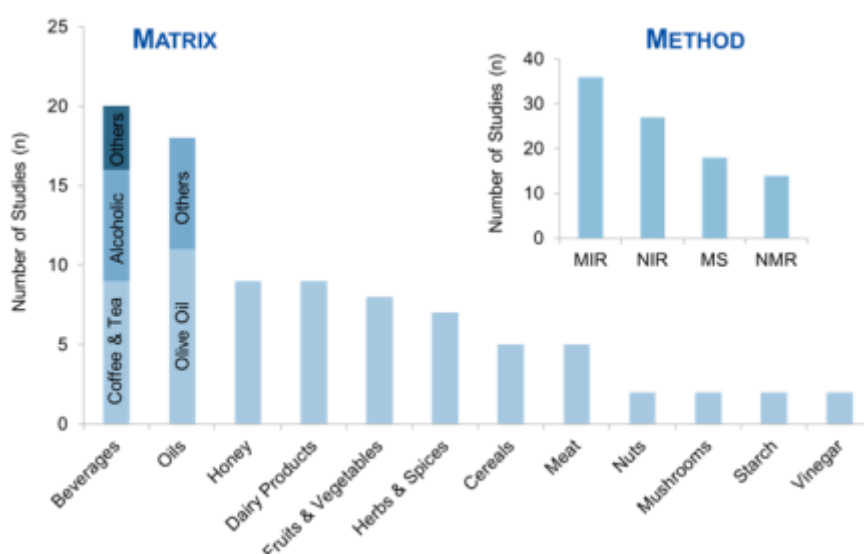


Figure 1: Matrices and methods used in the examined 89 studies (2015-2017).

A detailed analysis of the publications revealed a very heterogeneous picture. Internal and external validation (IV and EV) were frequently used. However, different performance parameters of the models were used for their evaluation. Although the use of quality control (QC) samples increased slightly compared to the last review (2011-2013) [1], QC samples were still only measured in 13 out of 89 studies (Figure 2). System challenges, which examine the applicability of the method under varying conditions and with new samples, were also only carried out in a few cases (Figure 2). Original raw data was also rarely fully provided. Overall, it must be concluded that although a respective number of recommendations for quality assurance and validation already exist, these are often not yet applied in practice. Thus, in order to establish the use of food fingerprinting methods in routine analysis and food surveillance, further progress towards standardisation is needed.

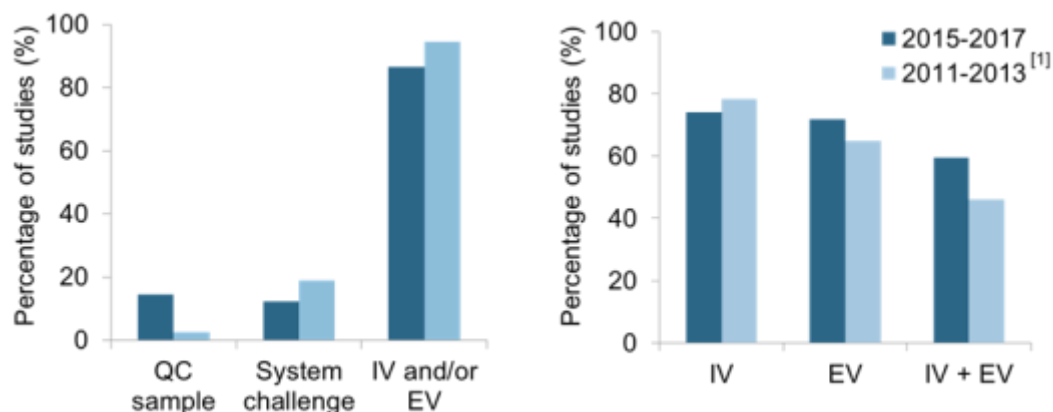


Figure 2: Percentage of studies using quality control (QC) samples, system challenges and internal (IV) and/or external validation (EV) in the current review (2015-2017) compared to older data (2011-2013) [1].

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P3.20

FOOD AND BEVERAGE FRAUD PREVENTION USING ISOTOPE FINGERPRINTS

Bonanomi, M.^{1,*}, Brodie, C.¹, Hilkert, A.¹, Kracht, O.¹, Radke, J.¹

1-Thermo Fisher Scientific, Bremen, Germany

*corresponding author e-mail: maddalena.bonanomi@thermofisher.com

Keywords: Isotopes, Fingerprints, IRMS

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. Examples will be shown for wine, vegetables, honey and alcoholic beverages.

P3.21

FOOD FRAUD DETECTION: A CHALLENGE BETWEEN DNA EXTRACTION AND QUANTIFICATION METHODS IN THE EVALUATION OF THE REAL AMOUNT OF SPECIES COMPOSITION IN MEAT PRODUCTS

Cravero, D.^{1,*}, Cerutti, F.¹, Maniaci, M.G.¹, Barzanti, P.¹, Scaramagli, S.², Riina, M.V.¹, Ingravalle, F.¹, Acutis, P.L.¹, Peletto, S.¹

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

2-COOP ITALIA, Bologna, Italy

*corresponding author e-mail: diego.cravero@izsto.it

Keywords: meat food, species quantification, DNA extraction, DNA quality score, qPCR

Food fraud represents an economic damage and a religious issue for consumers as well as a potential health risk in case of allergy problems. For these reasons, the lack of an accurate method for species quantification in food is the main problem to properly distinguish between fraud and unintended processing contamination in mixed meat food. DNA analysis-based methods are more performing than those based on proteins analysis. Here we compared four commercial DNA extraction kits on minced meat, ravioli filling and ragout experimental 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). Then, on the DNA extractions, three different qPCR methods were compared for meat amount detection: quantification by nuclear targets (nqPCR, pig β -actin gene, bovine growth hormone gene and highly conserved myostatin sequence as universal meat reference gene, Ren et al., 2015), by mitochondrial targets (mqPCR, kit of quantification pork/beef Speciation Kits Genesisig, PrimerDesign) and by targeting SINE (SqPCR, 1.711B *Bos taurus* repeat element and *Sus scrofa* PRE-1 SINE, Walker et al., 2006).

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 purity was 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. Variance analysis on the extraction data was performed with the ANOVA test with Bonferroni correction using the Stata statistical software. Scores were assigned in order to obtain a ranking (DNA Quality Score, DQS) for the selection of the best DNA evaluating the significant differences among the extraction kits for the considered parameters. qPCR was performed in triplicates for each sample. qPCR quantification values were obtained by $\Delta\Delta C_t$ algorithm. The accuracy was calculated as the ratio between obtained and attended values (optimum accuracy value: 1). Considering a single extraction kit, five experimental replicates were performed for each percentage and matrix thus obtaining a total of 25 values for accuracy calculation. All the qPCRs were performed following the protocol provided by Authors and manufacturers (nqPCR: Ren et al., 2015; mqPCR: Genesisig; SqPCR: Walker et al., 2006). Gentra, Mericon and Nucleospin extractions were considered for nqPCR, while only DNA obtained by Mericon was analysed for mqPCR and SqPCR analyses. Accuracy mean values, related to the

extraction kits, quantification methods and matrices, were compared by the non-parametric test of Wilcoxon and ANOVA test with Bonferroni correction.

DNA yield: Gentra purification kit showed the best results for minced meat, ravioli filling, and ragout (p-value<0.05). In contrast, the Wizard proved to be the less performant. Nucleospin and Mericon Food showed similar results (p-value >0.05) for all matrix related samples. **DNA purity:** for 260/280 ratio, no significant differences were observed among the extraction kits used on minced meat and ravioli filling (p-value >0.05). Also, absorbance ratios for all considered extractions were not related to the optimum value (1.8, p-value <0.05). For ragout, only 260/280 absorbance ratios obtained by Mericon and Wizard were related with the optimum (2.0 p-value >0.05). For 260/230 absorbance ratio, Mericon kit extractions on all food matrices showed the best results (p-value <0.05). **DNA integrity:** DNA fragment size of minced meat and ravioli filling processed with Mericon was the best in terms of length (p-value <0.05). Wizard kit confirmed to be the worst on all considered matrices (p-value<0.05). DQS identified extractions performed by Gentra as the best for all the considered matrices, together with Mericon extractions for ravioli filling. Considering the three food matrices, nqPCR performed on Mericon derived DNA resulted more accurate compared to those on Gentra and Nucleospin samples (p-value <0.05). In general, no significant correlation to the optimal accuracy value was found (p-value <0.05) for nqPCR analyses, while SqPCR method revealed to be accurate on all the percentages referring from all food matrices (p-value >0.05). mqPCR showed to be accurate only on ragout sample measurements (Figure 1). Precision of mqPCR and SqPCR measurements resulted to be better on ragout than less processed matrices, such as ravioli filling and minced meat (p-value <0.05, Figure 1). nqPCR demonstrated to be less precise than the others two qPCR methods on all considered matrices (p-value <0.05).

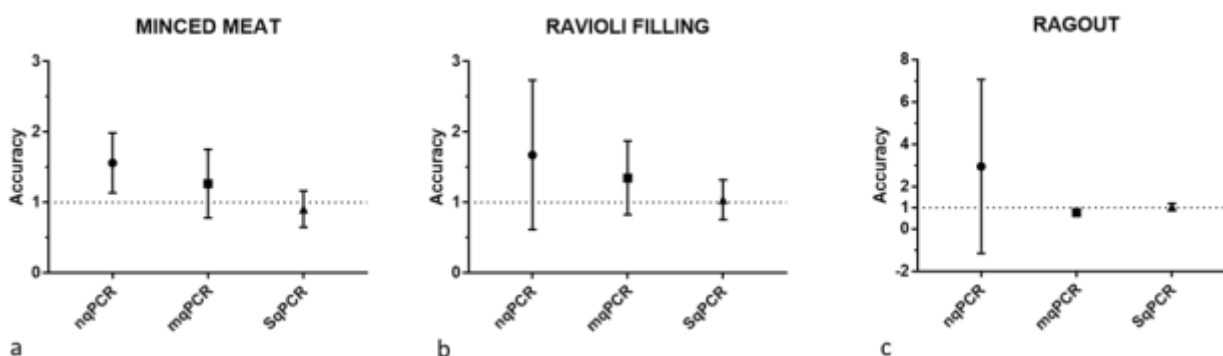


Figure 1: comparison of accuracy mean-values with standard deviations of qPCR according to the different gene targets. a) qPCR comparison on minced meat; b) qPCR comparison on ravioli filling; c) qPCR comparison on ragout. X-axis: qPCR methodologies, Y-axis: relative accuracy scale. Dot line: optimum accuracy value (1).

The DQS, integrating DNA yield, purity and integrity analyses, may be an effective tool in determining the quality of the extracted DNA also allowing comparing the nucleic acids isolated by different extraction methods in order to select the "best DNA". Furthermore, compared to the other qPCR approaches, SINE-based quantification proved to be more accurate for the measurement of the real amount of species composition in meat food. Optimal DNA quality coupled with an accurate qPCR method is the key features to properly distinguish food fraud from random contamination in meat products.

This work has been funded by the Ministry of Health (grant Ricerca Corrente IZS PLV 13/10 RC).

P3.22

DIFFERENTIATION BETWEEN WILD AND FARMED SEA BREAM USING HIGH RESOLUTION MASS SPECTROMETRY AND TWO-DIMENSIONAL ELECTROPHORESIS

Mazza, M.¹, Sciuto, S.¹, Esposito, G.¹, Brusadore, S.¹, Dell'Atti, L.¹, , Acutis, P.L.¹

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

*corresponding author e-mail: Maria.Mazza@izsto.it

Keywords: farmed, wild, sea bream, food fraud

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. Among all the existing species, the most farmed fish in the world are carp, salmon, sea bream, sea bass, croaker, amberjack, eel, trout, tuna, sturgeon, crustacean and molluscs. European legislation requires consumers to be informed about the origin and the history of the products they purchase. 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). Consumers often do not have instruments or competence to visually discriminate if a product corresponds to what is indicated on the label and the assessment of seafood origin assumes an enormous importance in the perception of quality by the final consumer, therefore a specific control to protect them from commercial fraud is desirable.

Different studies have confirmed that the fatty acid composition of farmed and wild fish is different and diet is the main reason for these differences (2). In the same way, the different diet between farmed and wild fish has an influence on the metabolism and on the proteomic profile, as already reported in other proteomic studies on cod muscle and rainbow trout liver (3, 4). Moreover, water temperature has an influence on the hepatic protein profile of gilthead sea bream (5).

The aims of the present study were: 1) to develop an easy and fast method to extract and simultaneously detect fatty acids (FA) in wild and farmed sea bream by high resolution mass spectrometry; 2) to identify liver protein markers to distinguish wild and farmed sea bream with two-dimensional electrophoresis (2DE). For mass spectrometry investigations, a total of 60 muscle samples of wild and farmed Sea bream (*Sparus aurata*) from Mediterranean Sea were bought and stored at 4 °C before the Fatty Acid (FA) extraction. The FA investigated were Docosahexanoic acid (DHA), Eicosapentaenoic acid (EPA), Linoleic acid (LA) and Arachidonic acid (AA). 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 by Direct Sampling Analysis (DSA) coupled with a Time of Flight Mass Spectrometer (AxION2 TOF). MS-TOF analysis was 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, and 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®). For proteomic analysis, a total of 30 liver samples of wild and farmed Sea bream (*Sparus aurata*) from Mediterranean Sea were bought and stored at -80 °C before proteins extraction. Soluble proteins were obtained from 0.2–0.3g of liver in 5 volumes (w/v) of lysis buffer. After homogenization, samples were incubated, centrifuged and supernatant was collected and stored at -80 °C until analysis. Protein amount was determined by Qubit™ Protein Assay Kit. A reducing buffer was added to reach 2.4mg/mL and samples were incubated at room temperature (RT) overnight then centrifuged before cup-loading on rehydrated IPG strips (18cm pH 3–10 NL) for isoelectric focusing (IEF), then underwent SDS–PAGE and stained with Oriole™ Fluorescent Gel Stain. The protein maps were acquired with a ChemiDoc™ Touch (Bio-Rad) and the images were analyzed with Delta2D software (Decodon) to find statically significant differences between wild and farmed samples.

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. In order to compensate species variability and obtain 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 in the ratio between [DHA-H]⁻/[AA-H]⁻, that was between 2.9 and 12 for farmed species and between 0.9 and 2.7 for wild species. The non-parametric regression model applied for statistical analysis showed that the farmed sea bream has on average DHA/AA values higher than 3.25 respect to the wild sea bream. This difference appears to be statistically significant (p value<0.001).

The DHA/AA ratio between fatty acids makes it possible to differentiate the farmed sea bream from those wild.

Proteomics preliminary analysis showed a protein spot, with a molecular weight of ~25 kDa and an isoelectrical point of ~6, present only in wild samples and absent in all the farmed sea bream, representing a good potential biomarker. Protein identification analysis, that are currently ongoing, will provide further information on the protein of interest opening the way for future studies to develop a rapid and accurate test, providing a useful tool for producers and official authorities. Since quantitative differences, up- and down-regulation, have been observed, further analysis are ongoing on a larger number of animals and replicas, to verify other potential biomarkers eventually associated to the 25 kDa protein spot marker. Both the approaches are valid for differentiate wild and farmed Sea Bream and can help in fighting commercial frauds in fish market, increasing the number of controlled samples and giving the possibility to make control throughout the entire commercial chain.

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P3.23

APPLICATION OF GC-IMS TO DISCRIMINATE VIRGIN OLIVE OILS ACCORDING TO THEIR SENSORY GRADES

Rossini, C.^{1*}, Casadei, E.¹, Panni, F.¹, Valli, E.¹, Bendini, A.¹, Cevoli, C.¹, García-González, D.L.³, Gallina Toschi, T.¹

1-Alma Mater Studiorum - Università di Bologna, Bologna, Italy

2-LabService Analytica s.r.l, Bologna, Italy

3-Instituto de la Grasa, Sevilla, Spain

*corresponding author e-mail: cesare.rossini@labservice.it

Keywords: GC-IMS, sensory analysis, virgin olive oils, chemiometric

Companies that sell virgin olive oils must make quick decisions on the purchase and bottling of large lots of products; in some cases, up to 20,000 samples, for which the authenticity of the label-declared commercial category needs to be ensured, must be controlled per year. For this reason, it is of great interest the availability of fast, robust and simple screening methods to support the sensory analysis for establishing the quality grade of virgin olive oils possibly through the use of easily calibrated and cheap instruments.

Sixty virgin olive oils were analyzed by using a gas chromatography coupled to an ion mobility spectrometer (GC-IMS) with a tritium source. The samples, without any preparation step, were injected by a headspace device, after a thermoregulation at 40 °C for 20 minutes. The obtained spectral data were analyzed by using chemometric techniques to discriminate the samples on the base of different quality grades (extra virgin, virgin, lampante) previously sensory assessed (EU Reg. 1227/2016). Particularly, PCA, PLS-DA and ANN statistical methods were investigated.

The statistical elaboration evidenced a promising capacity to discriminate the analysed samples according to their quality grade through a sustainable screening tool. 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). The information expressed in this abstract reflects the authors’ views; the EC is not liable for the information contained therein. The authors are grateful to the six sensory panels of the OLEUM consortium for the sensory evaluation of samples.

P3.24

NIR HYPERSPETRAL IMAGING ANALYSIS OF INDIVIDUAL KERNELS: A TOOL HELPING TO DETECT CONTAMINATION/FRAUD IN CEREALS

Vermeulen, P.^{1,*}, Lecler, B.¹, Fernández Pierna, J.A.¹, Baeten, V.¹

1-Food and Feed Quality Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

*corresponding author e-mail: p.vermeulen@cra.wallonie.be

Keywords: NIRS, NIR imaging, fraud, contaminant, cereal

In the food and feed sectors, the Near Infrared (NIR) technology is nowadays considered as an essential analytical tool that greatly contribute to enhance the quality and safety of agricultural products. Moreover, it has been implemented with success at different stages of the production chains, allowing collecting larger number of analysis, saving, then, time and money. The NIR technology is actually used for quality control of raw materials and end products, for the detection of undesired products and for the detection of presence of fraud in the food/feed chains.

To meet the quality product specifications required by the world grain markets and by the agro-food industries, the NIR technology has been adapted for the analysis at the kernel level. In this way, NIR hyperspectral imaging has been developed in order to detect contamination and fraud in cereals.

To illustrate the kernel by kernel analysis, four case-studies have been selected: i) a sorting problem based on quantification of protein content in a cereal batch [1]; ii) a sampling issue in the framework of the ergot detection in cereals [2]; iii) an authentication issue with the detection of common wheat kernels in durum wheat [3] and iv) an homogeneity issue by the assessment of the cereals seeds coating [4].

These studies using NIR Hyperspectral imaging have proved that by combining spectral and spatial information, it is possible to tackle challenges facing the cereal industry.

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Acknowledgments

These projects have received partially funding from the European Union's research programmes (Confidence and FoodIntegrity). The authors also thank the technical staff of the CRA-W, Stéphane Brichard and Nicaise Kayoka, as well as the students, Arthur Dijon and Pauline Flemal, for their help in the realization of the experiments presented in this poster.

P3.25

DIGITAL LABELLING FOR ACORN IBERIAN HAM BASED ON NIRS TECHNOLOGY

Pérez-Marín, D.C.^{1,*}, Garrido-Varo, A.¹, Adame, J.¹, Riccioli, C.¹, de Pedro, E.¹, Fearn, T.², Piotrowskiy, C.³, Doña, R.³, Blanco-Valero, A.⁴

1-Faculty of Agriculture & Forestry Engineering (ETSIAM), Universidad de Cordoba, Campus de Rabanales, Carretera de Madrid km. 396, 14071 Cordoba, Spain.

2-Department of Statistical Science, University College London, Gower Street, London WC1E 6BT, UK

3-AUNIR . The Dovecote, Pury Hill (Business Park), Nr Alderton, Towcester, UK

4-Ibérico de Bellota, SA (IBESA). Polígono Cárnico, s/n, 14440 Villanueva de Cordoba, Spain

*corresponding author e-mail: dcperez@uco.es

Keywords: NIRS, labelling, Iberian pig, Decision Support Systems

The advantages of Near Infrared Spectroscopy (NIRS) have resulted in more than 50% of the spectroscopic techniques currently used for the control of processes in the industry being based on the near infrared spectral region [1]. Technological advances in NIRS instrumentation have allowed the evaluation of on-site quality control applications of Iberian pork products as demonstrated in previous studies [2].

However, the implementation of this technology requires support platforms for decision-making that facilitate the analysis and interpretation of the results by the users. In addition, these platforms would allow NIRS technology to be a quality control system in real time.

Most NIRS applications depend on different software for the treatment of collected data. Currently, there is a large variety of multivariate data processing software on the market, but many of them are not completely compatible with data from different equipment.

The portable/miniature instrument MicroNIR Onsite Lite (Viavi Solutions Inc., Santa Rosa, CA) evaluated in the WP 19&21 (FI project) has very limited data processing software, so it is advisable to design and develop its own adapted software. In this way, non-expert users could make, analyse and interpret measurements in a simple and fast way. One possible option is based on designing a graphical user interface (GUI) consisting of a series of elements that helps the user to communicate easily with a system that may incorporate different treatment algorithms.

Considering these aspects, a GUI in MATLAB® environment was designed for the routine analysis of Iberian pig carcasses using NIRS portable instruments at the slaughter line. This enables non-expert operators to get predictions for carcass characterization. In addition these predictions can then be integrated into a database for the design of a decision support system based on fatty acid composition and authentication according to the feeding regime of the Iberian pigs.

Figure 1 shows the flow diagram of the procedure applied for the development of the GUI based on qualitative and quantitative prediction models used for the quality control of Iberian pig carcasses. The different stages of spectral data processing involve: 1. - import the NIR spectral data. 2. - evaluate the spectral repeatability between replicates. 3. - quantitative prediction of the fatty acid profile of the samples analysed. 4. - qualitative discrimination according to commercial categories. 5. - obtaining statistics that inform on the reliability of the prediction.

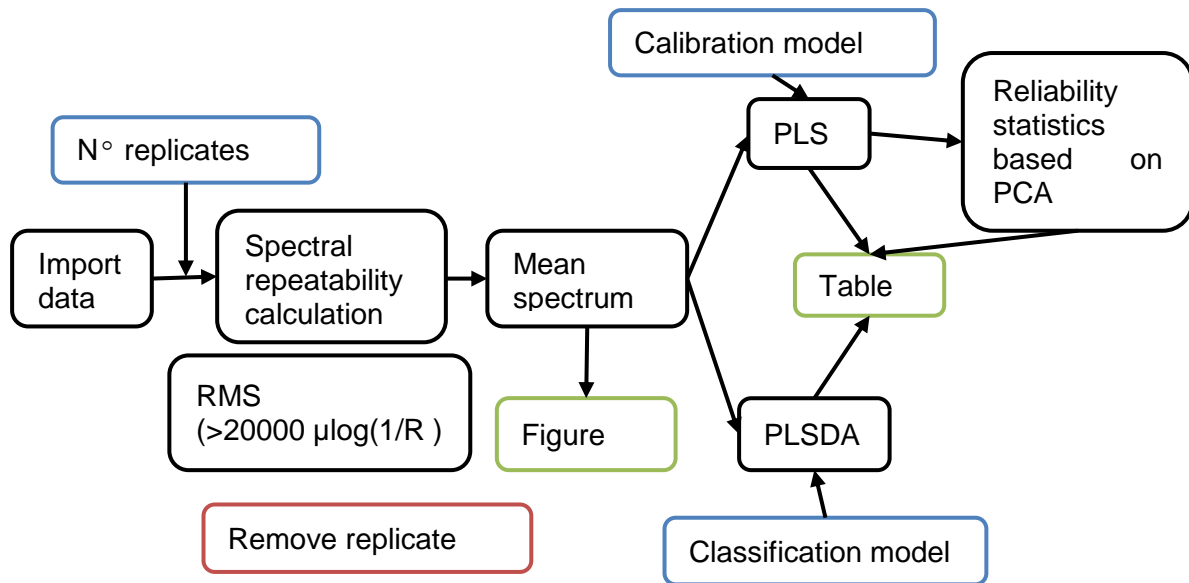


Figure 1. Flow diagram of the graphical user interface designed for the analysis of spectroscopic data from portable NIRS instruments.

