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Safety
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2ⁿᵈ ISO-FOOD
Symposium FROM
FOOD SOURCE TO HEALTH

BOOK of ABSTRACTS

Portorož, Slovenia **April 24 – 26, 2023**

Institut
"Jožef Stefan"
Ljubljana, Slovenija

BOOK of **ABSTRACTS**

2nd **ISO-FOOD Symposium FROM FOOD SOURCE TO HEALTH**

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Content

Welcome

Dear readers and delegates, it is a pleasure to present you with this Book of Abstracts consisting of contributions to the second ISO-FOOD International Symposium: "*ISO-FOOD from food sources to health*". The ERA Chair ISO-FOOD for isotope techniques in food quality, safety, and traceability has organised this event in affiliation with the Jožef Stefan Institute in Ljubljana, Slovenia, and Jožef Stefan International Postgraduate School. The symposium is scheduled to take place at the Grand Hotel Bernardin in Portorož, which is situated near the historic and picturesque town of Piran on the Adriatic coast.

The global food market with increasing international food trade and the impact of the Covid-19 pandemic on the global food supply chain brings many challenges, not least maintaining and assuring the integrity of foodstuff. Ensuring food safety, quality, and authenticity, detecting food fraud and its impact on consumer health, and addressing the effects of climate change on food production and quality are all essential. Achieving these goals requires the development of innovative and cutting-edge analytical approaches and shared responsibility among all stakeholders in the food production chain.

The 2ⁿᵈ ISO-FOOD Symposium will bring together an interdisciplinary array of researchers, scientists, policy makers, and stakeholders to present advances in isotopic and chemical methods in food safety, quality and authenticity and to foster a lively and rewarding exchange of ideas and information.

The sessions have been organised around ten themes which integrate the following topics:

- Food authenticity and traceability
- Food safety and quality
- New alternative food sources
- Nanomaterials and nanotechnology
- Foodomics
- Food databases and semantics
- Nutrition & Health
- Risk assessment & risk management
- Metrology in food
- Quality and sources of water

We want this to be an opportunity for people to strengthen existing relationships and foster future collaborations. A special effort was made to support and encourage early-stage researchers to participate in this event. The hope and wish behind this endeavour are that younger generations will carry on the spirit of ISO-FOOD in the future.

We look forward to the varied program with three plenary speakers, 12 invited speakers, and 32 oral and 49 poster presentations to be delivered over the next few days. The symposium features strong international representation, with authors of accepted abstracts from 17 countries in Europe and around the world.

In addition to an intensive scientific program, part of the event is dedicated to presenting and promoting Slovenian food SMEs and industries, which will be achieved through the degustation of their products and foodstuffs. This event will be organised on Tuesday and followed by MERFISH Science Café, where we will find out what mercury is and the health risks associated with mercury exposure. The traditional ISO-FOOD Science Slam will take place at the social dinner. With this new form of scientific communication, the presenters will be able to present the work in an entertaining and easily understandable way to a non-expert audience in a short time frame.

Finally, we would like to take this opportunity to thank all of the participants in the Symposium — plenary and invited speakers, presenters, moderators, and sponsors. We would also like to thank all the organising and scientific committee members for their efforts in planning this event.

Dear participants, we hope that with the wide range of interesting papers, activities, and networking opportunities, you find the symposium to be exciting, informative, and relevant to your research field. Since the topics covered at the symposium, such as food quality, safety, and security, will remain crucial for the foreseeable future, we anticipate that this event will be the second of many to come.

Prof. dr. Nives Ogrinc

Chair of the ISO-FOOD Organising committee

SPONSORS

The organising committee is deeply appreciative of the sponsorship generously provided by the following companies:

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Related projects

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PLENARY SPEAKERS

Alejandro **Cifuentes**

Foodomics study on the activity of bioactive compounds from plants, algae and agrifood by-products against Alzheimer's disease

Alejandro Cifuentes is a Full Research Professor at the National Research Council of Spain (CSIC) in Madrid, Head of the Laboratory of Foodomics and Director of the Metabolomics Platform (International Excellence Campus CSIC + University Autonoma of Madrid). He has been the Founding Director of the Institute of Food Science Research at CSIC. Alejandro's activity includes advanced analytical methods development for Foodomics (including transcriptomics, proteomics, lipidomics and metabolomics), food quality and safety, and isolation and characterisation of natural bioactive compounds and their effect on human health. He is on the editorial boards of 17 international journals. He is the Specialty Chief Editor of Frontiers in Nutrition (Nutrition and Food Science Technology), Editor of TrAC-Trends in Analytical Chemistry, Editor of Electrophoresis, Editor-in-Chief of Open Life Sciences and Editor-in-Chief of Exploration of Foods and Foodomics. He has published over 300 SCI papers (plus 30 books/book chapters and nine patents) that have received over 23000 citations. Dr Cifuentes has given more than 200 invited lectures in different meetings in Europe, Asia, Africa, America and Oceania. He has defined for the first time in an SCI journal the new discipline of Foodomics and has been included in the Top 1% of World Scientists by the Stanford University Ranking.