Figure 2 shows an image of the GUI designed and compiled to be able to run on different devices without the need to install MATLAB® or other mathematical data processing programs.

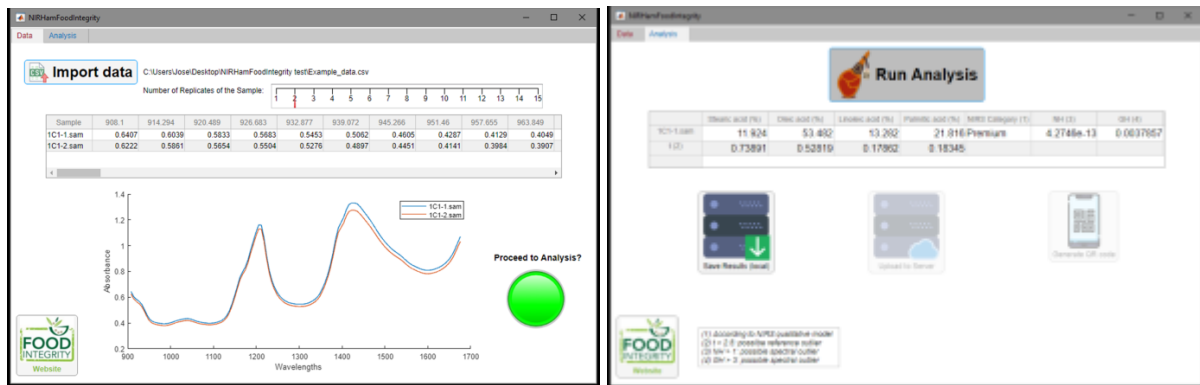


Figure 2. Layout diagram of the graphical user interface.

Also developed was an android application for smart mobile devices that allows, among different functions, upload of files to virtual servers facilitating computing through the Internet (cloud computing), and a web environment that allows easy access to all the generated information remotely.

In conclusion, this GUI allows an analysis of spectral data from NIRS portable equipment to be performed in a simple, clear, intuitive and coherent way. In addition, the type of designs and methodologies can be extrapolated to other NIRS applications.

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P3.26

IS VIBRATIONAL SPECTROSCOPY AN ADEQUATE TOOL FOR ASSESSING THE GEOGRAPHICAL ORIGIN OF HONEY?

Damiani, T.^{1,*}, Fernández Pierna, J.A.^{2,**}, Dall'Asta, C.¹, Baeten, V.², Aubone, I.³, Fuselli, S. R.^{3,4}, Alonso, R.M.^{3,5}.

1-Department of Food and Drug, University of Parma, Parma, Italy

2-Food and Feed Quality Unit, Valorization of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

3-Grupo Investigación Microbiología Aplicada (GIMA), Centro de Investigación en Abejas Sociales (CIAS), Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Provincia de Buenos Aires, Argentina

4-Comisión de Investigaciones Científicas (CIC), La Plata, Argentina

5-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

*corresponding author e-mail: tito.damiani@studenti.unipr.it

** j.fernandez@cra.wallonie.be

Keywords: honey, vibrational spectroscopy, geographical origin, chemometrics

Honey is defined as the natural sweet substance produced by *Apis mellifera* bees from nectar or excretions of plant-sucking insects. Honeys having a protected designation of origin (PDO) or protected geographical indication (PGI) can command a price premium. Hence, the potential for economic fraud is clear. Nowadays, pollen analysis (melissopalynology) is typically used for the honeys' floral origin identification, but it can hardly be used for geographical discrimination. Furthermore, this technique is extremely time-consuming and strongly affected by the analyst competence.

While a range of techniques have been proposed for the classification of monofloral honey, mainly based on the typical volatile signature and/or the phenolic profile, the correct classification of multifloral honey is still challenging.

The aim of the present study was to investigate the capability of vibrational spectroscopy as valid tool for the geographical origin discrimination of multifloral honey.

Multifloral honey samples were collected among the three main honey-producer regions of Argentina: Buenos Aires, Catamarca and Misiones. The sampling was repeated over four harvesting seasons (2014, 2015, 2016, and 2017). The total number of samples was $n = 508$. Analyses were performed by three different spectroscopic techniques: Raman, Near Infrared (NIR) and Mid-Infrared (MIR).

Partial Least Squares Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM) were used as supervised discriminant techniques. Cross-validation leaving one-year-out was performed and the resulting model has been used for the prediction of an independent test set. In order to improve the model discrimination ability, different data preprocessing methods were tested and variable selection was carried out. Since data coming from three different instruments were available, data fusion approach was tempted. The purpose was to gain better classification and lower error rate.

Principal component analysis revealed clustering between the geographical regions over all the considered years (Fig. 1). The same discrimination was obtained with all three spectroscopic techniques. Discriminant analysis showed very good performances in discerning Misiones and Buenos Aires samples, whereas higher error classification rate was found for Catamarca (Fig. 2). This might be explained by the environmental effect, especially in term of climate conditions and geographical features. While Misiones has its own characteristic subtropical weather and vegetation, Catamarca exhibits several different microclimates.

The results from this study demonstrated the feasibility of vibrational spectroscopic techniques as fast and effective methods for honey authentication and provenance confirmation.

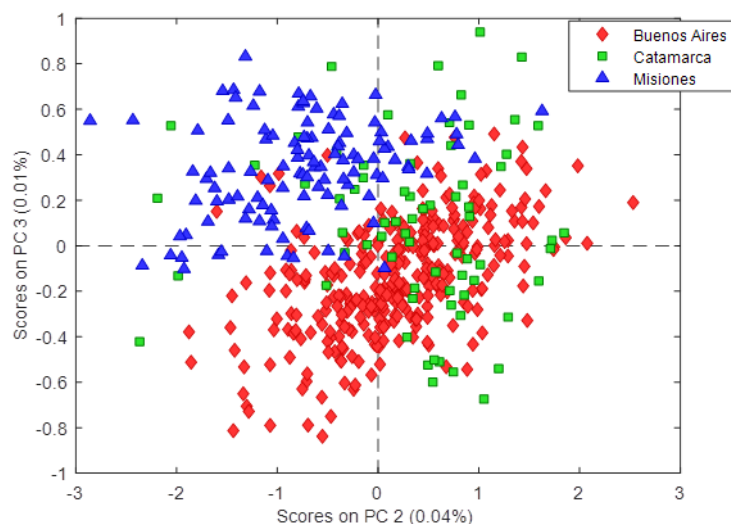


Figure 2. PCA scores plot of MIR data (all year considered). Red: Buenos Aires; Blue: Misiones; Green: Catamarca.

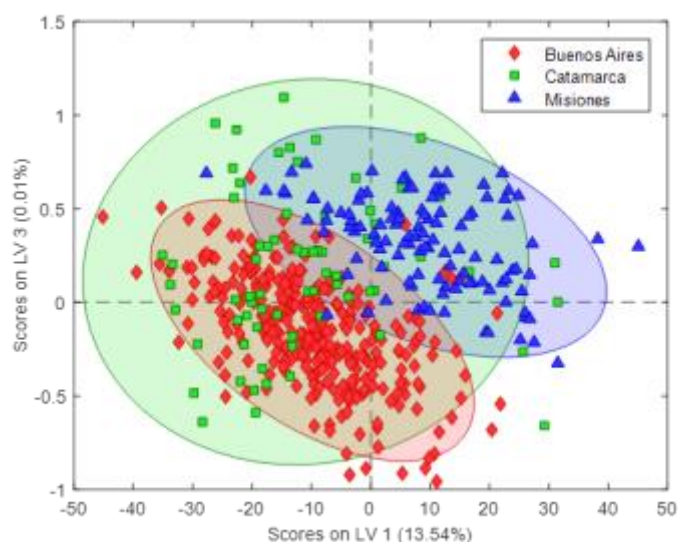


Figure 3. PLS-DA scores plot of MIR data (all year considered). Red: Buenos Aires; Blue: Misiones; Green: Catamarca.

P3.27 NEAR INFRARED MICROSCOPY AND HYPERSPECTRAL IMAGING FOR SPICES ADULTERATION: A FEASIBILITY STUDY

Damiani, T.^{1,*}, Baeten, V.^{2,**}, Dall'Asta, C.¹, Fernández Pierna, J.A.², Fahl-Hassek, C.³, Arnould, A.², Kayoka, N.².

1-Department of Food and Drug, University of Parma, Parma, Italy

2-Food and Feed Quality Unit, Valorization of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

3-Product Identity, Supply Chains and Traceability Unit, Department Safety in the Food Chain BfR - Federal Institute for Risk Assessment, Berlin, Germany

*corresponding author e-mail: tito.damiani@studenti.unipr.it ** v.baeten@cra.wallonie.be

Keywords: adulteration, oregano, NIR, hyperspectral imaging

Herbs and spices are attractive categories for fraud, because of their high value/weight and complex supply chain involved. Common authenticity issue related to these foodstuffs is the addition of adulterant agents. Dried herbs are particularly vulnerable about this fraud and, among others, oregano is specially affected.

Oregano is one of the most widespread herbs and it is usually sold as dried ground leaves. The adulterant agent addition is often massive, and foreign leaves with similar shade of green (e.g. olive and myrtle leaves) are normally used. In some studies, it has been found that almost 25 % of commercial UK oregano tested was adulterated at level up to 70 %. Procedures based on FT-IR as screening assay and LC-MS/MS as confirmatory analysis have been already developed showing good analytical performances. However there is a need of a fast method able to analyse large quantity of samples and at the same time to decrease the limit of detection. The aim of the present work was to assess the potentialities of advanced NIR spectroscopy based techniques (microscopy and hyperspectral imaging) as rapid and robust detection methods for herbs adulteration and reduction of the limit of detection. Moreover untargeted methods will be studied in order to detect unknown contamination. For this study, oregano samples, as well as different adulterant samples, were analyzed by NIR microscopy and hyperspectral imaging in order to find band spectra suitable for the discrimination. In Figure 1 the spectra of oregano and its common adulterants are reported. Clear differences can be observed along the entire spectra and they might be exploited for the fraud detection.

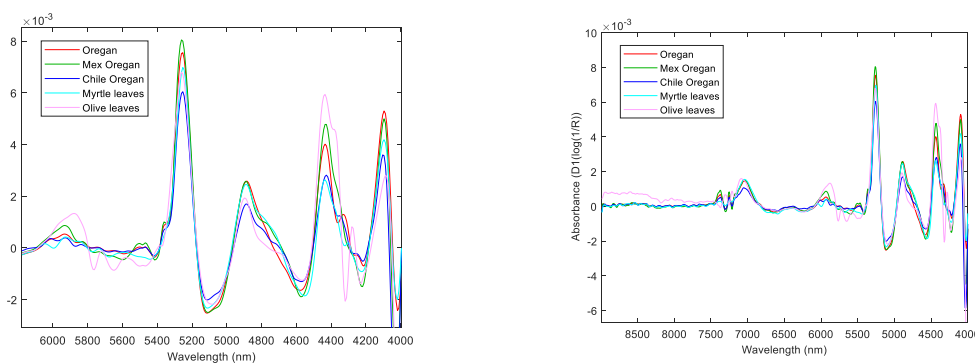


Figure 4. NIR spectra of oregano and common adulterants (left) and enlargement of the fingerprint region (right).

P3.28

POLYPHENOLS AS MARKERS OF AUTHENTICITY OF BAKERY PRODUCTS CONTAINING CHIA, FLAX AND SESAME SEEDS

Baroni, M.V.^{1,2,**}, Lucini Mas, A.^{1,2}, Brigante, F.^{1,2}, Pigni, N.^{1,2}, Wunderlin, D.^{1,2}

1-Food Science and Technology Institute Córdoba (ICYTAC-CONICET), Córdoba, Argentina.
2-Organic Chemistry Department, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina.

*corresponding author e-mail: vbaroni@fcq.unc.edu.ar

Keywords: chia, flax, sesame, HPLC-ESI-QTOF

Nowadays, new trends in food are incorporating specific ingredients to improve the quality of the product or the health of those who consume it. Because of this, people have special interest in what they eat and in the ingredients that constitute it. From this, it turns necessary to develop tools and methods to corroborate that the foodstuff complies with what is informed on the label and thus, determine the authenticity of the food and its ingredients. Chia, flax and sesame seeds are now widely used as ingredients of foodstuffs because of their beneficial effects on health. These seeds are an important source of unsaturated fatty acids, which have a positive effect on the circulatory system; and antioxidant compounds that prevent cell aging and the risk of having some diseases such as diabetes, cancer, Parkinson's and Alzheimer's. The compounds responsible of this last effect are polyphenols, organic compounds present in plants. This makes polyphenols candidates to determine the presence of certain ingredients of plant origin. However, it is necessary to consider that in the process of making a food product, the polyphenolic compounds of the ingredients could be modified; therefore, only the compounds that resist this processing could be used as markers.

The aim of this work was to generate a method to determine the presence of chia, flax and sesame seeds in bakery products using polyphenols as markers. For this, extracts of the seeds were obtained by sonication with solvent methanol: water (50:50) of the previously defatted seeds. Then, the markers found in seeds were searched in cookies made at our research facility to determine which compounds resisted the processing. After that, to evaluate the usefulness of the methodology, markers found in our cookies were searched in different commercial bakery products containing the seeds (sweet cookies, crackers, bread, puff pastry dough and breadsticks). In addition, in both the flax seeds and the entire commercial and samples that contain it, a basic hydrolysis step was also performed to release the compounds of this specific matrix. All these extracts were analyzed by HPLC-DAD-ESI-qTOF using MSⁿ spectra, UV-Vis spectra and exact mass comparison to determine the polyphenolic profile.

In chia seeds, 30 compounds were found belonging to the families of organic acids, flavonols, amino acids, and hydroxycinnamic acids; being the last ones the most characteristic and the majority. On the other hand, in sesame seeds, an amino acid, a hydroxycinnamic acid, a flavone and 28 lignans were found, being these the most characteristic. In addition, in flax seed, 28 compounds were found among lignans, flavonoids, hydroxycinnamic acids and amino acids.

Although there were ubiquitous components in all the seeds, some of them allowed differentiating between these seeds. In all the samples containing chia seed, 3 hydroxycinnamic acids (Salviflaside, Rosamrinic acid and Salvianolic acid) were found; in

those with sesame, 3 lignans (Sesaminol trihexoside and two isomers of Sesaminol dihexoside), while the only compound that allowed to differentiate flax was the flavanone Eriodictyol-7-O-hexoside. Even though other polyphenolic compounds were found in commercial samples such as Tryptophan, Quinic acid, Ferulic acid, Quercetin dihexoside, among others, these were not useful to differentiate between the different seeds because they are present in more than one of them.

In conclusion, there are certain polyphenols that can be used as markers for the seeds of chia, flax and sesame in food because they are characteristic of each of them and they resist processing.

Acknowledgment:

FOODINTEGRITY grant FP7-KBBE-2013-7-613688

P3.29 INTEGRITY OF COMPLEX FOODS: CONSUMER STUDY AND GUIDELINES TO STAKEHOLDERS

Montorsi, M.^{2,3,4,*}, Hyldig, G.¹, Sørensen, R.¹, Lorenzetti, R.²

1-National Food Institute, Technical University of Denmark (Lyngby, Denmark);

2-Consorzio Italbiotec (Milano, Italy);

3-Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University (Milano, Italy);

4-Institute of Molecular Bioimaging and Physiology, National Research Council (Segrate, MI, Italy).

*corresponding author e-mail: michela.montorsi@unisanraffaele.gov.it

Keywords: complex foods, QR codes, consumer study, guidelines to stakeholders

Introduction

Consumers want to be able to get easy access to essential information about relevant characteristics. Producers can provide this information and much more if wanted or if demanded by the authorities. European law demands that, for a batch of raw material, it must be possible to trace it through to all final products which it appears in. Similarly a final product must be able to have all its raw materials identified. Such information is not explicitly available to the final consumer, but the obligatory exposure of the brand owner and producer to such demonstrable evidence of authenticity is a major driver of transparency. By such means the food industry can prove, or not, that its presumption that the food chain is intrinsically safe. In this project however, the focus is on exploring what types of information exists along the food chain and the methods and forms of transmitting that information.

Materials and Methods

The two products that were used as examples on complex food products were: Dried Raw Ham Tortellini (Barilla); Bolognese sauce (Barilla). The consumer studies were set up in two focus groups one of which included the observation on the use of QR codes and the other a consumer survey conducted both in Denmark and Italy.

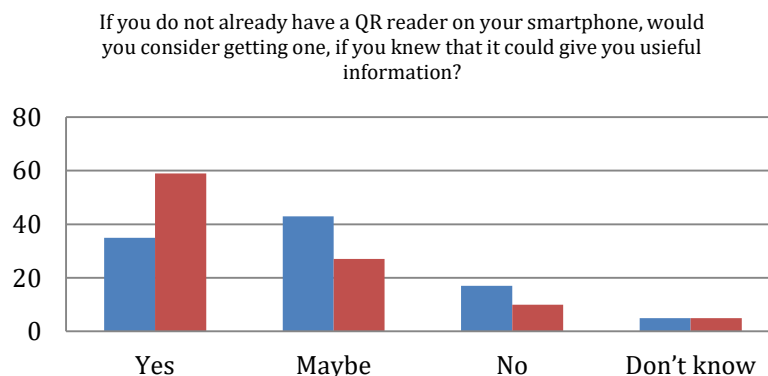
Focus group 1.	The test products With QR codes and the printed information	Examples of QR codes
Focus group 2.	The test products With QR codes and the printed information	
Consumer survey in Denmark	Questionnaire distributed on different social media	Incl. pictures of the test products With QR codes and the printed information
Consumer survey in Italy	Questionnaire distributed on different social media	Incl. pictures of the test products With QR codes and the printed information

The consumers in the first focus group were highly involved consumers whereas those in the second were mainly average consumers. In the first focus group together with the QR codes from the test products other products with QR codes were brought in. The participants were asked to scan and discuss if the information were useful. In the second focus group there was only the QR

code for the test products and a printed version on the information from the QR codes. For the consumer survey we developed a questionnaire which was diffused on different social media both in Denmark and in Italy. In Denmark 136 consumer (81% female, 19% male) completed the questionnaire and 138 (72% female, 28% male) in Italy.

Results and discussion

In general the interviewed consumers were not used to look for QR codes when they were shopping and they thought that it would take too much time. When they were shown the examples they was not convinced that they would scan the QR code if they saw one. They said that they should be sure that it was useful information and not that it only took them to a homepage. The following figure shows an example of the results of the consumer study about the interest for food. Both consumers in Italy and in Denmark consider them-self as persons that are interested in what the food contains and are interested to know the origin of the food. Among the Danish consumers there was a higher share of consumers that was searching for new food products.



Percent of the consumer in Denmark (blue) and Italy (red)

Guidelines to stakeholders

To make a good and useful QR code one should consider the following:

- Which country and which consumer group are the food products design for;
- Before the consumer scans the QR code they should be sure that it has relevant information, therefore it is important to state what information the QR code contains;
- The consumer should be sure that it is safe to scan and that they do not need to be concerned about their privacy;
- Relevant information for the consumer is not “this is a good product”;
- Give the overall information first and the more detailed only after;
- It should be easy to read and updated and working on “all” types of smartphones;
- The best is that it is a text and not a link that demands a connection to the internet;
- If it is a link to a homepage, it should bring the consumer to the relevant text at the homepage right away and not to the front page telling the history of the producer;
- It should only give additional information as essential information, such as information about allergy, should be on the packaging.

To make the use of QR codes more effective on a longer perspective, there is a need for investigations on the type of information that consumers from different countries/markets are demanding.

Acknowledgments

This work was supported by the FOODINTEGRITY Project, funded from the European Union’s Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement No. 613688.

P3.30

QUANTITATIVE AND QUALITATIVE PCR ASSAYS TO ASSESS QUALITY AND INTEGRITY OF COMPLEX FOODS

Montorsi, M.^{1,2,3,*}, Maestri, E.⁴, Abbruscato, P.⁵, Bosco, D.¹, Gorni, C.⁵, Marmioli, N.^{1,4}, Mrakic-Spota, S.³, Pira, G.¹, Scarpato, N.^{1,2}, Vezzoli, A.³, Lorenzetti, R.¹

1-Conorzio Italbiotec (Milano, Italy);

2-Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University (Milano, Italy);

3-Institute of Molecular Bioimaging and Physiology, National Research Council (Segrate, MI, Italy);

4- Interdepartmental Centre SITEIA.PARMA, University of Parma (Parma, Italy);

5-PTP – Science Park (Lodi, Italy).

*corresponding author e-mail: michela.montorsi@unisanraffaele.gov.it

Keywords: DNA-based methods, complex foods, database, production process

Introduction

Food safety and food quality have increasingly come to the forefront of consumer concerns, industry strategies and government policy initiatives. Thus in recent years various analytical techniques have been developed for quality and authenticity evaluation of food. In general projects on food traceability and authenticity focus on commodity products or products with a single ingredient such as meat, dairy or olive oil whereas in this study we focused on two multi-ingredient foods namely Bolognese sauce and dried raw ham tortellini.

Annotated Database analysis

The current knowledge related to the analytical methods standardized by international authorities or developed within the frame of R&D projects has been reviewed and an updated survey on the current state-of-the-art of know-how, methodologies, and reference materials applied to ingredients of the two complex foods under study has been produced (M. Montorsi et al., 2017, Annotated literature database: a review on analytical DNA methods applied to complex food matrices. Foodintegrity 2017 Conference Abstract Book P30: 142 - M. Montorsi et al., 2018, Food authentication by DNA based methods: a document classifier of the literature database. Asset 2018 Summit, Belfast, Abstract book ref. n. 100).

Methods based on polymerase chain reaction (PCR) amplification with species-specific primers are relatively fast, simple and accurate. Although more sophisticated molecular techniques (e.g. NGS, digital PCR) have been developed, these are still too expensive and/or difficult to be routinely implemented in an industrial process line. Thus in the present study we focused on traditional and real time PCR methods to provide industry with a reliable and feasible set of methods able to verify the compliance of tested food with the animal, plant and spice species listed in the labels.