Simon **Kelly**

Food authentication using synthetic transformations coupled with isotope ratio mass spectrometry (FAST-IRMS)

Simon Kelly is a Food Safety Specialist at the United Nations Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture in Vienna, Austria. He has been actively involved in applied and adaptive research using stable isotopes, elemental analyses, and complementary techniques for over 25 years to determine the authenticity of food, including the presence of adulterants and their geographical and production origin. Dr Kelly previously participated in several major European food authenticity projects. He coordinates several international projects to build food authenticity research and technical capacity in developing countries, emphasising simplifying and improving accessibility to analytical methods. He previously held positions at the UK's Food and Environment Research Agency and the Institute of Food Research. Dr Kelly is a member of the UK's Department for the Environment, Food and Rural Affairs Food Authenticity Methodology Working Group. He gained his degree in Chemistry from Anglia Polytechnic in 1992. He completed his PhD in the School of Environmental Sciences at the University of East Anglia in 2002, where he holds an honorary lectureship. Dr Kelly is a Chartered Chemist and a Fellow of the Royal Society of Chemistry.

Nicola **Pirrone**

Future perspectives in using earth observation data technologies for sustainable agriculture in Europe: the HE Partnership on Agriculture of Data

Nicola Pirrone graduated in Nuclear Engineering at the University of Pisa, took fellowships at IIASA (Austria) and the University of Michigan, and was a visiting scholar at the Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS). He now holds the position of Research Director at the National Research Council of Italy (CNR) and Adjunct Professor at the School of Public Health of the University of Michigan. Most recently, he was the Director of the Institute of Atmospheric Pollution Research of the CNR (2008-2018). He is an environmental research and policy development expert for major international and national public organisations, was vice-Head of the Italian delegation at the Conference of the Parties (COP1 and COP2) of the Minamata Convention and, for the past decade, Head of the Italian Delegation for the UNECE-LRTAPEMEP Executive Board. Currently, he is the Italian Representative in the Intergovernmental program of the Group on Earth Observation (GEO) and member of the HLWG and ExCom of GEO, co-chair of the Horizon Europe Partnership on "Agriculture of Data", Representative of the EU Presidency in the Open Ended Science Group (OESG) for the Effectiveness Evaluation of the Minamata Convention, and coordinator of the Italian Node of the European Infrastructure (ESFRI RI) EIRENE RI (European Infrastructure for Environmental Exposure Assessment in Europe). He has published over 300 papers in peer-reviewed literature, books, international reports and conference proceedings. Dr Pirrone has received several awards recognising his outstanding contributions to research and environmental policy, including the Kathryn R. Mahaffey Lifetime Achievement Award in 2017 for his exceptional research work, the UNEP award in 2017 for his commitment and contribution to the UN Environment Global Partnership for the Minamata Convention, and the international award from Green Cross Italy in 2013 for his contribution to environmental research and policy development.

INVITED SPEAKERS

Urška **Blaznik**

National representative food consumption data (SI.Menu study) in dietary exposure and intake assessments

Urška Blaznik obtained PhD in Biomedicine at the Medical Faculty, University of Ljubljana. She is employed at the National Institute of Public Health, Environmental Health and Analysis and Development of Health as a senior researcher on food safety, nutrition and public health. Her research focuses on exposure assessment to chemical contaminants in food, food consumption and nutritional intake at the population level, food reformulation, and the evaluation of public health measures related to diet. She is a member of European and national research projects and a member of the national research programme "Nutrition and Public Health". She is also a member of several forums, working groups and boards, including the Advisory Forum of the European Food Safety Authority (EFSA) and its Scientific Networks on Novel Food and Food Consumption Data, the National food reformulation working group at the Ministry of Health and the National Board for Food Safety at the Ministry of Agriculture, Forestry and Food.