PCR based analyses

To provide a complete set of assays assessing the presence of the expected animals, plants and spices ingredients in the dried raw ham Tortellini and Bolognese sauce 6 different methods were tested as follows:

1. Endpoint PCR in singleplex for animal species;
2. Endpoint PCR in multiplexing for animal species;
3. Real-time qPCR (SybrGREEN) for the quantification of bovine species;
4. Real-time qPCR (SybrGREEN) for the quantification of porcine species;
5. Endpoint PCR in singleplex/multiplex for plant species;

6. Endpoint PCR in singleplex/multiplex for spices.

Ingredients	Bolognese sauce	Dried Raw Ham Tortellini
Swine (meat and Ham)	11.0% ± 0.5%	16.0 % ± 1.2%
Bovine (meat or cheese)	10.2% ± 1.8%	5.3% ± 0.8%
Chicken (Eggs)	No	Yes
Bovine (Grana Padano)	Yes, from meat	Yes
Onion	Yes	No
Celery	Yes	No
Carrot	Yes	No
Tomato	Yes	No
Sunflower	method not applicable to oil	method not applicable to oil
Pepper	Below detection limit	Below detection limit

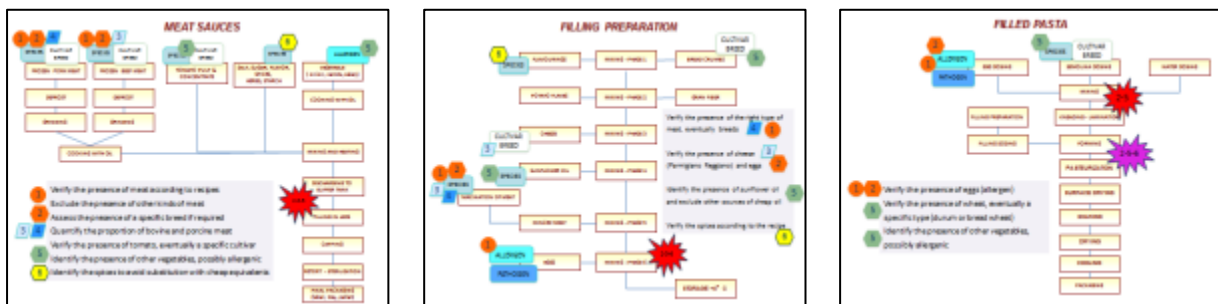
These methods were applied to DNA extracted from the two aforementioned to assess the presence of chicken eggs, bovine food stuff (Grana Padano), onion, celery, carrot, tomato sunflower oil, and quantify bovine and swine meat. Results are summarized in the table beside.

In order to evaluate the detection limit of a selection of these PCR methods and their ability to detect potential contaminants, both Bolognese sauce and Tortellini were spiked with horse and chicken, two well-known species commonly used in food fraud, while celery, tomato and carrot, that are ingredients of Bolognese sauce, were used to spike Tortellini. All together the PCR and qPCR results obtained on Bolognese sauce and Tortellini confirmed the

usefulness and reliability of these methods on industrial products (C. Gorni et al., 2018, PCR based analyses for safety and quality assessment in the production processes of complex foods. Asset 2018 Summit, Belfast, Abstract book ref. n. 129).

Production Chains Analysis

Process Flow Diagrams (PFD) of preparation of the two complex foods analyzed: ready-made sauce (Bolognese sauce) and stuffed pasta (Tortellini filled with meat) were constructed, in order to provide a clear and simple description of the steps involved in the production processes. The following PFDs resulted from the application of network analysis to the two food supply chains identifying the connections among the various steps and depicting movements of materials, inputs, and outputs. The key analytical steps have been positioned on the maps. The principles of process analytical technology (PAT) and fuzzy logic analysis of flow diagrams along with the obtained experimental results have been implemented to identify the critical steps that have the most relevant effect on the integrity of the final products.



Acknowledgments

We are grateful to Dr. Michele Suman, Food Safety Research Manager Barilla G. e R. Fratelli S.p.A., for his support to elaborate the drafts of the production maps and for his precious suggestions and comments.

This work was supported by the FOODINTEGRITY Project, funded from the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement No. 613688.

P3.31

DIFFERENTIATION OF PRODUCTION METHOD AND GEOGRAPHICAL ORIGIN OF SALMON BASED ON THE ANALYSIS OF FATTY ACID COMPOSITION

Amaral, J.S.^{1,4,*}, Grazina, L.¹, Nunes, M.A.¹, Mafra, I.¹, Rodrigues, P.J.², Igrejas, G.³, Oliveira, M.B.P.P.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;

2-ESTiG, Instituto Politécnico de Bragança, Bragança, Portugal;

3-CeDRI, Instituto Politécnico de Bragança, Bragança, Portugal;

4-CIMO, Instituto Politécnico de Bragança, Bragança, Portugal.

*corresponding author e-mail: jamaral@ipb.pt

Keywords: salmon, fatty acids profile, GC-FID, linear discriminant analysis, authentication.

Currently, aquaculture production supplies almost 50% of the global fish market, as a response to the increasing global demand for fish [1]. However, several consumers prefer wild over farmed fish, with the former generally attaining higher prices when the same species is considered. Therefore, there is the need to assure correct information, not only about the species, but also about the production method (farmed vs. wild) and the catch origin of fish. Salmon, a high-trophic-level carnivorous species with high economic value due to its popularity, is among the fish species that is frequently produced in aquaculture. Although the feed given to farm-raised salmon is designed to meet its nutritional requirements, it can present differences compared to the diet of wild salmon that can be reflected on the muscle composition of farmed versus wild salmon. Therefore, in this work, the use of fatty acid composition combined with chemometrics was evaluated as a potential tool to authenticate salmon samples. In particular, the work aimed at identifying the geographical origin and production method (farmed vs. caught in the wild) of salmon.

For that purpose, several salmon specimens were analysed, namely specimens caught in the wild in West of Vancouver Island, Canada (n=25) and farm-raised specimens from Canada (n=25), Chile (n=24) and Norway (n=25). Two lipid extraction methods (Soxhlet extraction with n-hexane and an adaptation of the Bligh and Dyer extraction method) and two derivatization procedures (alkaline transmethylation using KOH and acid-catalysed transmethylation using BF₃/MeOH solution) were tested. Fatty acid methyl esters (FAME) were analysed by gas chromatography (GC) in a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu AOC-20i auto-injector, a flame ionisation detector (FID) and a CP-Sil 88 silica capillary column (50 x 0.25 mm i.d, 0.20 µm). The injector and detector temperatures were 250 and 270 °C, respectively. The compounds were identified by comparison with standards (FAME 37, Supelco). Based on the obtained results, the modified Bligh and Dyer method was chosen for lipid extraction since it allowed obtaining higher amounts of long chain unsaturated fatty acids, particularly of docosahexaenoic acid (DHA). Similar results were obtained for both tested derivatization methodologies.

In general, the four groups of salmon showed different profiles (Figure 1), with wild specimens presenting significantly higher contents of health beneficial omega-3 fatty acids, in particular DHA and eicosapentaenoic acid (EPA), while farmed salmon presented significantly higher amounts of oleic and linoleic acids. Linear discriminant analysis evidenced a clear separation of four groups. Among the three groups corresponding to the farmed salmon with different origin, salmon from Chile and Canada were more similar, with

salmons from Norway being more distinctive mainly due to their lower levels of saturated fatty acids and higher of α -linolenic acid.

The results of this study emphasize the effectiveness of chemometrics tools for fish traceability purposes since both production method and geographical origin could be distinguished by the analysis of fatty acids profile coupled with chemometrics.

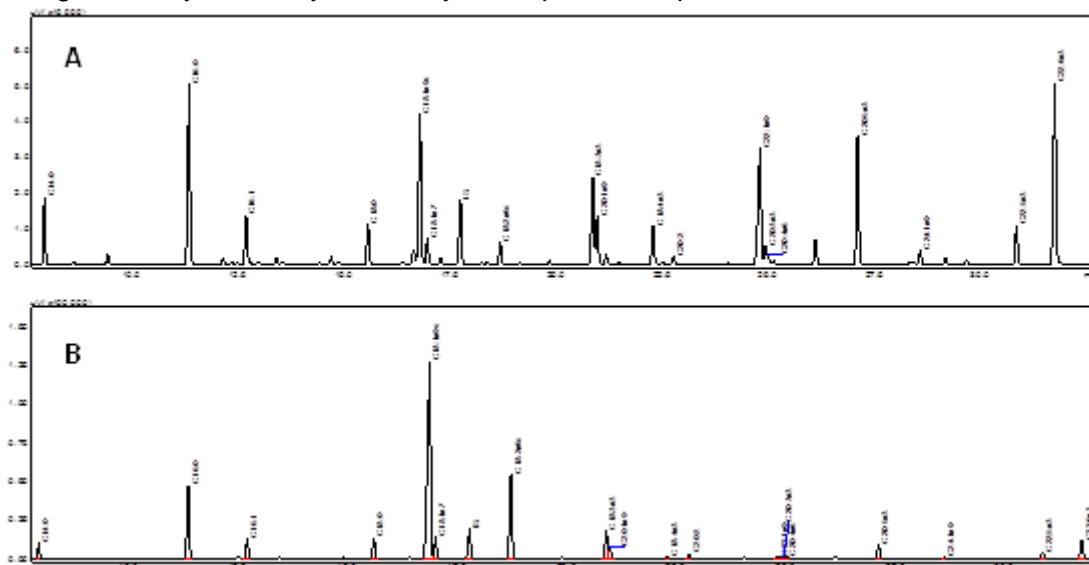


Figure 1. Chromatogram obtained from the GC-FID analysis of a wild (A) and a farmed (B) salmon sample.

The results of this study emphasize effectiveness of chemometrics tools for seafood traceability purposes.

Acknowledgments

This work has been supported by the European project FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688), 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. L. Grazina and M.A. Nunes acknowledge the PhD fellowship (SFRH/BD/132462/2017 and SFRH/BD/130131/2017) from FCT financed by POPH-QREN (subsidised by FSE and MCTES).

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P3.32

CHARACTERIZATION ABILITY OF MAJOR AND MINOR COMPOUNDS IN VIRGIN OLIVE OIL FOR CHEMICALLY DEFINING PROTECTED DESIGNATION OF ORIGIN IN SOUTHERN SPAIN

García-González, D.L.^{1,*}, Aparicio-Ruiz, R.¹, Díaz Montaña, E.J.², Tena, N.¹, Lobo-Prieto, A.¹, Morales, M.T.², Aparicio, R.¹

1-Instituto de la Grasa (CSIC), Campus Universidad Pablo de Olavide - Edificio 46 Ctra. de Utrera, km. 1, 41013, Sevilla, Spain.

2-Department of Analytical Chemistry, Faculty of Pharmacy, University of Seville, C/ Prof. García González, 2, 41012, Sevilla, Spain.

*corresponding author e-mail: dlgarcia@ig.csic.es

Keywords: protected designation of origin, virgin olive oil, minor compounds, chemical characterization, and traceability.

The legal framework of protected designation of origin (PDO) in Europe keeps pace with consumer expectations and improves the protection of virgin olive oils (VOOs) with particular botanical, chemical and sensory characteristics. Nevertheless, sometimes the registration of new PDOs associated to new demarcations of geographic areas is almost based on administrative aspects rather than objective chemical data. In this study we analysed the ability of chemical compounds of virgin olive oils with PDO that are not labile and their concentrations do not vary over time - fatty acids, alcohols, hydrocarbons, methyl-sterols and sterols - to differentiate them from those produced in their neighbouring geographical areas. Thus, the aim of this work is centred on the VOOs from Andalusian PDOs approved by the European Commission. There are twelve PDOs at the current time in Andalusia (Southern Spain): Antequera, Baena, Estepa, Lucena, Montes de Granada, Montoro-Adamuz, Poniente de Granada, Priego de Córdoba, Sierra de Cádiz, Sierra de Cazorla, Sierra de Segura, and Sierra Mágina. Nine of these twelve PDOs have been selected to be characterized chemically and the current problems in the chemical characterization of potential PDOs were illustrated by analysing three different cases.

These three cases combine pedoclimatic characteristics and variations of cultivar have been studied (9 areas with PDO and 3 non-PDO areas). With a modified procedure of ANOVA analysis - the Brown-Forsythe test, chemical compounds that showed good characteristics for the classification of samples were selected, and this ability was later checked by applying the unsupervised algorithm of Principal Component Analysis. Differences in their chemical composition were found when comparing the PDO oils with those produced in the neighbouring olive tree groves despite chemical criteria are not among the most decisive factors for registering PDOs. Then, in some cases, where the chemical compositions of major and minor compounds prove to be different, this composition could be applied to provide a support to certify the singularity of new registered PDOs. Nevertheless, the number of PDOs increases year by year, which can reach a point that the singularity of PDOs will not be supported by chemical or sensory variables but only by administrative controls.

P3.33

THE UK FOOD AUTHENTICITY PROGRAMME - COMBATting FOOD FRAUD THROUGH SCIENTIFIC INNOVATION

**Kidan, N.¹, Aguilera, V.¹, Foster, L.¹, Harding, D.¹, Rollinson, S.¹, Ndede, P.¹,
Hargreaves, W.¹, Furrage, A.¹**

1-Defra, London, United Kingdom

The prevention of food fraud features highly on the UK Government's policy agenda. Protecting consumers, maintaining the resilience of the food chain and preventing fraudulent practices are significant challenges facing policy makers, regulators, enforcers and the food industry. Maintaining our strong reputation in delivering high standards of traceability is vital to ensuring consumer confidence and making the UK a food nation renowned across the globe.

The *UK Food Authenticity Programme* develops fit for purpose analytical methods for use by official control laboratories and other competent laboratories engaged in food authenticity testing. The world-leading programme has been instrumental in spearheading the development of novel scientific methods and analytical technologies such as proteomics, thereby enabling laboratories to provide food law enforcement authorities with robust intelligence to verify non-compliances with food labelling law. Food fraud covers a broad spectrum of labelling misdescription issues including misleading claims about food quality, composition, geographic origin and method of production; this presents a plethora of technical challenges in terms of the analytical tools needed to verify food authenticity and support food law enforcement.

Defra continues to be committed to tackling future scientific challenges in the development of cutting edge technology and methods that are practical, transferable and cost-effective for food law enforcement authorities and the food industry. These methods also need to overcome challenges around analytical uncertainty, quantitation and demanding processing conditions. Better harmonisation of methods and databases and method standardisation is also needed to tackle food fraud.

P3.34

GC-IMS SCREENING TO ASSESS SALMON AUTHENTICITY

Rossini, C.^{2*}, Monaci, L.¹, Guidotti, S.², Fornaro, A.²

1-CNR-ISPA, Bari, Italy,

2-LabService Analytica s.r.l, Anzola Emilia (Bologna), Italy

*corresponding author e-mail: cesare.rossini@labservice.it

Keywords: GC-IMS, volatile profiling, salmon authenticity, chemiometric

The technical capability to distinguish between farmed and wild salmon can be a valuable tool for assessing the right value in commercial transactions. In this work, the use of a gas chromatography coupled to an ion mobility spectrometer with a tritium source was investigated for the discrimination between wild and farmed salmons. The samples, without any preparation step, were injected by a headspace device and, after the gas chromatographic separation, the ion mobility data coming from the eluted volatiles were processed with chemometric tools. The preliminary elaboration evidenced a promising capacity to discriminate the samples according to their characteristics; the proposed approach is also sustainable and fast. It lends itself for a routine analysis in a QC environment.

P3.35 AUTHENTICATION OF HERBAL TEAS / FOOD SUPPLEMENTS WITH DECLARED CONTENT OF GINSENG

Hrbek, V.^{1,*}, Kvirencova, J.¹, Navratilova, K.¹, Tomaniova, M.¹, Hajslova, J.¹

1-University of Chemistry and Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Analysis and Nutrition, Technicka 3, 166 28 Prague 6, Czech Republic

*corresponding author e-mail: vojtech.hrbek@vscht.cz

Keywords: ginseng, food supplements, authentication, UHPLC-HRMS

Food supplements are widely used by consumers around the world and represent one of the relatively expensive commodities. They are also very often the target of counterfeiting practices. For this reason, it is necessary to have an effective analytical tool to control the authenticity of food supplements and to detect their counterfeiting, which may be, for example, in the form of a lower content of the active substance than the declaration on the packaging or complete substitution of the ingredient used versus the claim on the packaging.

This study has been focused on authentication of ginseng-based food supplements. For this purpose various ginseng species (*Panax ginseng*, *Panax japonicus*) were investigated by metabolomic fingerprinting strategy employing ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS). Application potential of this approach for construction a statistical model allowing differentiation of ginseng based food supplements has been examined. In addition to ginseng, *Gynostemma Pentaphyllum* was further analysed, because it contains the same characteristic compounds (ginsenosides) which are present in ginseng. Due to this fact *Gynostemma Pentaphyllum* could be easily used by fraudsters as a ginseng substitute.

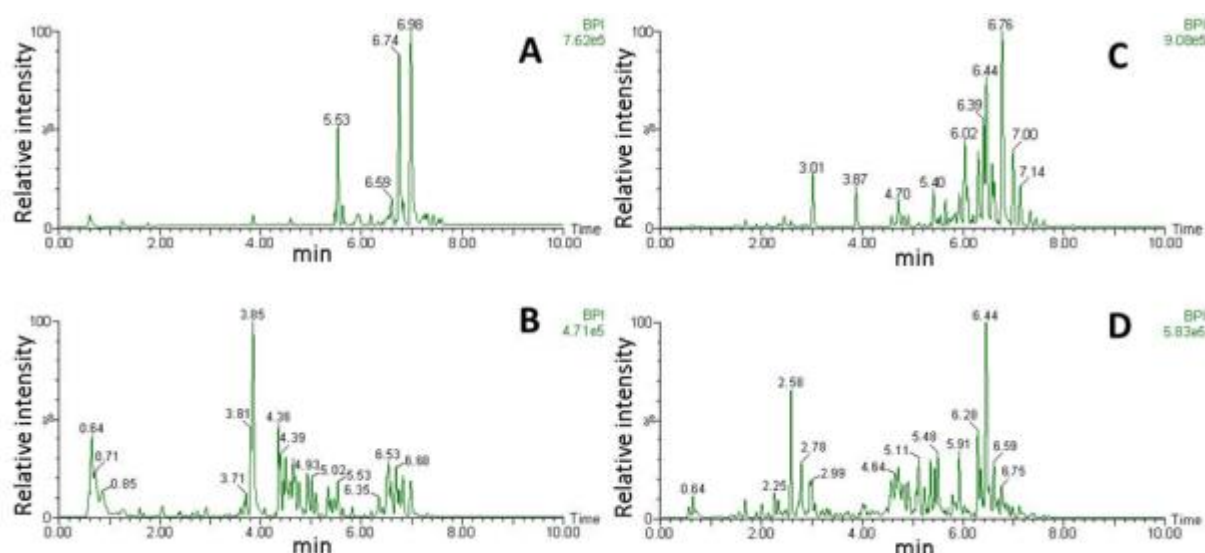


Figure 1: Example of UHPLC-HRMS fingerprints of ginseng MeOH:H₂O (4:1, v/v) extract in ESI+ (A), in ESI- (B) and gynostemma extract in ESI+ (C), in ESI- (D).

Within the development of analytical procedure, sample preparation step was optimised considering a range of factors including extraction solvent and those influencing chromatographic separation. Also MS detector settings were adjusted to achieve as many as possible 'features' (ions, *m/z*) characterizing respective sample. An example of

chromatographic records obtained by analysis of aqueous methanolic (MeOH: water, 80:20, v/v) extract of ginseng and gynostemma is shown in Figure 1. for both ionization modes.

4 samples of ginseng and 4 samples of gynostemma were analysed. Model mixtures of ginseng and gynostemma (ginseng: 100%, 99%, 98%, 95%, 93%, 90%, 80%, 70%, 60%, 50%, 25% in mixture with gynostemma) were prepared and analysed in three replicas by means of non-target screening employing UHPLC-HRMS.

The generated data were processed by multivariate chemometric analysis, principal component analysis (PCA). PCA models were constructed from data obtained by individual ionization modes as it is documented in Figure 2.

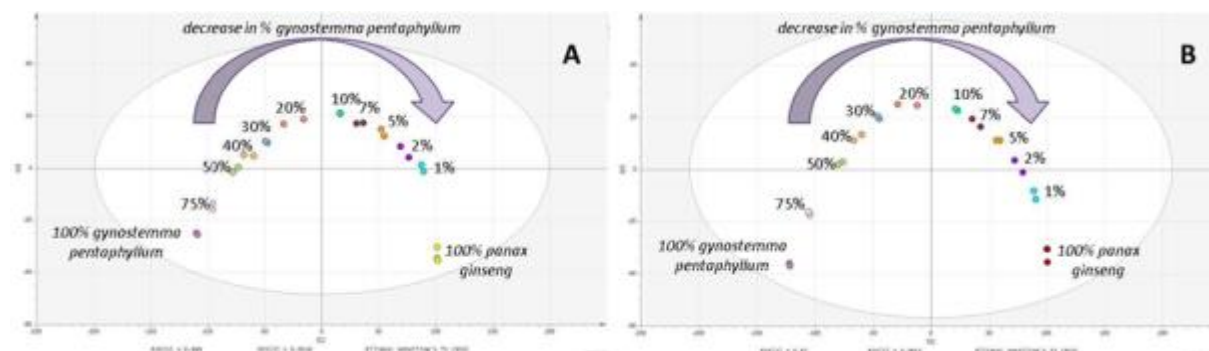


Figure 2: PCA models created from data obtained by analysis of ginseng, gynostemma and their various mixtures aqueous methanolic extracts in positive (A) and negative (B) ionization modes.

Advanced statistical methods (principal component analysis or partial least square discriminant analysis) allowed identification of characteristic compounds of *Gynostemma Pentaphyllum* e.g. ion m/z 431.1916. It was found, that using the model mixtures of gynostemma and ginseng, already 1% addition of *Gynostemma Pentaphyllum* to ginseng was possible recognized. Real food supplements with ginseng content, available on the market e.g. herbal tea with ginseng content, are going to be investigated as a follow-up of this study.

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).

P3.36

ISOTOPIC PROFILING IN ALCOHOLIC BEVERAGES AUTHENTICATION

Klajman, K.^{1,3,*}, Ciepielowski, G.^{1,2,**}, Góreczna-Skrzyńska, E.¹, Pacholczyk-Sienicka, B.², Albrecht, Ł.², Paneth, P.³

1-Product Authentication Laboratory, Bionanopark Ltd, Lodz, Poland

2-Lodz University of Technology, Institute of Organic Chemistry Lodz, Poland

3-Lodz University of Technology, Institute of Applied Radiation Chemistry, Lodz, Poland

*corresponding author e-mail: k.klajman@bionanopark.pl,

**grzegorz.ciepielowski@p.lodz.pl

Keywords: authentication of ethanols, isotopic methods, wine, accreditation

Product Authentication Laboratory, a new laboratory located in Lodz, is mostly involved in the food control by employing isotopic profiling. Product Authentication Laboratory has just obtained an international accreditation according to the norm ISO/IEC 17025:2005, confirming its competence in the area of testing the authenticity of wines and thus becoming the first Polish center that specializes in detecting adulteration in wines as a result of the illegal addition of water and sugar. In the case of isotopic profiling there are two methods for the isotope ratio measurement: isotope ratio mass spectrometry (IRMS) and nuclear magnetic resonance spectroscopy (NMR). Our laboratory is equipped with isotope ratio mass spectrometer (MAT 253) with elemental analyser (Flash 2000 HT) and nuclear magnetic resonance spectrometer (Bruker 500 MHz) for liquid analysis (one probe with ¹⁹F lock channel is dedicated to the deuteron measurement).

Currently at the Lodz University of Technology in the Institute of Organic Chemistry and Laboratory of Isotope Effects Studies, isotopic methods enabling quality control and detection of frauds in alcoholic beverages were implemented. The production of 'Polish Vodka' is restricted by the law to ethyl alcohol of the agricultural origin obtained from rye, wheat, barley, oat, triticale and potatoes grown on the territory of the Republic of Poland. Unfortunately, authentic 'Polish Vodka' is the most often counterfeited by the addition of cheaper and more accessible maize spirits. These illegal practices significantly reduce costs of the spirit production. Therefore, determination of the botanical origin of alcohol in Poland is highly relevant. Quantitative ²H nuclear magnetic resonance and isotope ratio mass spectrometry were used to investigate the authenticity of 30 samples of Polish spirits. Several isotopic parameters were used to determine the botanical origin of 10 unknown samples. Both approaches led to the same conclusions regarding the percentage of maize-derived ethanol addition [1].

Strong cooperation between these three laboratories results in possibility of developing new isotopic authentication procedures as well as interlaboratory comparisons of the obtained results. An innovative control method of rye spirits in order to ensure the quality of Polish vodka was introduced. The approach was holistic by using advanced technology such as NMR and IRMS.

[1] G. Ciepielowski, B. Pacholczyk-Sienicka, T. Frączek, K. Klajman, P. Paneth, Ł. Albrecht, J. Sci. Food Agric. (2018) DOI 10.1002/jsfa.9168

P4.1

IN-SITU APPLICATION OF NEAR-INFRARED SPECTROSCOPY IN BELL PEPPER QUALITY DETERMINATION

Torres, A.^{1,*}, Entrenas, J.-A.¹, Sánchez, M.-T.¹, de la Haba, M.-J.¹, Garrido-Varo, A.², Pérez-Marín, D.C.²

1-Food Science and Food Technology, University of Córdoba, Córdoba, Spain

2-Animal Production, University of Córdoba, Córdoba, Spain

*corresponding author e-mail: g72toroi@uco.es

Keywords: NIR spectroscopy, bell pepper, quality, in-situ determination.

Bell pepper (*Capsicum annuum*) is among the most grown vegetables in greenhouses worldwide. From quality determination, sugar content and acidity are of key importance. These parameters are routinely evaluated using methods that are time-consuming, destructive, and costly and contaminant. Therefore, there is a clear need for fast, accurate and non-destructive analytical techniques that can be used both in the field and by the industry. Near-infrared Reflectance Spectroscopy meets these requirements. The potential of this technology coupled with chemometric techniques based on modified partial least squares regression was assessed by comparison with the currently-used traditional method for determining soluble solid content and titratable acidity in bell pepper. A total of 147 bell peppers were used in the construction of calibration models for the parameters previously cited, testing various spectral signal pretreatments. The technology was useful to screening and sorting bell peppers into high, medium and low values on the basis of soluble solid content and titratable acidity. The results show that NIRS technology is a very promising tool for in-situ quality monitoring of the bell pepper, a complex vegetable with an irregular form.

P4.2 SPECIES IDENTIFICATION OF FISH FILLETS USING PORTABLE NIR

Acutis, P.L.^{1,*}, Rossi, F.², Sciuto, S.¹, Esposito, G.¹, Ubaldi, P.³, Magnani, L.³, Benso, A.²

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

2-Politecnico di Torino, Turin, Italy

3-Esselunga SpA, Biandrate, Italy

*corresponding author e-mail: pierluigi.acutis@izsto.it

Keywords: fish fillets, species substitution, NIR

Species substitution is the most frequent seafood fraud that consists in selling a fish species not corresponding to that declared on the label. Commonly this fraud involves the substitution of a valuable species with one that has a lower commercial value; it thus damages economically the consumer but an impact on public health is also possible, in case of toxic, allergenic, unhealthy or contaminated species imported from not properly controlled geographic areas. Fish fillets are more prone to be substituted given the difficulty in recognizing the species they belong to. At present, the identification of fish species is based mainly on two different methods: visual identification of morphological characters and DNA analysis. The first is based on a subjective and visual control, which requires expert personnel and it is hardly applicable on fish fillets; DNA analysis is instead very accurate but it requires invasive sampling, it is relatively expensive, it must be carried out in a specialized laboratory and it is not suitable for large scale and fast analysis.

A species identification method, low-cost and applicable on a large number of fillets, is therefore needed. Within the Food Integrity project, the aim of this work was to carry out a study able to explore the application of a Near-Infrared Spectroscopy (NIR) to identify the fish species to which a fillet belongs and that can be used also by inexperienced consumers. To measure the NIR spectrum and to perform a classification model of the fish fillets we adopted the SciO molecular sensor, developed and distributed by Consumer Physics3. In connection with the instrument the SciO Developer Toolkit enables to customize and collect the spectra of the desired materials via mobile application. Moreover, in the SciO-Lab web page is then possible to create predictive models based on acquired data to produce discriminative classification methods.

We selected 8 fish species: *Merluccius merluccius*, *Pollachius virens*, *Epinephelus costae*, *Gadus morhua*, *Pleuronectes platessa*, *Sebastes norvegicus*, *Scomber scombrus* and *Synaptura cadenati*. Acquisition was divided in two sets: training and validation set. In the first group, 40 samples for each species were analysed and for each sample 3 scans were realized on different points of the fillet. Scans were analysed with the four algorithms present in the instrument and compared in order to select the best model able to separate the different species. In the validation set, 5/6 samples for each species were acquired (3 scans each), for a total of 45 samples.

The NIR acquisition was performed directly on site, at Esselunga plant. The acquired scans were realized on different points of the fillet in order to evaluate possible variation with respect to the portion of the fillet side. Species was assigned on the basis of the results obtained by at least 2 out of 3 scans.

The 45 fillets used as validation set have been classified with the classification model generated with Selected Wavelength from 740 to 1070 nm. For method 1 "Processed" the

obtained accuracy was 100%; for method 2 “Normalized” was 100%, for method 3 “Processed and Normalized” 97%, for method 4 “LogR and Normalized” was 97%. Instead if the four methods are not considered separately but combined, the global accuracy was 98%. This study suggests the SciO is a promising tool to use in the context of on-site controls as portable devices.

This project has been funded by Food Integrity.

P4.3

NIR BASED TECHNOLOGICAL INNOVATION FOR ASSESSING THE QUALITY AND AUTHENTICATION OF AGRICULTURAL AND FOOD PRODUCTION

Fernández Pierna, J.A.^{1,*}, Vermeulen, P.¹, Lecler, B.¹, Minet, O.¹, Chamberland, N.¹, Pissard, A.¹, Baeten, V.¹

1-Food and Feed Quality Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

*corresponding author e-mail: e-mail: j.fernandez@cra.wallonie.be

Keywords: NIRS, handheld instrument, fraud, transfer

Near infrared spectroscopy, which has long been used mainly in the laboratory, is evolving to bring its use closer to the sample, and closer to the user of the results provided. In such direction and since a decade the number of portable instruments based on NIR has been drastically increased. Two main questions arise from this rapid development: How good are these instruments compared to classical benchtop devices? Are all of them of equal quality?

The main characteristics of portable instruments are their compact appearance, the ease of use, the fact that they can be controlled via a smartphone (or even a watch) with a wired or wireless connection, and their low cost compared with conventional infrared devices. Some can also be adapted to include predictive models for the simultaneous determination of various quality parameters, or can be coupled to a GPS device enabling geolocation of measurements. Some can also be connected to a cloud in order to offer custom-made solutions or directly embedded into a smartphone. These instruments make use of sensors that bring new technologies and strategies in order to generate part of the near-infrared spectrum resulting from the interaction of light with matter.

This new kind of instruments must be subject to rigorous evaluation to ensure that they represent a real opportunity for those working in the world of agriculture and food. Furthermore, they require optimisation of the measurement protocols in order to take into account the heterogeneity of the products, adaptation regarding sample presentation and the development of adequate calibration strategies. For this reason, the CRA-W has evaluated some of these portable instruments available in the market for several years.

Moreover, the CRA-W is also working on the most suitable methodology for transferring databases constructed during decades, with benchtop devices, to this new generation of portable instruments [1, 2]. These portable devices will, in a near future, obviously play an increasing part in our farms and businesses, to determine the key parameters necessary to bring quality control as close as possible to the place where the sample is produced. They will form an integral part of future decision-making tools, including precision food production, raw material quality control, batch segmentation, product traceability and fraud detection. They will increasingly support the ongoing digital revolution in our farms and businesses.

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Acknowledgments

The authors thank the technical staff of the Food and Feed Quality Unit, Stéphane Brichard, Nicolas Crasset, Eric Fontaine and Sandrine Mauro, as well as the students for their help in the realization of the experiments and measurements.

P4.4

OPTIMIZING ACORN (*QUERCUS ROTUNDIFOLIA*) SHELLING PROCESS: A FIRST STEP TO PROMOTE INNOVATIVE ACORN-BASED FOOD PRODUCTS

Alegria, C.^{1,2}, Máguas, C.¹, Abreu, M.²

1-cE3c–Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; csmalegria@gmail.com

2-UTI–Unidade de Tecnologia e Inovação, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

Keywords: modelling drying kinetics, shelling operation, fracturability, *Quercus rotundifolia* acorn.

Within the Mediterranean Basin, namely in the Portuguese *Montado* region and as part of the Mediterranean diet heritage, 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 heritage. The *Montado* agrosilvopastoral system is a diverse habitat, typically open woodlands (20-80 tress/ha) with different acorn producing oaks (*Quercus suber* and *Q. rotundifolia*). Stereotypically perceived as animal feed, acorns are an integrant part of the renowned Iberian black pig feeding system, responsible for the meat and derived products prime-quality (fatty acid and aromatic composition). For this reason, acorns are used as tracers of geographic origin of the Iberian black pig through respective isotopic signatures. Moreover, acorns are also an important part of both gastronomical and medicinal folklore of the regions where *Quercus* species are found. Acorn flour is part of the *Montado* traditions to produce bread and cakes. However, acorns and its flours are still underexploited *Montado's* products and are being evaluated for their potential as a food resource. In fact, acorns nutritional value and high contents in phytochemical compounds with biological activity have raised interest in integrating acorn into the human diet.

Overall, acorns can be described as a new “healthy food”, with high contents in carbohydrates, mainly starch (48% to 50%), 2% to 5% protein (gluten free), and the fat content may vary from percentages as low as 2% up to 30% (depending on the species), with oleic, palmitic, and linoleic acids as the major fatty acids. This composition makes acorns a highly attractive product for celiacs and as an alternative to traditional starchy raw materials, as profit can arise from the flours functionality regarding the high starch content and its unique swelling characteristics. More recently, the potential to use acorn for its antioxidant composition has also raised interest due to its richness in phenolic compounds, responsible for physiological, biological, and biochemical functions. Despite the variability among species, phenolic acids, flavonoids, and tannins are ubiquitous in all *Quercus* species. Since acorn nutritional and bioactive composition is very interesting, the sustainability of this *Montado* product can be promoted by innovating acorn foods. To develop acorn-based food products, proper postharvest handling is crucial to achieve a maximum yield of good quality acorns. However, for any type of acorn-based food processing technology, the peeling/shelling process is a critical and time-consuming manual operation.

A tentative mechanization of the process, occasionally used by small-scale producers, is the use of a flame-peeling system with the use of high temperature (>720 °C). In both, the imposed stresses during peeling can induce responses which can negatively affect the quality of the kernels, leading to e.g. undesirable browning reactions and loss of bioactive

compounds. To establish a technological procedure to promote *Q. rotundifolia* acorn shell brittleness while maintaining maximum fruit quality, two drying regimes (static vs. dynamic) were modelled and drying conditions effects ($T=30^{\circ}$ - 70° °C, $t=2$ - 74 h) on physical-mechanical properties of whole acorns evaluated. Shell fracturability (~ 1.8 mm) requires a temperature value $\geq 50^{\circ}$ °C and $\geq 60^{\circ}$ °C under dynamic and static regimes, respectively. Significant changes in shell colour ($TCD \geq 10$) only occurred in the first 2-4 h, regardless tested conditions. The influence of dynamic drying regime on drying rates and fracturability promotes a time-saving process ($\sim 1-3$ h at 60° and 70° °C). However, process effects on kernel quality needs to be evaluated considering acorn technological applications.

Acknowledgements

We would like to acknowledge financial support for author Carla Alegria through a post-doctoral fellowship (SFRH/BPD/126703/2016) from Fundação para a Ciência e a Tecnologia (Portugal).

P4.5 PROJECT PARTNERSHIP TO DEVELOP NEW METHOD FOR ON LINE SCREENING FOR RANCIDITY IN COCONUT CREAM

Averdieck, W.^{1,*}

1-Arosa Instruments Ltd

*corresponding author e-mail: waverdieck@arosainstruments.com

Scientists at the National Measurement Laboratory (NML) at LGC have been working in partnership with the Coconut Collaborative Ltd (CCL), the market leading coconut yogurt brand in the UK, and the Science and Technology Facilities Council (STFC) under the Innovate UK funded 'Analysis for Innovators' (A4I) programme. The A4I programme provides companies with access to state-of-the-art measurement and analytical technologies through partnership with National Measurement Laboratories (LGC, NPL, and NEL), the Science and Technology Facilities Council and Innovate UK.

For six months, supply specialists, engineers and scientists from CCL, STFC and LGC have been assessing the feasibility of using Raman spectroscopy and multispectral imaging to detect traces of rancid coconut cream ahead of its use in the production of coconut yogurt. This poster reports on the work conducted for this project and the results of this study. It also discusses plans for the resulting technology to be implemented in instruments being developed by Arosa Instruments Ltd

The quality control team at CCL is excited with the encouraging results of the project, which have effectively demonstrated proof of principle for using multispectral imaging as the basis for an enhanced level of quality control and screening in CCL's manufacturing plants. This screening approach will avoid annual costs in excess of £500k through reduced production and material charges. In addition, the project has also highlighted the importance of developing a repeatable and systematic sample preparation and handling procedure, which will also form the basis of future work.

P5.1

GUIDE TO QUALITY CONTROL FOR SMALL AND MEDIUM FOOD OPERATORS

Navarro, C.^{4,*}, Cugat, G.¹, Esser, L.², Gomar, E.³, Ribas F.⁵

1-Subdirector Inspection and Quality Control, Generalitat de Catalunya, Barcelona, Spain

2, 4-Food quality inspector: Generalitat de Catalunya, Girona, Spain

3-Food quality inspector: Generalitat de Catalunya, Barcelona, Spain

5-Training manager in agri-foods: Escola Agrària de l'Empordà, Girona, Spain

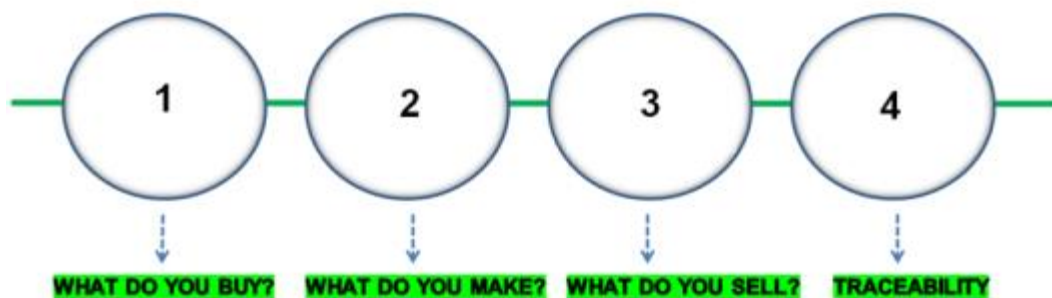
*corresponding author e-mail: carmen.navarro@gencat.cat

Keywords: food quality, prevent fraud, consumers, food business operators

The purpose is to give an effective tool to use for Small and Medium Food operators to guarantee the quality of the food products being produced or traded within the food chain. The objective is giving a better knowledge of the regulation related to quality to the food business operators. The Guide to quality control for small and medium food operators is useful to accomplish the regulation framework, to give guarantees to consumers and clients, to become more competitive, to allow continuous improvement such as detecting errors and correct its causes, to improve the organization and procedures, to have better knowledge of the products and processing and to establish the objectives based on indicators. The final aims are to protect the loyalty of commercial transactions and the rights of consumers.

Two dissemination events have been done and there is one already scheduled. It's a useful tool to get some advice, it's flexible and live and very practical. It contains more than 100 examples and an effort has been made to ensure that every food sector would be represented. There is a short video on the website about the contents of the guide and there are two versions of the guide, one short and one extended.

The structure of the guide is based in 4 pillars:



Each part tackles one of the pillars. At the end of each part, there is a suggestion of the documentary evidence which must be kept proving their own checks have been done. Also, at the very end of the guide there is a questionnaire to check the accomplishment of the quality control system that the food business operator is working with.

To conclude it's necessary to say that this kind of documents are very useful to make food business operators being aware of the need of introducing aspects of quality, fraud prevention, and authenticity rather than being focused only in the safety of the products. This document has been welcomed as a useful tool which gives a general guideline.

Acknowledgments



Generalitat de Catalunya
Departament d'Agricultura, Ramaderia, Pesca i
Alimentació

P5.2

ISOTOPE RATIO MEASUREMENTS FOR FOOD AUTHENTICATION AND PROVENANCING

Atkinson, H.^{1,*}, Welsh, S.¹

1-Sercon Limited, Crewe, U.K.

*corresponding author e-mail: helen.atkinson@sercongroup.com

Keywords: stable, isotopes, data, authentication

The stable isotopes of carbon, nitrogen, hydrogen, oxygen and sulphur are used in food authenticity and provenancing studies to determine geographical origin, compliance with stated growth and manufacturing processes and to detect post-growth adulteration. Both bulk and compound specific isotopic measurements are of interest, and instrumentation which is highly sensitive and precise enables food suppliers, consumers and researchers to have confidence in food forensic data. Here we present the developments which have recently been made to our instrumentation which is relevant to the food industry and data from a range of food and drink samples to demonstrate how stable isotopes are used in food authentication studies.

P5.3

IN SITU ASSESSMENT OF SAFETY OF VEGETABLES BY MEASURING THE NITRATE CONTENT USING NEAR INFRARED TECHNOLOGY

Torres, I.^{1*}, Sánchez, M.-T.¹, Garrido-Varo, A.², Pérez-Marín, D.C.²

1-Food Science and Food Technology, University of Córdoba, Córdoba, Spain

2-Animal Production, University of Córdoba, Córdoba, Spain

*corresponding author e-mail: g72toroi@uco.es

Keywords: safety, nitrate content, portable instruments, in-situ analysis

Over recent years, consumers have become increasingly aware of the risk of the presence of nitrates in foods, among which are vegetables. In response to this growing public concern, the European Union passed Commission Regulation (EC) No 1258/2011 of 2 December 2011 setting maximum levels for nitrates in vegetables, according to their final destination. As a result, growers are increasingly anxious to provide consumers with assurances regarding the safety of these products. Near Infrared (NIR) Spectroscopy by means of the use of portable and compact instruments and in conjunction with the application of multivariate analysis strategies, is an appropriate non-destructive technology for the in situ determination of the nitrate content in vegetables. The main challenge of this work was to develop robust and accurate models for the prediction of the nitrate content in two vegetables: summer squashes and spinach plants.

For this purpose, 157 summer squashes and 128 spinach plants were scanned in situ in the 1600–2400 nm regions, using a portable handheld MEMS-NIR spectrophotometer (Phazir 2400), working in reflectance. Nitrate content (mg NO₃⁻/kg) was measured by reflectometry. Modified Partial Least Squares regression was used to develop calibrations and several spectral signal pretreatments were also tested, combining derivation and scatter-correction treatments. The results showed that NIRS technology has a great potential for measuring nitrate content in intact summer squashes during on-vine ripening; in the case of spinach leaves, the models allow to distinguish between low, medium and high values of the parameter tested. Therefore, NIRS may be a potential tool for the rapid, accurate and non-destructive measurement of nitrate content, with a view to guarantee the safety of vegetables for human consumption.

Acknowledgments

This research was under the Research Projects 'Quality determination of spinach grown in Santaella (Córdoba)' and 'Quality determination of summer squash grown on an open-air plantation in Santaella (Córdoba)', funded by Gelagri Ibérica, S.L. The authors are grateful to Mrs. M^a Carmen Fernández of the Animal Production Department for her technical assistance.

P5.4

FRAUD IN CHICKEN MEAT LABELLING FOUND IN BARCELONA AREA RETAILS (SPAIN)

Montoro, A.^{1,3}, Aregall, N.², Vidal, C.¹, Sibera, M.¹, Latorre, M-L.³

1-Premiumlab, S.L., Barcelona, Spain;

2-Degree food Science and Technology, of Universitat de Barcelona, Barcelona, Spain. 3-Departamento de Nutrición, Ciencias de la Alimentación y Gastronomía, INSA XaRTA, Universitat de Barcelona, Campus de l'Alimentació de Torribera, Barcelona, Spain.

Keywords: food fraud, poultry, mandatory claim, labelling, agrifood sector.

Introduction

The chicken meat, is the most economical protein source after eggs, therefore, it is one of the most basic in the diet. A product being so necessary for such large part of population should never be subject to fraud or illicit actions that may affect the consumer. Unfortunately, food fraud is increasingly occurring, as the mere fact of obviating one of the mandatory mentions in the label, involves a fraud in itself. There are two main reasons that facilitate fraudulent actions:

1. Not having a clear definition of the concept of food fraud in European legislation. Therefore no protocol or preventive procedure against fraud is available.
2. Increasing product value and, as a result, the price to consumer, by attributing inadequate characteristics to a product.

Fraud in the food sector, either by incorrect labeling or by conducting illicit actions, can compromise food safety. In this context, it is necessary to evaluate what is the real situation at the level of fraud in labeling in chicken meat.

Objective

Evaluate the degree of compliance of the chicken meat label with the mandatory mentions established in the European Legislation.

Methodology

In order to evaluate fraud in chicken meat labeling we followed the next steps:

1. To carry out a search and select relevant information, supporting documents and consolidated legislation at European and national level.
2. To elaborate a theoretical label with mandatory mentions, as a model for each of the two main groups into which chicken meat products can be classified, and that are not submitted to the same mandatory mentions:
 - Group I: unpacked meat from small establishments and local markets, handled directly at the point of sale.
 - Group II: Packed raw chicken meat, marketed in supermarkets.
3. To categorize non compliances based on the severity of the effects they can have on costumers.
4. Collect labels from final products presented to consumer.
5. Analyze all gathered information.

Legislation

- Commission Regulation (EC) No 543/2008 of 16 June 2008 laying down detailed rules for the development of Council Regulation (EC) No 1234/2007 as regards the marketing of poultry meat.
- Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on food information provided to the consumer.

Results and Discussion

A total of 81 labels of chicken meat were collected from small establishments, local markets and supermarkets in the area of Barcelona.

After label revision, only 23 labels were compliant with all the mandatory mentions, indicating that near 72% of the labels are fraudulent in the area of Barcelona. All the detected irregularities were related to omission of information on the product label.

Not all the mentions have the same impact when omitted. Fraudulent labels have been classified according to the repercussions that they can have on consumer and they have been analyzed for each of the two product groups described. It has been detected that most of the fraudulent labels in Group I (non-packaged chicken meat) do not include mentions of a serious nature such as: preparation method, batch number, storage conditions, and name of the product. By contrast, fraud in Group II (packaged meat distributed in large supermarkets) is mainly related to trademark, commercial category, and net weight, which is considered to have a minor impact on food safety.