Simon **Kelly**

Compound-specific analysis for the verification of food authenticity

Luana Bontempo is a researcher at Fondazione Edmund Mach (Italy) and is the Head of the Traceability Unit. She obtained her degree in Pharmaceutical Chemistry and Technology from the University of Padua in 2001. In 2004, she completed her postgraduate specialisation in Chemical Methodologies for Survey and Analysis at the University of Padua and obtained her PhD in Food Sciences from the University of Udine in 2011. Her current research mainly focuses on developing analytical methods to apply stable isotope ratio analysis to characterise and trace food products along the production chain, particularly high-value products (such as Geographical Indication products). She has recently been developing methods to apply stable isotope ratios to ecology, specifically in studying bird migration and the trophic niche of ungulates. Dr Bontempo has published more than 70 ISI papers.

Olivier **Donard**

Novel analytical strategies using non-traditional isotopic and multi-isotopic dilution signatures for enhanced geographical food traceability

Olivier F.X. Donard is a research director at the French CNRS (Directeur de Recherche Classe Exceptionelle, now "Emeritus"). He has created, directed and is working at the Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux in Pau (France). He has pioneered and developed speciation-related issues in analytical chemistry for many international environmental organisations (US EPA, NSF, Dutch Ministry of Water and Environment, French fisheries IFREMER). He has developed several approaches for sample preparation, derivatisation, and detection using AAS, AFS, ICP/AES, and ICP/MS (including MC-ICP/MS) that facilitate the routine use of elemental speciation. He has developed novel nontraditional isotopic approaches to unravel Hg environmental pathways and has, in the last ten years, applied these novel possibilities to food traceability. He has created two commercial companies dedicated to speciation analysis: UT2A - Ultra Traces Analyses Aquitaine and AIA - Advanced Isotopic Analysis, while continuing to collaborate with instrument manufacturers. Prof Donard is the author of more than 310 international publications and co-edited one book in analytical and environmental chemistry and food chemistry and has delivered over 170 plenary or invited lectures at international meetings and over 500 poster or oral presentations. Additionally, he is the co-founder and part of the executive committee of the "European Virtual Institute of Speciation Analysis (EVISA)" and was recently appointed to the French "Académie des Sciences."

Tome **Eftimov**

Towards AI-driven Food Science and Society: Opportunities and Challenges

Tome Eftimov is a senior researcher at the Computer Systems Department at the Jožef Stefan Institute. He is an assistant professor at the Faculty of Computer and Information Sciences at the University of Ljubljana and visiting assistant professor at the Faculty of Computer Science and Engineering, Ss. Cyril and Methodius University, Skopje. He was a postdoctoral research fellow at Stanford University, USA, where he investigated biomedical relations outcomes by using AI methods. In addition, he was a research associate at the University of California, San Francisco, investigating AI methods for extraction from electronic health records for rheumatology concepts. He obtained his PhD in Information and Communication Technologies (2018), focusing on information extraction normalisation methods of food and nutrition data. His research interests include natural language processing, statistical data analysis, metaheuristics, representation learning, and machine learning. He has organised AI workshops at high-ranked international conferences, including the Big Food, Nutrition and Environment Data Management and Analysis at the IEEE BigData conference in 2019, 2020, 2021 and 2022, AI & Nutrition Track at the Applied Machine Learning Days 2020, 2021 and 2022, and Semantic Food Q&A at the International Semantic Web Conference. He was a scientific coordinator of an EFSA-funded project on information extraction in food safety. He currently participates in several European projects related to AI and food and nutrition data.

INVITED SPEAKERS

Russell **Frew**

Implementing food origin verification systems

Russell Frew is an analytical chemist who started his career applying trace metal and stable isotope analyses to trace ocean processes. As a Professor at the University of Otago, Russell established the Forensic Analytical Science Major and provided forensic evidence to the courts. Russell founded Isotrace in 2003 and then co-founded Oritain in 2008. From 2012 to 2016 he was employed at the IAEA/ FAO to establish a food traceability programme. Russell retired from his academic position in 2021 and has since been full-time with Oritain as Chief Scientist.

Dimitra A. **Lambropoulou**

SMA®t-EVOO-tools: Providing insights into Greek extra virgin olive oil authenticity and geographical origin.

Dr Dimitra Lambropoulou is an Associate Professor of Environmental Chemistry in the Environmental Pollution Control Laboratory, Department of Chemistry, Aristotle University of Thessaloniki (AUTh). She is a member of the NANOHYBRID group of the Center for Interdisciplinary Research and Innovation (CIRI) of AUTh. Her main research interests are the development and application of novel sample preparation techniques coupled with advanced mass spectrometry approaches in the field of environmental and food chemistry, the design and application of new materials in analytical and separation sciences, occurrence, transport, fate and effects of emerging contaminants (endocrine disruptors and pharmaceutical products, illicit drugs, polar pesticides, transformation products, nanomaterials and microplastics) in the environment (waste and natural water), identification and structure elucidation of organic contaminants using high-resolution mass spectrometry, application of "omics" techniques to environmental problems. She is also interested in developing effective degradation and purification processes for mineralising organic micropollutants, such as Advanced Oxidation Processes (AOPs). She has published more than 167 ISI papers (plus two books and 18 book chapters) that have received more than 8000 citations. Since 2021, she has served as Associate Editor for the journal Science of Total Environment. She is included in the World's Top 2% of Scientists List compiled by Stanford University (USA) based on standardised citation indicators.