It should also be noted that in Group I in shops and small establishments, during the fieldwork, illicit procedures and actions have been detected, such as: maintaining the correct labeling of the product, attributable basically to the lack of training or awareness of the repercussions that entails.

Conclusion

The first conclusion from this study is that fraud in chicken labeling is striking high in the geographical area under study, and mainly associated to omission of mandatory information. Fraud is more severe in small establishments and local markets as compared to large supermarkets.

From our study it becomes clear that awareness should be raised at the label of producers, distributors and the sales point in order to control the entire value process chain.

P5.5

CONTROL OF FRESHNESS DECAY OF CRUDE AND REFINED OILS WITH A COMPARATIVE STUDY OF PHOTO-OXIDATION AND AUTOXIDATION BY MESH CELL-FTIR SPECTROSCOPY

Lobo-Prieto, A.^{1,*}, Tena, N.¹, Aparicio-Ruiz, R.¹, Morales, M.T.², Aparicio, R.¹, García-González, D.L.¹

1-Instituto de la Grasa (CSIC), Ctra. de Utrera, km. 1, Campus Universitario Pablo de Olavide - building 46, 41013 - Sevilla, Spain.

2-Department of Analytical Chemistry, University of Seville, Prof. García González, 2, 41012, Seville, Spain.

*corresponding author e-mail: ana.lobo@ig.csic.es

Keywords: mesh-cell-FTIR, freshness control, moderate storage conditions, photo-oxidation.

The development of new methods for freshness control of edible oils is necessary to increase the confidence of consumer who always expect the oils to be fresh. Edible oils can be considered as lipid matrices (refined or crude) and they are used as ingredient for the elaboration of processed food. However, a lack of control of the shelf-life of these oils in real conditions (not in drastic conditions of temperature or light) may compromise on the integrity of the product. The oxidation stability of these edible oils when they are exposed to moderated visible light intensity (400 lx) or room temperature (23°C, 35°C) is not so much studied yet. However, gaining knowledge about the chemical changes produces in these lipid matrices during their shelf life is of high interest to understand how oils lose their freshness, affecting the quality of the food product. In this study the time-course changes of the chemical composition of different lipid matrices has been monitoring by mesh-cell FTIR spectroscopy when they are exposed to the combination of low intensity of visible light and mild heating.

The storage conditions applied in this study are very similar to those that occurred during the shelf life of the fat food: in the dark at 35°C, 400 lx at 23°C and 400 lx at 35°C. To monitor and control the changes produced as consequence of these storage conditions, a novel accessory named 'mesh cell' combined with FTIR spectroscopy has been used. The spectra obtained every 24 hours allowed tracking the chemical changes on primary and secondary oxidation products. The changes observed in the spectra permitted to evaluate the relative effect of light, mild heating, and the combined effect of these two factors. The initial effect of moderate light intensity in the degradation of oils can be even more relevant than mild heating (23°C or 35°C) being responsible for the freshness decline. The results proved that the exposition to moderate light intensity (400 lx) were 4 times more relevant than the mild heating (35°C) in the degradation of monounsaturated lipid matrices (OOO).

On the contrary, mild heating (35°C) accelerated the formation of hydroperoxides 2 times faster than light (400 lx) in polyunsaturated lipid matrices (SO) which were more sensitive to mild heating. However, the combination of both factor light at 400 lx and mild heating at 35°C presented the fastest degradation in all the studies lipid matrices. So, the results prove the importance of light at moderate intensity (400 lx), even in those oils that are known for being relatively more stable. Thus, mesh cell-FTIR is presented as a useful tool to monitor

and control the freshness of fat food when they are exposed to light intensity commonly found on the supermarket shelf and room temperature.

Acknowledgments

Financial support by Fundación General CSIC (Programa ComFuturo) is acknowledged. This research has been also funded by AGL2015-69320-R project (Spanish State Secretariat of Research, Development and Innovation).

P6.1

OLIVE OIL, REFINED: A NEW *FOOD CHEMICALS CODEX* IDENTITY STANDARD

Clapper, G.^{1,*}, Laurvick, K.¹, Moore, J.¹

1-US Pharmacopeia, Rockville, MD, USA

*corresponding author e-mail: gina.clapper@usp.org

Keywords: olive oil, identity standard, standardization

Economically motivated adulteration is an ongoing, global issue. Decernis' Food Fraud Database includes more than 200 records of olive oil adulteration with substances including vegetable oils, lower-grade olive oils, chlorophylls and chlorophyllin, and non-food grade oils. USP's Food Ingredient Expert Committee convened an Olive Oil Authenticity and Quality Panel to address this opportunity. The panel has drafted an Identity Standard for benchmarking refined olive oil which will be published in December 2018 FCC Forum.

P6.2 STANDARDISATION OF METHODS - NEW INITIATIVES IN GERMANY

Stoyke, M.¹, Uhlig, S.², Mierke-Klemeyer, S.¹, Klemm, C.¹, Reimann, D.¹, Szabo, K.¹, Becker, R.¹

1-Federal Office of Consumer Protection and Food Safety (BVL), General affairs and method standardisation, Berlin, Germany; manfred.stoyke@bvl.bund.de

2-QuoData GmbH Quality & Statistics, Dresden, Germany

Keywords: official collection of methods, species identification, mass spectrometry, method validation

As coordination office for § 64 LFGB (German Food and Feed Act), the unit “General affairs and method standardisation” at the Federal Office of Consumer Protection and Food Safety (BVL) has among other things the statutory obligation to keep up-to-date the Official Collection of Methods of Sampling and Analysis (ASU) in Germany. In this context, the potential of modern methods is being considered, e.g. for species identification and for checks on the geographic origin or method of production [1].

In order to meet the requirements of the official authorities responsible for food surveillance in Germany and Europe, these methods must be validated and included in the Official Collection of Methods of Sampling and Analysis (ASU). Furthermore, they must be conveyed to the Comité Européen de Normalisation (CEN). Therefore, the BVL constitutes new working groups consisting of experts from the field of food authenticity.

Polymerase chain reaction (PCR) has been applied as a sensitive method for species identification in food of plant and animal origin for some time. In this field, several working groups already exist (“Speziesdifferenzierung“- *species differentiation*; “GVO-Nachweis“- *GMO detection*; “Allergene“- *allergens*).

Due to its multiplexing capacity, mass spectrometry is gaining increasing attention from food surveillance. In recent years, several methods have been developed that use liquid chromatography coupled to mass spectrometry (LC-MS) to identify species-specific marker peptides in food. These marker peptides can be used to verify food authenticity. Although these methods have great potential, none of them have been standardised or validated in inter-laboratory studies so far. In order to achieve this goal, the BVL has, as the first of its kind, constituted a new working group for the peptide-based mass spectrometric analysis of food and agricultural products [2]. The purpose of this group is to identify and validate methods based on LC-MS, which can then be used to control and enforce regulations dealing with food authenticity and food allergens.

A further technique, MALDI-TOF-MS, is already being used for the identification of, among others, microorganisms in food. Here, a working group is being founded, which aims on standardisation and validation of MALDI-TOF methods for species identification (i. e. methods for the identification of microorganisms in food and methods for the differentiation of animal species, plants, insects and fungi)

Further methods used for authenticity testing are next generation sequencing (NGS); nuclear magnetic resonance (NMR) spectroscopy, isotopic ratio mass spectrometry (IRMS) and

other so-called untargeted methods incorporating a multivariate classification. The constitution of new working groups regarding NGS, NMR and IRMS is already planned at the BVL. For the latter group, first of all validation approaches allowing a platform-independent evaluation have to be elaborated.

On the European level, the ASU coordination office supports the CEN coordination group "Food Authenticity" (FACG). After taking an inventory of the methods that need to be standardised, the methods will be prioritised. At the same time, the German Institute for Standardization (DIN) made a request to CEN to establish a Technical Committee (TC) on "Food Authenticity" and to host its secretariat. This request will be submitted to the members of CEN in the course of this year for comments and voting. The methods standardised and validated in the § 64 working groups will then be introduced into this TC.

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Acknowledgments

Members and stakeholders of the new working groups

P6.3

FRESH OR FROZEN-THAWED FISH? A HISTOLOGICAL TOOL TO REVEAL FRAUDS

Pezzolato, M.¹, Meistro, S.¹, Gili, S.², Varello, K.¹, Baioni, E.¹, Maurella, C.¹, Giarratana, F.³, Panebianco, A.³, Abramo, F.⁴, Vascellari, M.⁵, Bozzetta, E.¹

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy;

2-ASL Città di Torino, Torino, Italy;

3-Università di Messina, Messina, Italy;

4-Università di Pisa, Pisa, Italy;

5-Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

*corresponding author e-mail: marzia.pezzolato@izsto.it

Keywords: fish, histology, fresh, frozen-thawed

Seafood fraud is a serious concern. Selling fish products as fresh, intended as never subjected to sub-zero temperatures, when they have actually been frozen-thawed is a common fraudulent commercial practice; moreover, fish intended for raw or almost raw consumption must be previously frozen according to EU Regulation 1276/2011, in order to protect consumers from parasites and to avoid a potential sanitary fraud. Nevertheless it is important to note that in no case it is possible to automatically exclude possible negative effects on health even for commercial frauds and particular attention must be focused on all frauds. Since years at Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta the most performing techniques in distinguishing fresh from frozen fish have been evaluated in order to make available reliable tools. Aim of the work was to set up a valid standardised histological method for the identification of fish as fresh or frozen-thawed.

Preliminary investigations on samples frozen at -80°C were successfully conducted, even if these data were not included in the validation process. The study has considered time/temperature combinations considered by law, i.e. -20°C/24 hours and -35°C/15 hours, and also a very quick protocol not considered by law, i.e. -40°C/2 hours. In all, during successive experiments, 423 muscle samples of 35 fish species (both bony and cartilaginous, both saltwater and freshwater fish), never subjected before to temperatures below zero, were prepared. The samples were divided into 2 reference groups: group A (n=273), fresh samples (stored at 0-4°C); group B (n=150), experimentally frozen, and then thawed at 0-4°C. After, respectively, refrigeration and freezing/thawing, all samples were fixed in 10% neutral buffered formalin and routinely processed. Paraffin embedded blocks were cut on a microtome into 3-5 µm sections and stained with haematoxylin and eosin. Slide preparations were examined by optical microscopy at increasing magnification (X4, X10, X20, and X40). Different morphological parameters were evaluated even if sensitivity and specificity of the histological method were estimated only on presence/absence of vacuoles of various dimensions, optically empty or filled with eosinophilic material, caused by ice crystals, in the cytoplasm of muscle cells: the only parameter able in differentiating fresh from frozen-thawed samples.

Histological method resulted in 94.20% sensitivity (C.I.95%: 90.10-97.00%) and 97.70% specificity (C.I.95%: 94.20-99.40%) in differentiating between fresh and frozen-thawed samples and it was validated, irrespective of the fish species analysed, and accredited. For the purpose of the histological interpretation, no significant differences from fish to fish were noticed. The method is now applicable to all fish species, to not-transformed and to prepared

fishery products derived from fish and also to transformed ones, e.g. marinated fish and smoked fish. Furthermore, a ring test recently performed in the Italian network constituted by the Istituti Zooprofilattici Sperimentali obtained a K-combined value of 0.94 (C.I.95%: 0.89-0.99), which represents an optimal value of concordance.

The histological method represents a reliable and cost-effective analytical tool, able to distinguish fresh from frozen-thawed fish, and it can be profitably used for revealing frauds regarding the storage conditions of the fish, achieving the goal of the protection of consumers from frauds. In 2015 Italy ranked second in EU in terms of volume of consumed fresh fish products and third in terms of economic value. In 2017, 13.2% of 53 batches of samples declared as “fresh” and analysed by histology was actually frozen-thawed. Still, many important challenges are standing in the seafood sector: e.g. fast and reliable methods that would differentiate between fresh and frozen-thawed cephalopods are being sought and we are working on it.

Acknowledgments

Research projects 11C03 and 17C10, funded by Italian Ministry of Health.

P6.4

STANDARDIZATION OF NON-TARGETED METHODS: GUIDANCE AND EXAMPLE

Xie, K.^{1,*}, Moore, J.¹

1-US Pharmacopeia, Rockville, MD, USA

*corresponding author e-mail: kyx@usp.org

Keywords: non-targeted methods, food fraud, standardization

Food fraud, also known as Economically Motivated Adulteration (EMA) is known to be present in many food and beverage products. It is estimated that up to 10% of the food supply could be affected by food fraud. To fight against food fraud, in addition to supply chain controls and a preventive control plan, advanced methods such as non-targeted methods should be implemented to mitigate the risks.

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 this approach.

Based on our experience working on non-targeted methods, USP has developed a guidance document to address this need. The guidance document covers: the collection and analysis of reference samples, development and validation of the non-targeted testing statistical models, monitoring and maintenance, as well as advice on handling of abnormal samples. The overview of the non-targeted methods, the guidance and a detailed example on QA screening of milk by FTIR will be presented.

P7.1

DIFFERENTIATION OF PENAEIDAE SHRIMP SPECIES BASED ON A NEW METHOD OF HRM ANALYSIS OF A COI MINI-BARCODE

Mafra, I.^{1,*}, Silva, C.R.¹, Fernandes, T.J.R.¹, Costa, J.¹, Oliveira, M.B.P.P.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Portugal

*corresponding author e-mail: isabel.mafra@ff.up.pt

Keywords: high resolution melting analysis, DNA barcode, crustaceans, authentication.

Penaeid shrimps (belonging to Penaeidae family of the Decapoda order) represent more than 30% of the worldwide demand of crustaceans, comprising many commercially relevant species, such as whiteleg shrimp (*Litopenaeus vannamei*), giant tiger prawn (*Penaeus monodon*), Indian white prawn (*Fenneropenaeus indicus*), jinga shrimp (*Metapenaeus affinis*), and striped shrimp (*Melicertus kerathurus* also known as *Penaeus kerathurus*) [1]. However, they share a noticeable morphological similarity, which makes them potential targets of adulteration. Therefore, mechanisms for authentication and certification of such crustaceans, frequently included in processed foods, constitute a benefit for the food industry. Molecular methods based on DNA biomarkers have been shown to be suitable tools for seafood species identification, particularly relying on short barcode regions [2]. This work intended to develop a new approach for the specific detection and differentiation of the referred five closely related shrimp species based on high resolution melting (HRM) analysis targeting a cytochrome oxidase subunit I (COI) mini-barcode.

In silico barcode analysis was performed for the design of universal primers targeting a COI region of the selected species, namely *P. monodon*, *P. indicus*, *M. affinis*, *L. vannamei* and *P. kerathurus*. A total of 58 animal and plant species were used to evaluate the specificity of the proposed method, including fish, crustaceans and molluscs (n=25). In addition, 33 frozen and/or processed seafood products were acquired at local markets, comprising typical Portuguese (shrimp with beans “feijoada”, shrimp “açorda”) and other pre-cooked (risotto, pizza, paella) dishes, seafood soups and sauces (powders), shrimp kernels, among others. DNA was extracted using NucleoSpin Food kit according to manufacturer instructions, with minor changes. The concentration and purity of the DNA extracts were measured by UV spectrophotometry in a micro-volume plate accessory. Specificity and sensitivity of the designed primers were assessed by qualitative PCR, prior the development of a real-time PCR assay using Evagreen dye coupled with HRM analysis.

The results of conventional melting analysis showed that COI amplicons presented two groups of melt peaks: one at 73.6°C (*L. vannamei*) and the second ranging from 75 to 76°C (*P. monodon*, *P. indicus*, *M. affinis* and *P. kerathurus*) (Fig. 1A). The application of HRM analysis allowed the discrimination of all five species that were included in five distinct clusters with a level of confidence above 99% (Fig. 1B). The method was effectively applied to analyse processed seafood samples, from which *F. indicus* and *L. vannamei* were the main identified species (Fig. 1). When verifying labelling compliance, four samples suggest adulterations based on the complete or partial substitution of declared species. The proposed method proved to be a potential tool for the rapid and cost-effective differentiation of penaeid shrimp species.

Acknowledgments

This work was 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, by the project NORTE-01-0145-FEDER-000011 and the European project FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688). Telmo J. R. Fernandes and Joana 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). The authors acknowledge the kind supply of shrimp species by Marfresco (Loures, Portugal) and Brasmar Seafood Companies (Trofa, Portugal).

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P7.2

QUANTITATIVE DETECTION OF SHRIMP CRUSTACEANS BY A NOVEL REAL-TIME PCR METHOD

Costa, J.^{1*}, Fernandes, T.J.R.¹, Oliveira, M.B.P.P.¹, Mafra, I.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Portugal

*corresponding author e-mail: jbcosta@ff.up.pt

Keywords: crustaceans, real-time PCR, 16S rRNA gene, quantification of shrimp.

Crustaceans, such as shrimps, crabs or lobsters represent a major food resource with high commercial value, being also responsible for eliciting the majority of the shellfish-allergic reactions [1]. About 60% of all crustaceans (13.9 million tonnes), on the global capture and production, belong to shrimps/prawns, from which *Litopenaeus vannamei* is the main crustacean species with a production of 3.9 million tonnes in 2015 [2]. To protect people from experiencing adverse allergic reactions, reliable methodologies are necessary to verify the labelling of processed seafood. Considering the scarce methods on crustacean quantification by DNA analysis, the development of a quantitative real-time PCR system specific for a wide range of shrimp species was proposed in the present work.

Sequences of the 16S rRNA mitochondrial gene were selected from NCBI database from a set of 17 different crustacean species, which were aligned with BioEdit v.7.2.5 software (Ibis Biosciences, Canada) using ClustalW (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and examined for suitable regions for primer and probe design to amplify as much species as possible. Several crustacean specimens from different species (n=18) were acquired, together with 62 non-crustacean species, including fish, molluscs, meats and plants. Several seafood products (n=18), including shrimp patties, seafood broth/soups, surimi and pre-cooked dishes, were also acquired at local markets for assay applicability. A set of model mixtures was prepared to simulate a processed shrimp stuffing/filling, containing 50-0.0001% of shrimp in béchamel. The DNA from all crustacean species and mixtures was extracted using the SureFood® Prep advanced kit (CONGEN Biotechnologie GmbH, Berlin, Germany), according to the manufacturer instruction of Protocol 1.

Two new DNA-based approaches targeting the 16S rRNA gene were proposed to detect crustaceans in foods using a qualitative PCR assay specific for crustaceans (shrimps, lobsters and crabs) and a quantitative real-time PCR assay specific for shrimp crustaceans. The real-time PCR system allowed the detection and quantification down to 0.1 pg and 0.0001% (w/w) of shrimp DNA and shrimp in model mixtures, respectively. The method exhibited high performance for quantitative analysis in the range of 0.0001% to 50% as inferred by the calibration curve parameters (Fig. 1), being effectively validated with blind mixtures. The assays were successfully applied to processed seafood samples, allowing estimating the shrimp contents and verifying the absence of crustaceans in those with precautionary labelling [3]. The qualitative PCR assay can provide a simple, fast and high throughput tool for screening the presence of crustaceans in processed foods, while the proposed real-time PCR method proved to be a useful tool for the accurate detection and quantification of shrimp in foods at trace levels.

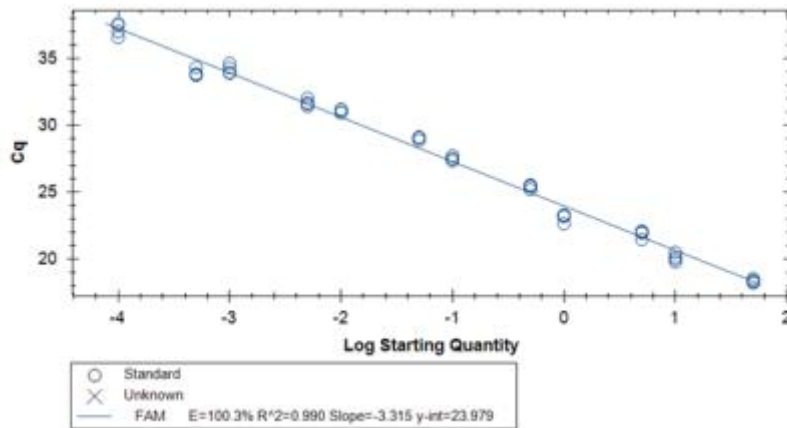


Fig. 1. Calibration curve of the real-time PCR assay with TaqMan probe targeting the 16S rRNA gene using from binary model mixtures of cooked shrimp in béchamel sauce (50-0.0001% (w/w)).

Acknowledgments

This work was supported by FCT (Fundação para a Ciência e Tecnologia) through projects AlleRiskAssess – PTDC/BAA-AGR/31720/2017, 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. T. J. R. 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). The authors acknowledge the kind supply of shrimp species by Marfresco (Loures, Portugal) and Brasmar Seafood Companies (Trofa, Portugal).

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P7.3

A NOVEL DNA-BASED APPROACH FOR ARGAN OIL AUTHENTICATION: DETECTION OF OLIVE OIL AS A POTENTIAL ADULTERANT

Mafra, I.^{1,*}, Raja, F.Z.^{1,2}, Costa, J.¹, Amaral, J.S.^{1,3}, Grazina, L.¹, Villa, C.¹, Kartah, B.E.², Charrouf, Z.², Oliveira, M.B.P.P.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Portugal

2-Faculté des Sciences, Université Mohammed V, Rabat, Morocco

3-CIMO, Instituto Politécnico de Bragança, Bragança, Portugal

*corresponding author e-mail: isabel.mafra@ff.up.pt

Keywords: *Argania spinosa* L., authenticity, real-time PCR, *matK* gene

Argan oil is a non-refined vegetable oil produced from the argan tree (*Argania spinosa* L.), a species endemic only in the South-western Morocco. Argan oil has been used in this country for centuries, either as food or for cosmetic/medicinal purposes. Depending on the use of roasted or raw argan kernels, food or cosmetic grade oil is obtained [1]. The use of roasted kernels affords edible oil with a nutty and roasty flavour that for long years has been prepared exclusively by Berber women according to an ancestral laborious process. Currently, as a consequence of its high cost and increasing demand, this traditional product from Morocco is highly prone to illegal practices to increase profits, such as adulteration by the addition of cheaper vegetable oils. Therefore, considering the economic, social and cultural importance of argan oil in Morocco, it is important to develop methodologies that can be used in control and inspection programs in order to guarantee argan oil authenticity and quality. In particular, there is the need for methodologies that allow the accurate identification of vegetable oils illegally added to argan oil. The present work aims at developing novel approaches based on DNA markers to detect the presence of adulterants, using olive oil as case study.

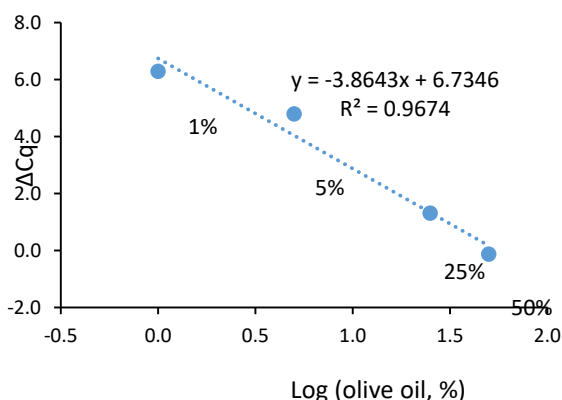


Fig. 1. Normalised calibration curve of real-time PCR with EvaGreen dye targeting the *matK* gene of olive, using binary mixtures of olive oil in argan oil (50, 25, 5, 1%, w/w).

In silico analysis was performed for the design of *Olea europea* L. and *A. spinosa* L. specific primers targeting the chloroplastial *matK* gene and the ITS2 region, respectively. Samples of authentic argan oil were acquired from producers in Morocco, while olive oil samples were obtained from local stores in Portugal. Other edible and oil producing plant species were also

used for assay specificity testing ($n=17$). Binary model mixtures were prepared with the addition of known amounts of olive oil in argan oil in the proportions of 50, 25, 5, 1% (w/w), followed by concentration by centrifugation. DNA was extracted using the Nucleospin Plant kit, protocol B (Macherey-Nagel, Düren, Germany) according to the manufacturer instructions. The concentration and purity of the DNA extracts were measured by UV spectrophotometry in a micro-volume plate accessory. Specificity and sensitivity of the designed primers were assessed by qualitative PCR. Species-specific PCR assays were successfully developed, producing amplicons of 109 and 117 bp for olive and argan, respectively, down to 0.01 pg of DNA for both species. The application of the olive-specific PCR assay to DNA extracts of binary mixtures enabled the clear detection of 1%. Subsequently, a real-time PCR assay with EvaGreen dye was developed for quantitative analysis using the normalised ΔCq method (Fig. 1). The assay confirmed the limit of detection of 1% of olive oil, in a dynamic range of 1-50%, with acceptable correlation coefficient and PCR efficiency (81.1%), considering the type of food matrix. Both, qualitative and quantitative PCR assays can provide a simple, fast and high-throughput tool to detect the presence of adulterant oils in argan oil.

Acknowledgments

This work was supported by FCT (Fundação para a Ciência e Tecnologia) through projects FCT/CNRST (Portugal/Morocco) (FCT/6460/6/6/2017/S), 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; by projects NORTE-01-0145-FEDER-000011 and FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688). Joana Costa, Liliana Grazina and Caterina Villa are grateful to FCT grants (SFRH/BPD/102404/2014, SFRH/BD/132462/2017 and PD/BD/114576/2016, respectively) financed by POPH-QREN (subsidised by FSE and MCTES).

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P7.4

MITOCHONDRIAL GENE SCREENING TO IDENTIFY COW'S DNA MARKERS FOR MEAT AUTHENTICATION AND MILK ALLERGEN DETECTION

Villa, C.^{1,*}, Costa, J.¹, Oliveira, M. B. P. P.¹, Mafra, I.¹

1-REQUIMTE-LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal.

*corresponding author e-mail: caterinavilla@hotmail.com

Keywords: meat authenticity, milk allergens, mitochondrial genome, real-time PCR.

Meat is among the most vulnerable foods to fraudulent adulterations for economic gain, especially in what concerns ground and comminuted meat products. Consequently, the demand for more transparency in the meat food chain has been increased. A correct and truthful labelling has become imperative to guarantee consumer protection from fraud, as well as from health hazards. In fact, cow's milk proteins are common food allergens present in uncounted meat products as technological aids (sausages, ham), exposing the allergic individuals to a constant threat of experiencing cutaneous, respiratory or gastrointestinal reactions and, in some extreme cases, anaphylaxis, even with the ingestion of trace amounts [1]. In recent years, DNA-based techniques have been successfully applied in species identification in meat products, by the use of specific molecular markers able to detect low quantities of the target [2]. However, the analytical methods for allergen detection need to be more sensitive and able to identify trace levels of the offending food. The aim of this work was screening different genes of cow mitochondrial genome in order to develop a specific, sensitive and accurate method to detect trace quantities of cow DNA in complex and processed foods, which can be applied to meat product authentication and milk allergen detection.

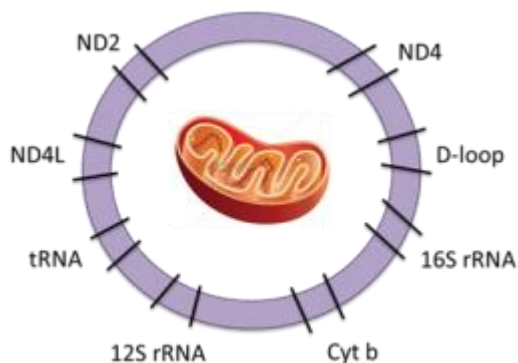


Figure 1. Genes of the cow's mitochondrial genome selected for the design of specific primers and probes.

Model mixtures containing known quantities (10-0.00001%) of cow's milk protein concentrate (technological aids) in turkey meat were prepared and submitted to two processing methods, simulating the production of ham (67°C, 5 h) and sausages (121°C, 15 min). DNA extraction was performed using the NucleoSpin Food kit (Macherey-Nagel, Düren, Germany). Sequences of mitochondrial genes (Figure 1) from cow and other animal species used in ham and sausages preparation were retrieved from NCBI database. Specific primers and TaqMan probes were designed and tested in model mixtures by qualitative PCR and real-time PCR. Sequencing of the same genes of different animal species were also performed for cross-reactivity study.

The results showed that most of the tested PCR assays presented absolute limit of detection (LOD) between 10 pg and 1 pg of cow DNA and relative LOD of 0.01% and 0.1% of cow's milk protein concentrate in raw and processed ham respectively. However, with such high sensitivity levels, the specificity study revealed cross-reactivity with some meat species, including turkey, pig, goat and chicken. The cow amplicons from the assays that presented less cross-reactivity and the respective regions in the reactive animal species were sequenced in order to identify any nucleotide differences that to increase the specificity by the designing of new primers and probes to be applied in real-time PCR. Until now, real-time PCR targeting the *cyt b* gene showed a LOD of 0.01% with adequate performance parameters (PCR efficiency=98.7%, $R^2=0.980$, Slope=-3.355) in the range 10-0.01% of cow milk protein concentrate in non-processed ham, but still with a background signal of some cross-reaction with turkey meat.

Currently, other cow mitochondrial genes are under evaluation and will be tested by real-time PCR with specific probes in order to improve the sensitivity and specificity of the method. The development of useful and effective tools for cow meat and milk detection and quantification in meat products, to verify labelling compliance and to guarantee consumer's protection will be the final objective of this work.

Acknowledgments

This work was supported by FCT (Fundação para a Ciência e Tecnologia) through projects AlleRiskAssess – PTDC/BAA-AGR/31720/2017, 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. Caterina Villa and Joana Costa are grateful to FCT grants (PD/BD/114576/2016 and SFRH/BPD/102404/2014, respectively) financed by POPH-QREN (subsidised by FSE and MCTES).

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P7.5

APPLICABILITY OF HRM ANALYSIS FOR CARNAROLI RICE AUTHENTICATION BASED ON POLYMORPHISMS OF THE WAXY GENE

Grazina, L.^{1,*}, Costa, J.¹, Amaral, J.S.^{1,2}, Garino, C.³, Arlorio, M.³, Oliveira, M.B.P.P.¹, Mafra, I.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;

2-CIMO, Instituto Politécnico de Bragança, Bragança, Portugal;

3- Dipartimento di Scienze del Farmaco & Drug and Food Biotechnology Center, Università del Piemonte Orientale "A. Avogadro", Novara, Italy.

*corresponding author e-mail: li_grazina@hotmail.com

Keywords: *oryza sativa*, DNA markers, high resolution melting analysis, variety differentiation.

Rice (*Oryza sativa* L.) is a staple food and one of the most important cereals in the worldwide. Italy, the leading rice producer in Europe, holds nearly 200 different varieties in the available germplasm [1]. The Carnaroli rice is a high quality and priced variety belonging to the group of japonica ecotype, 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, thus, ideal for the preparation of traditional risotto. Italian rice varieties have different characteristics, from which the starch composition is a highly relevant parameter. Together with amylopectin, amylose is the main component of starch, whose ratio is determinant for the rice cooking properties. After cooking, varieties with high amylose content have dry, firm and separate grains, while low amylose ones usually have tender, cohesive and glossy texture [2]. Amylose synthesis is catalysed by the granule bound starch synthase (GBSS) that is encoded by the Waxy gene (*Wx*), being located on the chromosome 6. Various nucleotide polymorphisms have been associated with the *Wx* gene, namely (CT)_n repeats and several single nucleotide polymorphisms (SNP) [2]. The aim of this work was to propose a new method based on high resolution melting (HRM) analysis, exploiting those polymorphisms to differentiate Carnaroli rice from other closely related varieties.

The Italian rice varieties sold as Carnaroli (Carnaroli, Carnaval, Caravaggio, Koepe, Poseidone, Karnak, Carnise, L202 and L252), as Roma-Baldo (Roma, Baldo, Cammeo, Galileo and Casanova), as Arborio (Volano, Telemaco and Generale), as Thaibonnet (Gladio) and as Sant'Andrea (Sant'Andrea) were acquired from producers. DNA from rice grains was extracted with NucleoSpin food kit (Macherey-Nagel, Düren, Germany). In silico analysis was performed in the *Wx* gene to design primers targeting the (CT)_n microsatellite and the G/T in the first intron. Two sets of primers were designed to amplify fragments of 183 bp and 341 bp for HRM method development and sequencing analysis, respectively. The sequencing results showed that all the varieties commercialised as Carnaroli have 17 CT repeats on the microsatellite, while the others have 18 and 20, in the case of Gladio. Additionally, the first intron SNP is G for Carnaroli, while is a T for all varieties except Gladio. The development of a real-time PCR assay targeting the 183 bp fragment with EvaGreen dye combined with HRM analysis enabled the differentiation of Carnaroli rice varieties in Cluster 1, Roma-Baldo, Arborio and Sant'Andrea varieties in cluster 2 and Gladio in cluster 3, with levels of confidence above 98% (Fig. 1). The results were well corroborated with the sequencing data. Therefore, the proposed new HRM method can be a simple, specific and high-throughput tool for the authentication of Carnaroli rice.

Acknowledgments

This work has been supported by the European project FOODINTEGRITY (FP7-KBBE-2013-single-stage, No 613688), 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. L. Grazina and J. Costa are grateful to grants (SFRH/BD/132462/2017 and SFRH/BPD/102404/2014, respectively) from FCT financed by POPH-QREN (subsidised by FSE and MCTES).

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P7.6

ENTOMOLOGICAL AUTHENTICATION OF HONEY BASED ON DNA MARKERS: DIFFERENTIATION OF HONEY PRODUCED BY *APIS MELLIFERA* AND *APIS CERANA*

Amaral, J.S.^{1,4,*}, Soares, S.¹, Grazina, L.¹, Mafra, I.¹, Costa, J.¹, Pinto, M.A.², Duc, H.P.³, Oliveira, M.B.P.P.¹

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Portugal

2-CIMO, Instituto Politécnico de Bragança, Bragança, Portugal

3-Bee Research Centre, Hanoi, Vietnam

*corresponding author e-mail: jamaral@ipb.pt

Keywords: *apis cerana*, *apis mellifera*, authenticity, real-time PCR

According to the European Union legislation, honey is the natural sweet substance produced by *Apis mellifera*, also known as European honeybee. However, in other regions of the world, honey is traditionally obtained from other bee species. Among those, *A. cerana* (also known as Asian honeybee) is also of economic importance since it is used in apiculture. Due to the decline of the wild populations of the *A. cerana* in some countries, such as Japan and parts of China, there is an increasingly interest in preserving the native Asian honeybee, being its honey increasingly valued. Owing to the growing demand for this traditional product, the honey produced by *A. cerana* attains a much higher market value compared to that of *A. mellifera*, thus being prone to adulteration. So far, only a few protein-based methods have been proposed to assess honey entomological origin [1], which in fact is related to its geographical origin since bee species generally occupy different geographical ranges according to their evolutionary lineages [2].

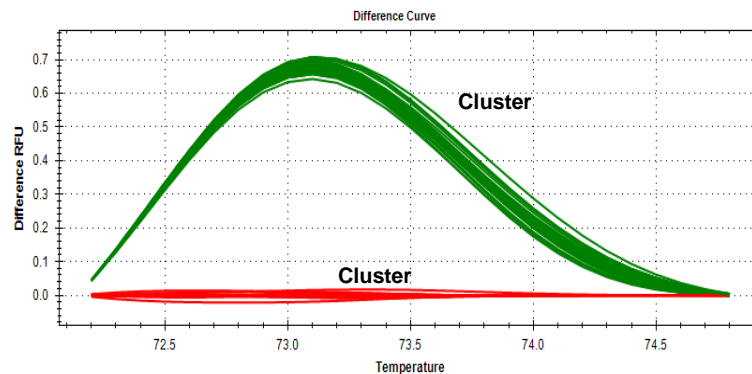


Fig. 1. Difference curves obtained by real-time PCR amplification with EvaGreen dye and HRM analysis targeting 16S rRNA gene (n=3 replicates). Legend: cluster 1, *A. cerana* from Vietnam and authentic honey samples produced by *A. cerana* from Vietnam; cluster 2, *A. m. carnica*; *A. m. iberiensis*; *A. m. ligustica*, authentic honey samples produced by *A. mellifera* from Vietnam, and commercial honey samples from Italy, France and Portugal

In this work, DNA methods were developed for the specific identification of *A. mellifera* and *A. cerana* DNA in honey. For this purpose, bees of *A. cerana* from Thailand, China and Vietnam and honeybees of 4 different subspecies of *A. mellifera* (*iberiensis*, *mellifera*, *ligustica* and *carnica*) from EU countries were used. Different sets of primers were designed targeting the tRNA^{leu} - COII intergenic region and the 16S rRNA gene. For both cases, the

specificity and sensitivity of the designed primers were assayed by qualitative polymerase chain reaction (PCR). DNA was extracted from honey samples as previously described [3]. PCR with primers targeting the tRNA^{leu} - COII intergenic region allowed the specific detection of *A. cerana*. The applicability of the proposed new PCR method was assayed with authentic *A. cerana* and *A. mellifera* honey samples, which enabled the identification of *A. cerana* honey. PCR targeting the 16S rRNA gene successfully amplified both honeybee species, but without being able to differentiate them. However, the use of real-time PCR with 16S rRNA primers coupled with High Resolution Melting (HRM) analysis allowed the differentiation of both species in distinct clusters (Fig. 1). The developed new HRM methodology was further applied to the analysis of authentic honey samples from Vietnam (produced from *A. cerana* and *A. mellifera* honeybees) and from Portugal (produced from *A. mellifera* honeybees), as well as commercial samples of honey labelled as produced in the EU, allowing its successful entomological origin identification [4]. Both developed techniques proved their effectiveness for establishing the entomological origin of honey and can be considered as useful tools for authentication/control purposes.

Acknowledgements

This work was 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 project NORTE-01-0145-FEDER-000011. S. Soares, L. Grazina and J. Costa are grateful to FCT grants (SFRH/BPD/102404/2014, SFRH/BD/132462/2017 and SFRH/BD/75091/2010) financed by POPH-QREN (subsidized by FSE and MCTES).

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P7.7

MONITORING FOOD AUTHENTICITY USING AN ADVANCED GLYCAN ARRAY PROFILING PLATFORM

Smith, A.^{1,2,*}, Seal, C.¹, Tétard-Jones, C.¹, Iqbal, W.¹, Donarski, J.², Willats, W.¹

1-Newcastle University, Newcastle, United Kingdom

2-Institute for Agri-Food Research and Innovation, York, United Kingdom

*corresponding author e-mail: a.smith29@newcastle.ac.uk

Keywords: authentication, polysaccharide, wholegrain, monoclonal antibodies

Constant advancements enabling the fraudulent manufacturing of food produce have dictated the need for the development of an efficient testing regimen. Public awareness of food fraudulence and the need for robust monitoring notably increased after the 2013 'Horsemeat Scandal'. This, and other episodes, highlighted the need for a structured legislation framework within which authentication technologies can operate. These issues are likely to grow in importance as societies worldwide tackle the food production requirements of a growing population, whilst maintaining quality and nutritional traits.

Products based on cereal grains can potentially provide positive health effects and the benefits of wholegrain products are well documented, however a consensus regarding definitions of wholegrain content is not present. This uncertainty can be exploited by producers, such that a product carrying a 'wholegrain' label, may in fact contain very low levels of the grain components that confer health benefits. A recent recommendation (2017) issued by the Healthgrain Forum suggested that 'wholegrain' products should contain at least 30% wholegrain by dry weight.

Such quantitative descriptions are necessary – but to maintain consumer confidence suitable monitoring technology is required to ensure they are adhered to.

The work being conducted describes a new analytical platform that enables the wholegrain content of diverse foods to be analysed rapidly and in detail. The method combines the high-throughput capability of microarrays with the specificity of monoclonal antibodies, exploiting the fact that some polysaccharides only occur in certain anatomically distinct regions of grains. Once identified, these polysaccharides can be used as markers for the presence of germ, bran, endosperm etc., even when these components are homogenised via milling or product formulation.

This method adapts a technique that has been widely used for long range tracking of polysaccharides in complex natural and industrial systems, as shown recently by Fangel *et al.* in relation to brewing⁽¹⁾. In this project we are working with specialist millers and wholegrain interest groups, to develop this technology for improved wholegrain monitoring.

Reference

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P7.8

DETECTION OF MISLABELLING AND ENDANGERED SPECIES IN "GALEOS" SHARK MEAT FROM GREEK MARKETS USING DNA BARCODING

Imsiridou, A.^{1,*}, Gubili, C.², Pazartzis, T.¹, Siaperopoulou, S.¹, Maradidou, S.¹, Chatzisprou, A.³, Loukovitis, D.¹, Minos, G.¹

1-Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

2-Fisheries Research Institute, Kavala, Greece

3-Institute of Marine Biological Resources and Inland Waters, Athens, Greece

*corresponding author e-mail: imsiri@otenet.gr

Keywords: species identification, fish substitution, sustainability, mitochondrial DNA

Lack of morphological features due to food processing is common in elasmobranchs. Such methods increase the chances of species substitution with cheaper alternatives or endangered species, leaving consumers unprotected from unfair or deceptive practices. DNA barcoding has become the most commonly applied approach for species identification, as it is capable to overcome difficulties and detect fraudulent fish substitution. According to the Greek food and safety legislation (Official Government Gazette 475/27-3-2015, No. 1750/32219), only the *Mustelus* spp. products can be sold under the common name "galeos". In this work, we have evaluated species composition in "galeos" shark fillets from Greek markets, using DNA barcoding.

A total of 87 samples were collected from 33 fishmongers and open markets located in four different cities of Greece (Athens, Thessaloniki, Kavala, and Komotene), between October 2015 and May 2018. Genomic DNA was extracted according to a CTAB-based protocol and commercial kits (UltraClean Tissue & Cells DNA Isolation & Genomic Mini kit). Two different mitochondrial (mtDNA) genes were selected for the analysis in each sample. A universal primer pair was used for the amplification of a 600 bp fragment from the mtDNA 16S rRNA gene, and two universal primer pairs were used for the amplification of a 670 bp segment of the mtDNA COI gene. All produced sequences were checked and compared with those available in GenBank and BOLD databases. A non-parametric analysis of similarity (ANOSIM) utilising the Bray-Curtis distance measure was initially conducted to test if there were significant differences between cities, and a two-way ANOSIM was used to evaluate differences among cities with both retailer types as grouping factors. Moreover, a Principal Component Analysis (PCA) was used to visualise general patterns on the data, with each sampling location representing an individual data point in the ordination.

Overall, 13 species across ten genera were genetically identified, with legal species (*Mustelus* spp.) representing 44.19% of the marketed products (Figure 1). Moreover we were able to detect mislabelling by species belonging to the IUCN red list and species with prohibited landing by international and national legislation (55.81%). Most barcode searches produced clear matches, with confident assignment of $\geq 99\%$ similarity to records reported in both databases. The ANOSIM results indicated a highly significant difference among sampling locations ($R=0.063$, $p=0.006$), with *Scyliorhinus canicula* and *Mustelus* spp. contributing almost equally to the total dissimilarity (25% each). Additionally, differences in the species composition of the two main cities between retailer types were significant ($p=0.028$, two-way ANOSIM). The PCA analysis illustrated the differences between retailer types in Thessaloniki, corroborating the ANOSIM results. The CITES protected species

Alopias vulpinus, illegally landed species belonging to the Hexanchidae family, *Squatina squatina*, *Prionace glauca*, and *Squalus blainville* were mainly found in the open markets of Thessaloniki, followed by Athenian retailers, whereas they were absent from the rest of the sampling locations. Nearly 70% of the variance was covered by the first two principal components.

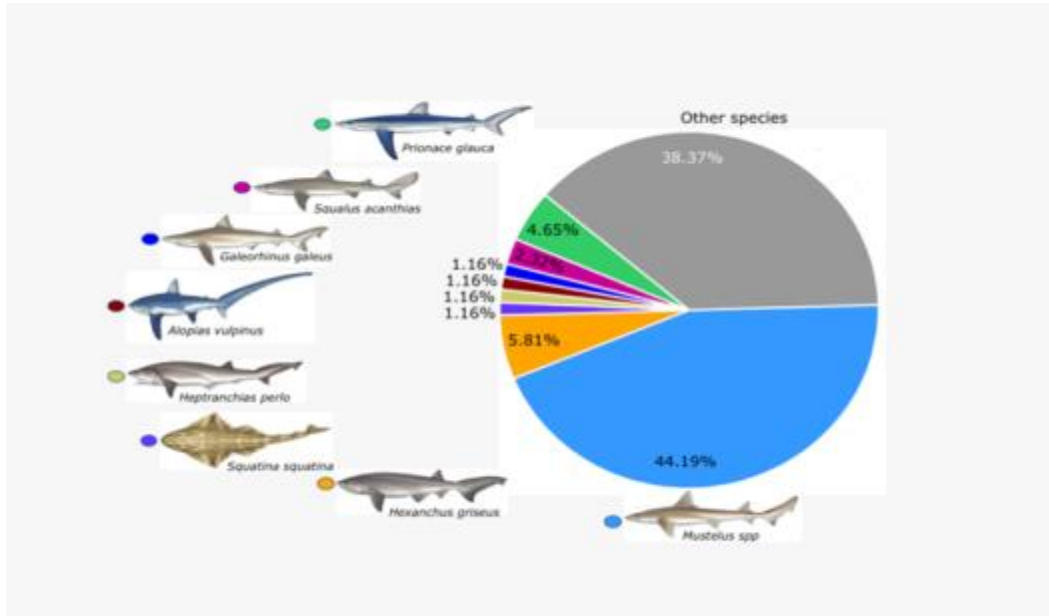


Figure 1: Species composition (i.e. number of individuals) as genetically identified from “galeos” shark meat samples, using DNA barcoding approaches

©Shark species designs from Shark Trust.