Aleksandra **Lobnik**

Optical sensors for food freshness and pesticides detection

Aleksandra Lobnik received her PhD in 1998 from the Institute of Organic Analytical Chemistry at Graz University, Austria. Following this, she pursued her postdoctoral education at the same university. Since 2009 she has been employed as a full professor at the Faculty of Mechanical Engineering at the University of Maribor, where she teaches chemistry, analytical chemistry, and nanomaterials courses. Prof. Lobnik's research focuses on the development of novel materials, such as nanomaterials, biomaterials, and sol-gels, for use in advanced optical, chemical, and bio-sensors for various applications. These applications include personal protection and security, smart textiles, environmental protection, and the fields of food and medicine. In 2006, she co-founded a spin-off company called IOS Ltd. Prof Lobnik has authored 35 patents/patent applications and 280 scientific and conference papers. She has delivered over 30 invited lectures, authored/co-authored five scientific monographs and seven scientific chapters, mentored 46 BA, MA, and ten doctoral students, and several postdoc students and has participated in over 50 national and international research projects. Prof Lobnik actively promotes science and is regularly invited to speak on a variety of topics, including sensors, nano-sciences, innovation, and knowledge transfer, to both experts and the public. In 2015 she lectured at TEDx in CERN on the topic of sensors. In 2021, Prof. Lobnik became the fourth female member of the Slovenian Academy of Engineering, joining 64 male colleagues.

Zoyne **Pedrero Zayas**

Hg and Se fate and interaction in fish: new insights and potential valorisation of tuna byproducts on sustainable aquafeeds

Zoyne Pedrero Zayas is a research scientist at CNRS, specifically at IPREM, Pau, France. She obtained her PhD in Analytical Chemistry at Complutense University of Madrid, Spain. Her research activity is principally related to using natural isotopic fractionation and developing analytical strategies by using MC-ICP-MS as a tool for understanding biogeochemical processes (metal/loids). She is primarily interested in Hg (and Se) analysis in biota and has published in different research areas: such as (bio)analytical chemistry and environmental sciences. She coordinates several national and international projects in that domain, including a European H2020-MSCA-RISE project. She currently leads an international (EU, Mauritius and Brazil) network of experts from cross-sectorial research areas (fish nutrition, analytical chemistry, environmental science, communication, toxicology & aquaculture) in industry and academia, with a long-term goal of furthering the understanding of the source, transport, fate and effects of Hg from fish to humans and the detoxification role of Se.

INVITED SPEAKERS

Francesca **Scandellari**

The signal of water: stable isotopes from rain to table

Francesca Scandellari obtained a degree in Biological Science from the University of Bologna, Italy, where she specialised in Applied Genetics. She has also worked at the University of California, Berkeley. She obtained PhD in Agricultural Chemistry at the University of Torino, Italy, after which she started a 4-years postdoc at the Universiy of Bologna, followed by a 2-years postdoc at the University of New Hampshire at Durham. She then moved to the Free University of Bolzano as a fixed-term researcher. Her expertise in stable isotopes started in 2004. She is a researcher at a private company U-Series Srl where she manages the stable isotope laboratory. The main topics are related to water analysis to detect mixing from unforeseen sources and studying food and other organic materials to detect their geographical origin and possible frauds. She leads the Working Group 4 Networking and dissemination within the COST Action WATer isotopeS in the critical zONe: from groundwater recharge to plant transpiration (WATSON https://watson-cost.eu/).

Arndt **Schimmelmann**

Can ionising radiation cause isotopic fractionation of organic compounds?

Arndt Schimmelmann received his M.Sc. Chemistry from the University of Braunschweig, Federal Republic of Germany 1979 and PhD in Geochemistry, University of California in Los Angeles, USA 1985. His scientific positions included a postdoc in 1985-1988 at the Scripps Institution of Oceanography and from 1988-1993 as a specialist at the Scripps Institution of Oceanography before becoming a Senior Scientist at Indiana University, Department of Geological Sciences. His current research activities include the development of organic hydrogen, carbon, and nitrogen stable isotope reference materials, research of shale gas, funded by the US Department of Energy, paleoclimatology of laminated sediments, radon isotopes in soil-built dwellings in developing countries, subterranean methanotrophic consumption of atmospheric methane.