P7.9

POPULATION STRUCTURE OF THREE GREEK MARINE SPECIES (*SARDINA PILCHARDUS*, *PENAEUS KERATHURUS*, *MULLUS BARBATUS*) AS A TOOL FOR THEIR FUTURE PGI DEFINITION

Imsiridou, A.^{1,*}, Papapetrou, M.¹, Alexandros, T.¹, Loukovitis, D.¹, Minos, G.¹, Gouva, E.², Chatzopoulos, A.², Skoufos, I.², Paschos, I.²

1-Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

2-Technological Educational Institute of Epirus, Arta, Greece

*corresponding author e-mail: imsiri@otenet.gr

Keywords: *sardina pilchardus*, *penaeus kerathurus*, *mullus barbatus*, PGI products

European Commission has established among others the Protected Geographical Indication (PGI) protection label in order to identify an agricultural product, raw or processed, of which quality, reputation or other characteristics are linked to its geographical origin. One hundred and twenty four Greek products have been recognized as Protected Designation of Origin (PDO)/Protected Geographical Indication (PGI) while there is only one marine product among them, botargo of Messolonghi (PDO-Council Regulations 1107/96 and 1263/96). Sardine (*Sardina pilchardus*, Walbaum 1792) is a small pelagic fish species of great interest to fisheries. In Greece it is one of the two most commercially important fish, with a total catch of 10,890.5 t in 2016. The caramote prawn *Penaeus kerathurus* (Forskäl 1775) is an ecologically and economically important penaeid species. In Greece the total catch for 2016 was 1,404.1 t, representing almost 2% of the total catch of marine species. The red mullet *Mullus barbatus* is one of the most commercial fish resources in the Mediterranean. In Greece it ranks among the most important demersal fish, with a total catch of 1, 758, 7 t for 2016 (2.4% of the total catch of marine species) (Hellenic Statistical Authority ELSTAT, 2016). The aim of this research was to assess the potential of using the population differentiation of the three marine species (*S. pilchardus*, *P. kerathurus*, *M. barbatus*) as a tool for their future PGI definition, applying the PCR and further Sanger sequencing analysis.

Specimens of the three species were collected by professional fishermen from different localities: *S. pilchardus* from Amvrakikos Gulf, Ionian Sea, Kalloni Bay (Aegean Sea), during July 2017; *P. kerathurus* from Amvrakikos Gulf, Ionian Sea, Thracian Sea (Aegean Sea), from September 2016 till May 2017; *M. barbatus* from Amvrakikos Gulf, Ionian Sea, Kalloni Bay (Aegean Sea) (from July 2016 till May 2017); twenty individuals per population per species. Total genomic DNA was extracted from muscle, with the UltraClean™ Tissue and Cells DNA Isolation Kit (MoBio) and with the CTAB methodology. Three mitochondrial segments were screened with the PCR analysis: cytochrome oxidase subunit I (COI), cytochrome b (cytb) and the control region (D-loop). Sequencing was carried out in both directions on a ABI 3500 Genetic Analyzer. Different haplotypes for each mitochondrial segment were detected in all three populations of each species with the DAMBE6 software package, and haplotype frequencies were estimated for each population. Genetic distances among the three populations per species were estimated based on both the nucleotide sequences of each segment and the total nucleotide sequences (COI, cytb, D-loop), using the 2-parameter Kimura distance model. Phylogenetic relationships among them were estimated with the MEGA7 software using three different algorithms: Neighbour Joining (NJ), UPGMA and Minimum Evolution (ME), considering both the nucleotide sequences of each

segment and the complete nucleotide dataset. For population differentiation estimates the total nucleotide sequences (COI, cytb, D-loop) for each individual were used and they were conducted using ARLEQUIN 3.5.2.2. The significances of both the F statistics and variance components were assessed with 1,000 permutations. The correlation between geographic distances separating sampling sites and genetic differentiation (F_{st}) was performed using Mantel's test.

For *S. pilchardus*: Thirty three haplotypes were detected for COI gene, twenty five for cytb gene and fifty three for the D-loop region. The NJ, UPGMA and ME dendrograms revealed the same structure: populations from Amvrakikos Gulf and Kalloni Bay were grouped together, whereas the population from Ionian Sea formed a separate clade. The total F_{st} value was $F_{st}=0.01232$. The highest F_{st} value (0.03119) was revealed between the populations from Amvrakikos Gulf and Ionian Sea and it was also significant ($P<0.05$), while there was no significant differentiation among the other pairs of populations.

For *P. kerathurus*: Thirty haplotypes were revealed for COI gene, forty one haplotypes for cytb gene and fifty one haplotypes for the D-loop region. The NJ, UPGMA and ME phylogenetic trees had identical topology: the first cluster included populations from Amvrakikos Gulf and Ionian Sea and the second one was formed from the Thracian Sea population. The total F_{st} value was $F_{st}=0.12096$. The highest significant F_{st} value was detected between the populations from Amvrakikos Gulf and Thracian Sea ($F_{st}=0.16438$) and statistically significant genetic differentiation among all the other pairs of populations ($P=0.0000$) was revealed.

For *M. barbatus*: Thirty two haplotypes were detected for COI gene and nineteen haplotypes were revealed for cytb gene. The NJ, UPGMA and ME phylogenetic trees had similar structure: they could not discriminate the *M. barbatus* populations as genetic distance had the same value between Amvrakikos Gulf-Ionian Sea and Amvrakikos Gulf-Kalloni Bay population pairs. The total F_{st} value was $F_{st}=0.03146$. The population from Ionian Sea was significantly differentiated from the Kalloni Bay population ($P<0.05$), while there was no significant differentiation among the other pairs of populations. A moderate but non-significant isolation by distance pattern ($r= 0.712366$, $P=0.365$) was detected for *P. kerathurus* populations according to the Mantel's test, whereas no pattern of genetic differentiation according to geographic distances was detected for *S. pilchardus* and *M. barbatus* ($r = -0.991484$, $P=1.000$ and $r = -0.497857$, $P=0.639000$ respectively).

P. kerathurus revealed to be the most structured species and all the populations were significantly differentiated, with the Thracian Sea population proposed as a PGI one. For *S. pilchardus* and *M. barbatus* a much lower differentiation was estimated among the populations, with the ones from Ionian Sea revealing a weak but significant genetic heterogeneity for both species. These results, if combined with further mtDNA or microsatellite analyses from more locations in the Ionian basin, could shed light on a possible definition of these stocks as PGI products.

P7.10

BIOACTIVE PEPTIDES IN FOOD OF PLANT AND ANIMAL ORIGIN

Maestri, E.^{1,*}, Pavlicevic, M.², Montorsi, M.^{3,4,5}, Imperiale, D.¹, Marmiroli, M.¹, Marmiroli, N.^{1,4}

1-SITEIA, PARMA, Interdepartmental Centre for Food Safety, Technologies and Innovation for Agri-food and Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy

2-Institute for Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Serbia

3-Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, Milano, Italy

4-Conorzio Italtotec, Milano, Italy

5-Institute of Bioimaging and Molecular Physiology, National Council of Research (CNR) Segrate, Italy

*corresponding author e-mail: elena.maestri@unipr.it

Keywords: bioactive peptides, food processing, functional food

Functional food by definition contains an added ingredient with confirmed health effects; however certain functional foods contain naturally occurring constituents with known bioactive effects. Bioactive peptides are short amino acid sequences, usually 2-20 residues that are released from a source protein either during technological processing of the food or during in vivo digestion. The presence of bioactive molecules provides a partial explanation for the health benefits of some fermented foods, which have been developed in diverse human cultures. Their beneficial effects on immunity, inflammation, infection, hypertension, hypercholesterolemia, diabetes, some cancers and various neurological problems are of great interest, since these diseases are becoming ever more prominent. In our work we have studied plant-derived bioactive peptides associated with a beneficial effect on human health, particularly in modulation of the immune system. Inspection of crop proteomic data revealed that at least 6000 proteins potentially present in food may harbour immunomodulatory bioactive peptides.

The analysis of these proteins using a Gene Ontology approach has provided a number of insights regarding their occurrence and relevance. In parallel, amino acid sequences of 807 bioactive peptides from food of animal origin were examined in order to correlate peptide structure with activity (antihypertensive, antioxidative, immunomodulatory, antimicrobial, hypolipidemic, antithrombotic and opioid) and stability in vivo. Food sources such as milk, meat, eggs and marine products show different frequencies of bioactive peptides exhibiting specific effects. Opioid peptides contain a high percentage of aromatic amino acid residues, while antimicrobial peptides show an excess of positively charged amino acids. Peptides which have activity in vivo contain a high percentage (67 %) of proline residues. We also discuss the influence of processing on activity of these peptides, as well as methods for predicting release from the source protein and activity of peptides. Peptides in food can be an element for studying authenticity and functionality of special foods, often related with tradition and culture.

Acknowledgements

This study was supported by the EU FP7 project, Grant Agreement No.316004 (REGPOT-AREA). Authors EM, MM and NM acknowledge the contribution of the project FOODINTEGRITY, which has received funding from the EU's Seventh Framework Programme for research, technological development, and demonstration under grant agreement No. 613688

P7.11

REVERSE COOKING: SPECIES IDENTIFICATION IN COMPLEX FOOD MATRICES BY MTDNA METABARCODING

Cravero, D.^{1,*}, Cerutti, F.¹, Beltramo, C.¹, Riina, M.V.¹, Acutis, P.L.¹, Peletto, S.¹

1-Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

*corresponding author e-mail: diego.cravero@izsto.it

Keywords: mtDNA metabarcoding, NGS, species identification, complex food matrices

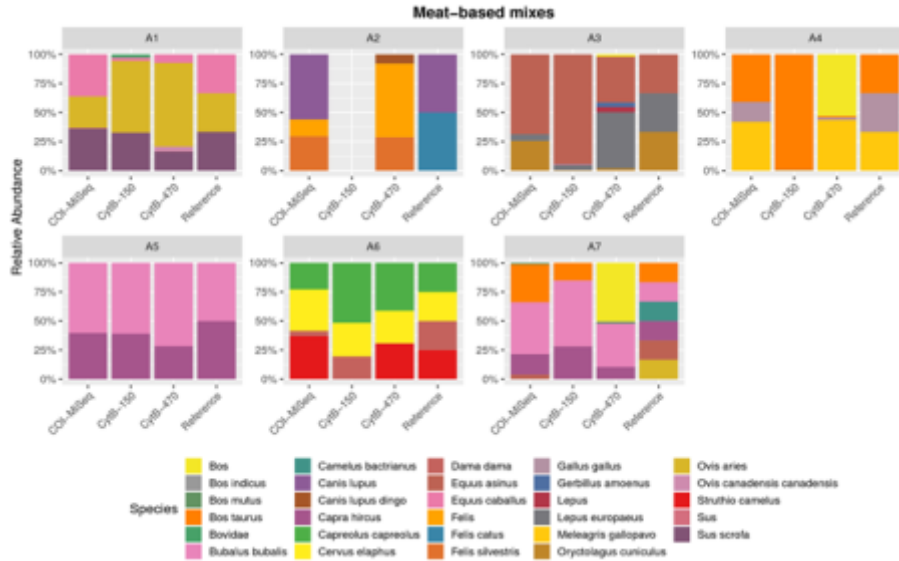
The identification of the species of origin in food products is a major concern to identify frauds that might have economic, environmental, ethical and health implications. To date, the most common methods for species identification are PCR-based (end-point PCR and Sanger sequencing, real-time PCR). The limit of these approaches is that only one or relatively few species can be identified. Next Generation Sequencing (NGS) can overcome this limit, since it merges untargeted DNA-based species identification and high-throughput DNA sequencing. Nevertheless, also the target gene region may be an issue, depending on length and nucleotide variability.

Here we tested five primer sets, reported in the Table below, to amplify different mitochondrial DNA (mtDNA) regions of known and unknown food samples. Meat-based food were 7 DNA mixes composed by equimolar amounts of known species prepared in the laboratory, while fish-based food were 6 commercial baby food of different species, 1 cod stick and 1 salmon dry dog food.

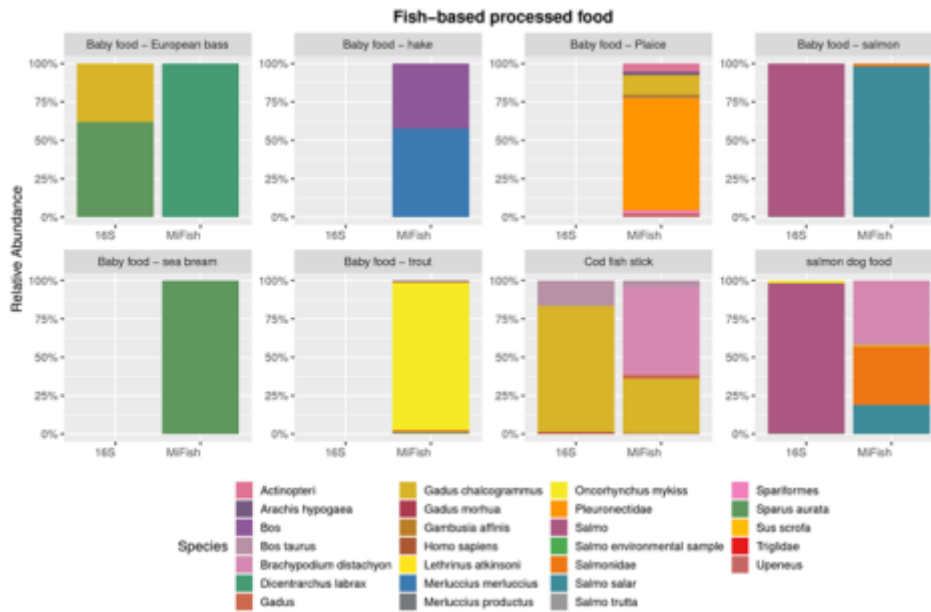
Primer pair	Forward primer	Reverse primer	Amplicon size	Target gene	Application	Reference
CytB-150	L15601	H15748	148 bp	Cytochrome B	Meat	Lopez-Oceja, 2016
MiFish	MiFish-U-F	MiFish-U-R	163–185 bp	12S rRNA gene	Fish	Miya, 2015
16S	16sf-var	16sr-var	250–260 bp	16S rRNA gene	Fish	Chapela, 2002
COI-MiSeq	mICOLintF	jgHCO2198	313	COI	Meat	Leray, 2013 Geller, 2013
CytB-470	mcb 398_F	mcb 869_R	472 bp	Cytochrome B	Meat	Verma & Singh, 2002

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 paired-end workflow (2x150 or 2x250, according to the amplicon size). The pipeline included a first step consisting in primer and quality trimming and mate pairing, then dereplication, chimera detection and removal, clustering at 98%, removal of minor clusters (<0.2%). Finally, the reads were compared to the GenBank nt database by local blastn search.

CytB-150 primers showed low affinity to birds, failing to identify both chicken and oyster; they also were less precise for the species definition of *Bos taurus*, probably due to the short size of the fragment and misclassification of sequences in the nt database.



COI-MiSeq and CytB-470 performed better, although results could be interpreted only qualitatively and not quantitatively. Less precise results were obtained when 6 species were mixed (sample A7).



For fish-based food, MiFish primers successfully amplified the target region for all samples, while 16S failed in four baby food. In all samples, the declared species was confirmed by the metabarcoding, although also other related species were detected at a lower abundance. This may be due to the small size of the amplicon, whose sequence is not variable enough to discriminate among taxonomically close species in a such processed food. Nevertheless, the use of longer fragments might be critical due to the high DNA fragmentation during the production process.

The outcome of the mtDNA metabarcoding on food matrices provided promising results by correctly identifying the major species.

This work has been funded by the Ministry of Health (grant Ricerca Finalizzata RF-2013-02359002).

P7.12

FOOD AUTHENTICITY TESTING WITH NEXT-GENERATION SEQUENCING

Prentice, N.^{1,*}, Karla, T.²

1-Thermo Fisher Scientific, Basingstoke, UK

2-Thermo Fisher Scientific, Vantaa, Finland

*corresponding author e-mail: nicole.prentice@thermofisher.com

Keywords: NGS, ion, authenticity, species

A complete process was developed to study the species authenticity of food products. The Thermo Scientific™ NGS Food Authenticity Workflow relies on next-generation sequencing technology to identify meat, fish and plant species from foods and feeds. With semi-automated workflow and extensive database thousands of species can be identified and more than a hundred samples can be simultaneously analyzed.

The advantage of the next-generation sequencing method is the unmatched capacity to identify species without the need to specifically target only a limited set of species. The method combines DNA extraction, library preparation and Thermo Fisher Scientific™ Ion Torrent™ technology for a streamlined workflow producing quality results. The workflow consists of sample and library preparation steps followed by automated templating, sequencing and result analysis processes. The utilization of Ion Torrent™ technology, Ion Chef™ Instrument and Ion GeneStudio™ S5 sequencer enables rapid sequencing with less than 30 minutes hands-on time during the templating and sequencing. The results are analyzed with SGS AllSpecies ID software which maps the sequencing reads for each sample against a regularly updated database producing comprehensive high-quality results in a user-friendly format.

Foods from different categories were tested to challenge the method including heavily processed foods, fresh and frozen foods, ready-to-eat meals, liquid foods and dried food products. After the DNA extraction, the sample libraries for sequencing were prepared with SGS AllSpecies Kits for meat, fish and plant, to cover all the components in a food product. Following the library prep samples were prepared for sequencing on Ion Chef Instrument and then sequenced on the S5 instrument. Results analysis and reporting was automated using the AllSpecies ID software.

P7.13

NGS UN-TARGETED APPROACH FOR HERBS AND SPECIES IDENTIFICATION

Pellesi, R.^{1,*}, Zanardi, S.¹, Mourinha Chaves, S.², Nogueira, S.², Gadanho, M.², Leporati, A.¹, Suman, M.¹

1-Advanced Laboratory Research, Barilla G.& R. F.lli SpA, via Mantova 166, 43122, Parma, Italy

2-SGS Molecular, Agrocolture Food and Life, Grupo SGS Portugal, Lisboa, Portugal

*corresponding author e-mail: roberta.pellesi@barilla.com

Keywords: Next Generation Sequencing, herb and spices, species identification, food frauds, Non-targeted NGS approach

In 2013, the EU produced just 137 thousand tons of herbs and spices, however this amount is only 2% of the world's herbs and spices yield, 81% were in Asia, 12% in Africa, and 3.7% in Latin America and Caribbean (CBI, 2015a). Spices are used in foods in the form of whole spices, ground spices and spice extracts; they can be added, also, to prevent lipid degradation and food rancidity, as source of natural food colors and for their nutritional properties [1]. For their mainly use in food productions, their high price and long supply chains, herbs and spices are in high rank of food frauds risk and the cost of one incident to a company can be between 2% and 15% of annual revenue [2].

Nowadays companies invest money in new technologies able to detect adulterations in raw materials and finish products in a fast and cheap way. In this scenario, next to common analytical methods like spectroscopy (FT-NIR, NMR, HSI) and mass spectrometry (GC-MS, LC-MS, IR-MS, ICP-MS, LC-MS/MS, LC-ESI-MS/MS,...), DNA analysis began to be used in the fight against food frauds because of their high specificity, sensibility, reproducibility and cost effective.

This work presents the possibility to identify herbs and spices species in a non-targeted way using NGS (Next Generation Sequencing) technology. NGS is able to sequence, in a single chip, still 200 different samples in a deep way, giving robustness to data. Mitochondrial DNA target regions usually used for discriminating each species in a target way, in this case are used as un-target thanks to their ancestrally. Data present in this work are proofs of a new strategy, which combine NGS platform, add to mitochondrial ancestral regions and to SGS Molecular database, creating a robust new DNA un-targeted approach. This could help food companies to detect food frauds and to monitor the supply chain in a rapid and cost-effective way.

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P7.14

DEVELOPMENT OF A NEXT GENERATION SEQUENCING (NGS) WORKFLOW FOR FOOD DNA ANALYSIS: HOW TO IDENTIFY MEAT AND FISH SPECIES IN COMPLEX FOOD PRODUCTS

Nogueira, S.¹, Manolis, A.², Gadanho, M.¹, Chaves, S.¹

1-SGS Molecular, Lisbon, Portugal

2-Thermo Fisher Scientific, Austin, USA

Next Generation sequencing (NGS) has been introduced in recent years as a very powerful method for species identification in food products. However, the use of NGS requires the development of the correct workflow to ensure the reliability of the results and to maximize the advantages of this high throughput DNA-based method. Taking advantage of the non-targeted and massive sequencing output obtained by NGS, a workflow was developed and tested to identify meat and fish species in food products. The workflow was defined and optimized to meet the following criteria: (i) barcoding of several specific DNA regions suitable for species identification (multi-barcoding); (ii) definition of consensus primer panels producing very small amplicons (multiplex) to ensure the use in highly processed food where DNA can be highly damaged; (iii) optimization of the Ion Torrent™ technology (Ion Chef™ System and Ion GeneStudio™ S5 System, Thermo Fisher Scientific); (iv) development of a software for automatic data analysis containing suitable databases with thousands of meat and fish species for species identification.

The workflow defined was tested on a group of 80 complex samples of fish and meat including 20 artificial DNA mixtures and 60 real food samples. The real food samples were selected to include different processing treatments, namely dry, canned, fresh, frozen and liquid.

The NGS workflow was effective at correctly identifying the species present in all the food samples regardless of their processing treatments. Furthermore, the workflow was successfully used simultaneously for meat- and fish-based products analysis in a single NGS run. The whole workflow from DNA extraction to species identification takes under 24 hours and requires limited handling due to the automation of the workflow using the Ion Chef and Ion GeneStudio S5 Systems together with the automatic software-based analysis for species. The rapid time to results and simple workflow make this the first credible solution for in-house screening of samples for species identification in routine food analysis laboratories. SGS are partnering with Thermo Fisher Scientific who will commercialize the solution later this year

P7.15

FASTFISH-ID: A MULTI-CENTRE EVALUATION OF A NOVEL METHOD FOR NON-TARGETED SEAFOOD IDENTIFICATION

Naaum, A.M.^{1,a,*}, Cusa, M.^{2,a}, Elliott, C.¹, Goodhead, I.², Helyar, S.¹, Mariani, S.², Sanchez, A.³

1-Institute for Global Food Security, Queen's University Belfast, Belfast, UK

2-School of Environment and Life Sciences, University of Salford, Salford, UK

3-Thermagenix, Inc., Natick, USA

a-these authors have contributed equally to this work

*corresponding author e-mail: naauma@gmail.com

Keywords: DNA barcoding, fish, species identification, mobile testing

Mislabelled fish products hiding lesser-value or lower-quality species risk the economic welfare of food companies and can damage brands and consumer trust. DNA analysis readily authenticates species in fish products, but the high costs and time required in sending specimens out for sequencing is a barrier to the large-scale testing needed to protect seafood industries. FASTFISH-ID offers a portable, turnkey solution for rapid and cost-effective on-site authentication of commercial fish species in a single-tube, which can be carried out in ~3 hours by anyone, anywhere along the supply chain. A key advantage to FASTFISH-ID is the single set of reagents needed for the authentication of many species in contrast to either requiring multiple species-specific tests to be run in parallel, or the time delay associated with sequencing.

The sequence of a segment of the mitochondrial COI gene is widely recognised as a universal molecular marker (DNA barcode) for standardized DNA identification of animal species. FASTFISH-ID enables convenient COI-based species DNA identification in a simple, one-step protocol without DNA sequencing. The test amplifies the complete COI DNA barcode region and converts this sequence into highly-accurate species-specific fluorescent signatures. Fluorescent signatures are automatically compared to a cloud-based reference database for immediate species identification. The test is performed in a portable MIC PCR Cycler for on-site applications. The ongoing FASTFISH-ID multi-centre study compares the performance of the FASTFISH-ID test for identification using a blinded test of commercial fish samples at four independent laboratories in Europe and North America. Here we present results from two study participants: Queen's University Belfast and the University of Salford. The results demonstrate the test's ease of use and utility in the identification of 75 blind samples from 18 commercially important fish species.

P7.16

GENETIC TOOLS FOR IDENTIFICATION OF FLAXES, CHIA, AND SESAME INGREDIENTS IN SEEDS AND PROCESSED FOODS

Bruno, C.¹, Posik, D.M.¹, Zappa, M.E.¹, Wunderlin, D.¹, Giovambattista, G.¹, Peral García, P¹

1-Instituto de Genética Veterinaria (IGEVET, CONICET-UNLP)

There is an increasing demand among consumers to know about the origin of their food and the accuracy of food labeling, which influences purchasing decisions. Thus, flax, chia, and sesame seeds, as well as processed food (e.g., cookies and cereals bars) containing these species became popular foods. For this reason, it is necessary to develop genetic tools to certificate the presence of these species. Taking this into account, the main objective when we started this work was developing and comparing different genetic tools that could be used to determine the origin and the authenticity of flax, chia, and sesame products, based on the analysis of two regions from MaturaseK (matK) and Ribulosebiphosphate carboxylase large chain (rbcL) genes through PCR-SBT, RT-PCR-HRM, and PCR-specie specific methods.

First, different DNA extraction methods were tested for different matrix (seeds, commercial cookies, cereals bars and experimental homemade cookies), which showed that the combination between a mechanic lysis of Seed and bakery samples using the FastPrep-24 (MP Biomedicals) and the DNA purification from the grinding material using the FastDNA™ Spin Kit for Plant and Animal Tissue were the most appropriate methods to obtain enough DNA quantity and/or quality to allow genotyping.

PCR-SBT analysis showed that matK and rbcL genes exhibited a high percentage of identity with its own specie but a low value of diversity in this region among them. However, the observed diversity was enough to further develop RT-PCR-HRM and PCR-specie specific assays in the future. The analysis of 100 bp and 71 bp rbcL fragments of Flax, Chia, Sesame, and Wheat by RTPCR-HRM allowed us to discriminate these species according to melting temperatures. Finally, the implementation of these tools based on the characteristics of each product and the evaluation of the cost and benefit was discussed.

P8.1

WHAT ABOUT THE PROGRESS OF NON-TARGETED SCREENING MASS SPECTROMETRY AND CHEMOMETRICS IN VERIFYING THE GEOGRAPHIC ORIGIN OF TOMATOES?

de Dominicis, E.^{1*}, Gritti, E.¹, Piva, M.¹, Menegon, V.¹, Saner, S.¹, Dameno, F.²

1-Mérieux NutriSciences R&D, Resana (TV), Italy

2-Mutti SpA, Parma, Italy

*corresponding author e-mail: emiliano.de.dominicis@mxns.com

Keywords: tomatoes, authenticity, mass spectrometry, chemometrics

Can food companies distinguish themselves in the global market, by analyzing the finished product and being able to identify the geographical origin of the ingredients involved? Mérieux NutriSciences and Mutti, after the completion of a previous work on Italian vs. Chinese tomato origin (presented in 2017 Food Integrity Conference), have now worked together analytically discriminate tomato products coming from different regions in Italy. According to that, 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.

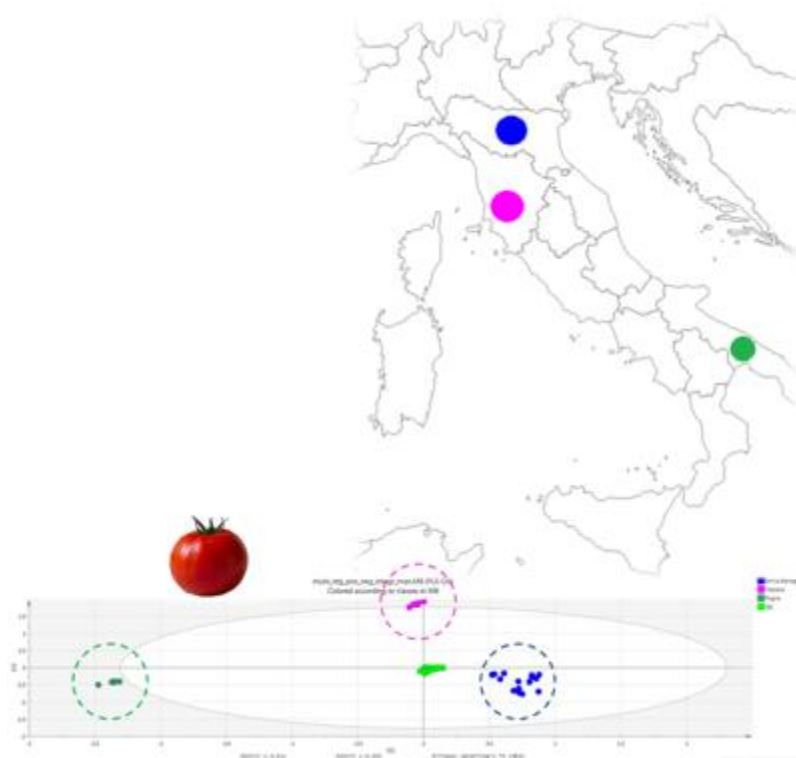
The experimental design is characterized by:

- Purpose: clear geographical origin distinction capacity of tomatoes used for the manufacture of tomato products (Emilia Romagna vs. Toscana vs. Puglia regions);
- Classification Method based on:
- LC/HRMS Non Targeted Screening;
- Chemometrics (SIMCA PCA-CLASS and PLS-DA) checked and verified through pre-defined acceptance criteria;
- Sampling for Reference Database:
- excellent traceability information
- many variables considered: seasonality, production process, storage etc.

Figure 1: LC/HRMS 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.

All authors thank Mutti SpA and Mérieux NutriSciences companies for the financing of this research, certainly important for the dissemination of scientific knowledge designed to verify the full transparency of final products in terms of Authenticity and Food Integrity.

Figure 1: LC/HRMS Non-Targeted Screening Discrimination



P8.2 THE FOOD AUTHENTICITY NETWORK: THE ONE-STOP-SHOP THAT CAN HELP PROTECT THE INTEGRITY OF YOUR FOOD

Elahi, S.^{1,*}, Ellison, S.¹, Woolfe, M.²

1-LGC Limited, Teddington, UK

2-Food Authenticity Consultant to LGC Limited, Thames Ditton, UK

*corresponding author e-mail: Selvarani.Elahi@lqcgrou.com

Keywords: Food authenticity, fraud, mitigation

The Food Authenticity Network (www.foodauthenticity.uk/) is a free open access 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 government funded initiative that was born out of the 2013 horsemeat issue and brings together all those with an interest 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.

Membership is free and it's very quick to join so if you're not a member then please visit www.foodauthenticity.uk and sign-up today for the latest information on food authenticity. The Network is now 3 years old and has nearly 900 people from 41 countries registered as members of the website and >1,260 followers of the Twitter account (www.twitter.com/fauthenticity). The Network also has a Google page rank score of number 1 for a search on the term 'food authenticity'. In addition to the focus on food authenticity testing best practice, links to the major global food fraud mitigation guides have been placed on the Network so that supply chain integrity (mitigation) and testing is covered. The Network is a one-stop-shop for all things related to food authenticity testing and food fraud mitigation as shown by Figure 1.



Figure 1: Key Features of the Food Authenticity Network

Our vision for the Network is to grow it internationally so that it becomes a truly global network aimed at fighting food fraud in today's global food supply chain. To aid this, the Network will transition to being industry led from January 2019 at which point it will cease to be solely UK government funded. This talk will highlight the key features of the Network and outline our plans to create a global self-sustaining network aimed at fighting food fraud in a unified and cost efficient manner that can also help capacity building in countries that cannot afford to have dedicated food authenticity programmes of their own.

Acknowledgments

Department of Environment, Food and Rural Affairs
Food Standards Agency
Food Standards Scotland
Department of Business, Energy and Industrial Strategy.

P13.1

NMR-BASED MULTIPARAMETRIC CHARACTERISATION OF FOODSTUFFS: THE PROS WITHOUT THE CONS OF TARGETED ANALYSES APPLIED TO FOOD INTEGRITY

Recht, R.¹, Fougy, L.¹, Stahl, V.¹, Hamon, E.¹

1-Aerial, Illkirch, France.

*corresponding author e-mail: e.hamon@aerial-crt.com

Keywords: multiparametric analysis, NMR, food characterisation.

Establishing food integrity encompasses safety aspects, in particular in terms of pathogen contamination. With this regard, scientists and quality managers resort to predictive models to anticipate the risk of a pathogen development throughout the product shelf life. These models rely on key intrinsic physico-chemical parameters of the food matrix: pH, water activity (A_w), organic acid concentration and total phenol content. So far, all these product features have been determined using one destructive analysis per parameter. Such approach prevents an overall view of these characteristics on a single sample and undermines the prediction quality of the models.

Nuclear Magnetic Resonance (NMR) is a versatile non-destructive spectroscopic technique that can simultaneously measure the physicochemical parameters of interest without extensive preparation. We designed an NMR approach using a single 10-mg sample and validated it on four food matrices: smear soft cheese, cooked peeled shrimps, smoked salmon and smoked ham. This proof of concept and application allows investigating key specific quantitative features without the usual drawbacks of targeted analyses. It also opens the door to (i) the systematic spatial characterization of foodstuffs at microlocal scale and (ii) the improvement and fine-tuning of predictive microbiology models, without curtailing the possibility to perform untargeted metabolomics.

P13.2 NEW CHEMICAL MARKERS FOR THE ASSESSMENT OF EGG PRODUCTS FRESHNESS

Cavanna, D.^{*1,2}, Catellani, D.¹, Dall'Asta, C.², Suman, M.¹

1-Advanced Laboratory Research, Barilla G.& R. F.lli SpA, via Mantova 166, 43122, Parma, Italy

2- Department of Food and Drug, University of Parma, Parco Area delle Scienze 95/A, 43124 Parma, Italy

*corresponding author e-mail: daniele.cavanna@barilla.com

Keywords: egg products, freshness, non-targeted mass spectrometry

Eggs, mostly in the eggproducts 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. Since now, scientific literature was mostly focused on the development of rapid techniques able to assess this parameter with the evaluation of global fingerprints, for example using electronic noses [1] [2] or with spectroscopic approaches [3]. On the contrary, generic “non-targeted” methods based on a metabolomic approach, that are an emerging instrument to detect different food frauds [4], are not largely used on this field. The identification of new markers could represent an important tool to highlight fraudulent eggproducts freshness declaration, with a reduction of the risk for the final consumer.

In this study, new compounds responsible of freshness and of not freshness of egg products are identified with an UHPLC-HRMS metabolomic approach, using an Orbitrap Q-Exactive instrument (Thermo Scientific) and different data processing software. Samples were collected directly from the production plant, extracted immediately after the receipt, left at room temperature and extracted again after 1 day and 2 days. A total amount of 79 samples was used for the model creation. The same molecules were detected in a group of new egg products batches subjected to the same experimental design but not used for model creation; this certifies that these compounds can be considered reliable freshness markers, regardless of the chemometric model used to identify them. In addition, appearance, disappearance or strong intensity variations of these markers was evaluated also in egg product batches stored at 2-8 °C [5].

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P13.3 NON TARGETED SCREENING FOR FOOD INTEGRITY PROJECTS: DEVELOPING A RAPID WORKFLOW FOR AN EFFICIENT COMBINATION OF ANALYTICAL TECHNIQUES AND CHEMOMETRICS

de Dominicis, E.^{1,*}, Gritti, E.¹, Piva, M.¹, Meli, V.¹, Saner, S.¹, Garino, C.², Locatelli, M.², Arlorio, M.², Leonardi, G.³, Portinale, L.³, Monaci, L.⁴, Lippolis, V.⁴, Gallo, V.⁵, Mafra, I.⁶, Amaral, J.S.⁶, Gottardi, F.⁷, Scaramagli, S.⁷, Faenza, C.⁷, Hollosi, L.⁸, Godula, M.⁸.

1-Mérieux NutriSciences Research, Resana (TV), Italy

2-Università del Piemonte Orientale, Novara, Italy

3-Università del Piemonte Orientale, Alessandria, Italy

4-ISPA - Bari, Italy

5-Innovative Solutions, Italy

6-ICETA, Portugal

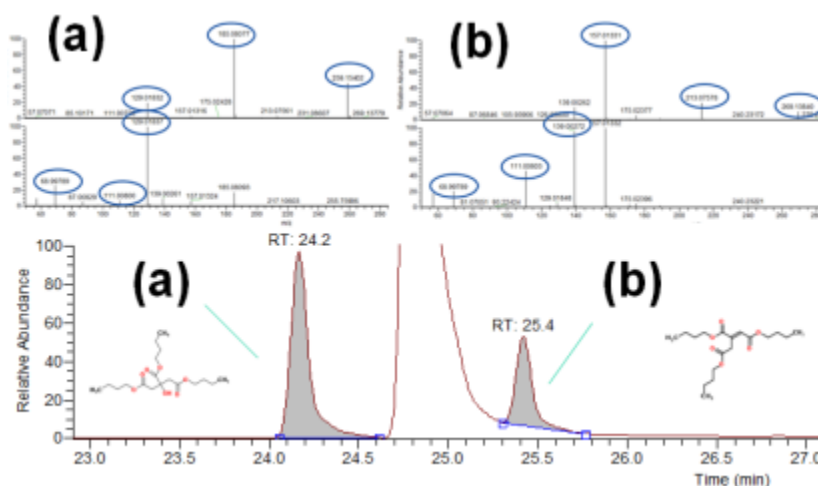
7-Coop Italia, Italy

8-Thermo Fisher Scientific, Germany

*corresponding author e-mail: emiliano.de.dominicis@mxns.com

Keywords: food integrity, non-targeted screening, projects, chemometrics, mathematical classifiers

To date it is well known that there is not a unique best analytical technique that alone is capable of answering the question whether a food is authentic or not, nor there is the best mathematical classifier to correctly interpret the results assigning one sample to one or another population.



A holistic and multidisciplinary approach benefitting from the knowledge and the skills/expertise acquired by different researches is therefore necessary to tackle this task.

In this regard, it is fundamental to know in details the product, the different variables involved, the raw materials and the production processes. It is important to have an in thorough knowledge of the storage conditions of the food along the process chain, and to

combine all the available information into an experimental design that should consider all the possible sources of variation.

The collection of all these preliminary information represents the starting point to develop tailor-made projects, specifically designed on the objective of the study.

In this regard, we need:

- representative sampling, with respect to the objective to be achieved;
- robust analytical results;
- appropriate data interpretation (method, theoretical classification and real classification) based on mathematical classifiers able to distinguish / classify according to the pre-established purpose;
- validation protocols.

All these issues will be schematized and concisely presented in the present communication, aiming at establishing a universal protocol to be intended as guideline by food industries, suppliers, control laboratories, etc.. to help developing non targeted based methodologies.

All authors thank Food Integrity Project, Università del Piemonte Orientale, CNR-ISPA, Innovative Solutions, ICETA, Coop Italia, Thermo Fisher Scientific and Mérieux NutriSciences for the opportunity to participate in this Research, of utmost importance for the dissemination of analytical services designed to verify the full transparency of final food products in terms of Authenticity and Integrity.

P13.4

ENHANCING THE DETECTION OF OLIVE OIL ILLEGAL BLENDS: SHORT LIQUID CHROMATOGRAPHY (LC) AND HRMS FRAGMENTATION FOR TAG FINGERPRINTING

Tres, A.^{1*}, Quintanilla-Casas, B.¹, López, J.¹, Bustamante, J.¹, Simón, M.², Guardiola, F.¹, Barrón, D.¹, Vichi, S.¹

1-Universitat de Barcelona, Barcelona, Spain

2-Federació de Cooperatives Agràries de Catalunya, Barcelona, Spain

*corresponding author e-mail: atres@ub.edu

Keywords: olive oil, illegal blending, High Resolution Mass Spectrometry, fingerprinting

Triacylglycerol (TAG) composition is a useful tool for detecting illegal blends of olive oil (OO) with oils of different botanical origin. Nevertheless, the current official analytical tools are time-consuming and not always able to reveal blends between OO and oils with similar fatty acid composition. Disposing of fast and high throughput methods for an efficient wide-ranging screening analysis would be supportive in the detection of OO illegal blends, contributing to avoid fraud within the sector, increasing its competitiveness and reducing the chances of food safety crisis.

Recently, the analysis of TAG profile by shotgun high-resolution mass spectrometry (HRMS) has been proposed as a promising tool to detect illegal blending of OO with low percentages of other vegetable oils, suitable for the screening of a large number of samples. This method allows obtaining, in the same analysis and in short times (<2 min), the exact masses of many components, including minor TAGs not detected by conventional techniques. However, despite the possibility to detect a high number of TAG species with different elemental formula, the direct injection of the sample preclude the possibility to distinguish TAG isomers, thus missing part of the information.

Therefore, the aim was to enhance the previous authentication models by enriching the HRMS TAG fingerprint with data from TAG isomers keeping the analysis time as short as possible. The introduction of a short liquid chromatography (LC) separation step before HRMS analysis and the treatment of data under an untargeted approach were assayed as a combined strategy. Moreover, to further increase the amount of information obtained by the analysis, an all ion fragmentation (AIF) experiment was added to the full scan mode in HRMS. Analysis time could be relatively short (<15 min) whereas a partial resolution of TAG isomers could be achieved.

A sample set composed by 30 genuine VOOs and their blends at 2 and 5% with 10 soybean oils and 10 sunflower oils (n=90) was analyzed by both analytical approaches (shotgun HRMS TAG and LC-AIF-HRMS TAG). Once TAG profile was obtained and the ion chromatograms of all possible TAG fragments were extracted and aligned, models were developed under a fingerprinting approach following the state-of-art in food authentication which relies on finding analytical patterns for the authentic products. This unique pattern is used as a fingerprint of the authentic product to distinguish it from the non-authentic ones. As an advantage, the fingerprinting approach does not require a full resolution of chromatographic peaks which in this case enabled to keep analysis time < 15 min.

For that, two Partial Least Square-Discriminant Analysis (PLS-DA) authentication models were developed and compared: one using shotgun HRMS TAG profile and one using LC-AIF-HRMS TAG fingerprint. PLS-DA prediction results by leave-10%-out cross validation were satisfactory for both models: all genuine oils were unequivocally distinguished from OO blends containing ≥ 2 % of soybean or sunflower oils. However, the model based on the LC-AIF-HRMS TAG fingerprint showed a higher predictive capacity than the shotgun HRMS TAG profile, according to the Partial Least Square regression performed.

These results indicate that taking advantage of the fingerprinting approach, the introduction of a short LC separation before HRMS analysis and an AIF step increase the information obtained by the analysis, enhance the discrimination capacity of the authentication model without increasing excessively the analysis time.

Acknowledgments

This project has been funded by ACCIÓ-Generalitat de Catalunya and the EU through the Programa Operatiu FEDER Catalunya 2014-2020 in the framework of the project Autenfood (Ref COMRDI-15-1-0035). This study has also been supported by the Spanish MINECO through JCI post-doctoral program (JCI-2012_13412) and FPU pre-doctoral program (FPU16/01744) from Spanish MECD.

P13.5 TARGET AND NON-TARGET SESQUITERPENE ANALYSIS TO AUTHENTICATE VIRGIN OLIVE OILS FROM CATALAN PDOS

Quintanilla-Casas, B.^{1,*}, Tres, A.¹, Bustamante, J.¹, Guardiola, F.¹, Simón, M.², Vichi, S.¹

1-Universitat de Barcelona, Barcelona, Spain

2-Federació de Cooperatives Agràries de Catalunya, Barcelona, Spain

*corresponding author e-mail: beatrizquintanilla@ub.edu

Keywords: olive oil, authentication, geographical origin, sesquiterpenes.

Through the EU quality schemes, the common agriculture policy provides tools to help highlight the qualities and tradition associated with registered products and to assure consumers that these are the genuine products. In this sense, food products that are linked to a specific geographical area, such as olive oil¹, can be identified and grouped under the same Protected Designation of Origin (PDOs) and protected geographical indications (PGIs). Moreover, both virgin (VOO) and extra virgin olive oil (EVOO) should state the production country or countries, according to EU Regulation No 29/2012. For this reason, the verification of the label-declared geographical origin of VOO and EVOOs has become crucial to protect consumers from misleading information. Recent studies have revealed that despite their low concentration in olive oil, sesquiterpene hydrocarbon (ST) – a group of semi-volatile secondary plant metabolites – profile is highly dependent on the olive trees variety and growing area, plus these compounds are scarcely influenced by other factors; allowing them to be used as markers to verify the geographical origin of VOOs^{2,3}.

Catalonia, located in the northeast of Spain, comprises five EVOO PDOs (*Siurana*, *Garrigues*, *Terra Alta*, *Baix Ebre-Montsià* and *Empordà*). Despite the proximity between them, these PDO present distinct pedoclimatic conditions and traditional olive cultivars; therefore, the resulting EVOOs show distinctive quality features according to these geographical factors. In fact, the present study aims to discriminate among 80 EVOOs from the previous Catalan PDOs by sesquiterpenes analysis through Solid Phase Micro-Extraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS). Given that the state-of-the-art strategy in food analysis consists in finding specific patterns in highly dimensional analytical data, known as fingerprints, targeted and non-targeted approach were applied and compared for the present purpose.

A Partial Least Square-Discriminant Analysis (PLS-DA) classification model was built and internally validated by leave 10% out cross-validation for each approach; the discrimination efficiency of the authentication models was compared. As a result, the classification power of the non-target model is higher than for the target one; moreover, the untargeted approach is a less time-consuming and automated process. Even EVOOs produced from the same cultivar and in neighboring regions could be classified according to their corresponding PDO. Although a wider sampling and an external validation are necessary, sesquiterpene fingerprinting represents a promising tool for the verification of origin of EVOOs produced in specific PDOs.

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This project has been funded by ACCIÓ-Generalitat de Catalunya and the EU through the Programa Operatiu FEDER Catalunya 2014-2020 in the framework of the project Autenfood (Ref COMRDI-15-1-0035). This study has also been supported by the Spanish MINECO through JCI post-doctoral.



SOCIAL PROGRAMME

Conference dinner

Where else in Europe could you see a giant elephant wandering around the city!

Actually, at 19:30 on 14 November 2018, the Elephant won't be wandering the streets. Instead, he will have settled in for the evening inside his nave on Ile de Nantes and will be hosting a special French aperitif for the FoodIntegrity Conference participants before their Conference Dinner. There, a surprise will await our guests as they enter the imaginary world of the Machines de l'Île.

Just across the former shipyard, dinner will be served on the Nantilus, a unique floating building moored 24 m from the quay on the River Loire and designed as a legacy to Nantes's ship building and maritime past, located at the heart of the city, opposite the Sea Worlds Carousel and the yellow Titan crane.

Reminder: this event is only for those who registered previous to the conference. Cocktail will start at about 7:30 p.m. and end around 11.30 p.m.



Visit Eurofins labs

Thursday 15 November – Afternoon

You have the opportunity to visit Eurofins Nantes, the company's first-ever laboratory and the largest single-site independent food testing laboratory in Europe.

Since the new 9,500 m² extension last year (investment of more than EUR 22m), this state-of-the-art food testing laboratory brings the total size of the campus to 23,640 m², offering its clients many fast and sensitive testing services like nutrition analyses, molecular biology, microbiology testing, DNA sequencing, contaminants testing and authenticity testing.

Reminder: this event is only for those who registered previous to the conference.



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