Janja **Vidmar**

The potential of single particle inductively coupled plasma mass spectrometry for studying micro- and nanoscale particles in water and food

Janja Vidmar received a PhD in Environmental Sciences from Jožef Stefan International Postgraduate School in 2017. She conducted postdoctoral research at the National Food Institute of the Technical University in Denmark. She is employed as a Research Associate in a Research Group for Trace element speciation at the Department of Environmental Sciences, Jožef Stefan Institute, Slovenia. She is also an Assistant Professor at Jožef Stefan International Postgraduate School, study programme Ecotechnology. Her research interests are in the field of inorganic analytical chemistry. She has been mainly focused on optimising and applying single particle ICP-MS method for quantifying and characterising nanoparticles and microplastics in environmental, food and biological samples. Dr Vidmar has worked on several European and national research projects, published 40 scientific papers and two book chapters, and co-authored one patent. In 2020, she received the Jožef Stefan Golden Emblem Award for her outstanding scientific contribution.

Claudia **Zoani**

Integrated Research Infrastructures' initiatives in support of food quality and safety and sustainability of agrifood systems

Dr Zoani is a researcher at the ENEA Biotechnology and Agroindustry Division and holds a PhD in Analytical Chemistry. Additionally, she has completed research activities for a second PhD in Agriculture, Food and Environment. As a specialist in atomic spectroscopic and mass spectrometry techniques and metrology, she conducts R&D activities on reference materials and methods, measurement uncertainty, food quality, safety, traceability, sustainability of agrifood systems, and chemical risk assessment. She is also the Scientific Secretary of the IMEKO TC23 Metrology in Food and Nutrition and a member of the Eurachem WG on Reference Materials and UNI Committee General Metrology. She has been selected as one of the ten Italian experts on Circular Economy to participate in the Comité des Dix-Italie - Sommet des Deux Rives initiative by the Italian Ministry for Foreign Affairs and International Cooperation (MAECI). In 2014, she received the Premio Leonardo UGIS for her research and communication skills. She is the coordinator of the Research Infrastructure METROFOOD-RI, which promotes metrology in food and nutrition and is included in the ESFRI Roadmap for the Health and Food domain. Recently, the METROFOOD-RI concluded its Preparatory Phase under the H2020 project METROFOOD-PP. She is engaged as PI for ENEA in several national and European ongoing projects, such as the HEu AgroServ and DGR4Food, the H2020 FNS-Cloud, iNEXT-Discovery and FoodSafety4EU and the ERANET SUSFOOD CORE ORGANIC "PROVIDE". She coordinates the METROFOOD-IT project as part of the Italian National Plan for Recovery and Resilience. The project focuses on strengthening the infrastructure for Metrology and Open Access Data to support the agrifood industry.

ORAL PRESENTATIONS

Sunday, 23.4.2023

15:00-19:00	**Registration**	
19:00	**Welcome party**	

Monday, 24.4.2023

	Moderators: N. Ogrinc, D. Heath	
9:00-9:45	**A. Cifuentes**	**Foodomics study on the activity of bioactive compounds from plants, algae andaAgrifood by-products against Alzheimer disease**
9:45-10:30	**S. Kelly**	**Food authentication using synthetic transformations coupled with Isotope Ratio Mass Spectrometry (FAST-IRMS)**
10:30-11:00	Coffee break	

FOOD AUTHENTICITY AND TRACEABILITY	**Moderator: S. Kelly, T. Zuliani**	
11:00-11:30	**R. Frew**	**Implementing food origin verification systems**
11:30-12:00	**L. Bontempo**	**Compound-specific analysis for the verification of food authenticity**
12:00-12:15	L. Strojnik	The authenticity of food flavourings and geographical traceability of food crops using a stable isotope methodology: synthesis of approach
12:15-12:30	B. Torres-Cobos	Nuts about authentication: using a handful of isotopic tools to crack the hazelnut origin mystery
12:30-12:45	S. Pianezze	GC-C-IRMS on single fatty acids and EA-IRMS on bulk lipid to study the fractionation processes in bovine organism and to detect differences in four matrices of Simmental cows fed on C3 and C4 diets
12:45-13:00	M. Horacek	Control of geographic origin of saffron using stable isotope analysis (IRMS)
13:00-14:00	Lunch	

QUALITY AND SOURCES OF WATER	**Moderator: H.A.J. Meijer, E. Heath**	
11:00-11:30	**J. Vidmar**	**The potential of single particle inductively coupled plasma mass spectrometry for studying micro- and nanoscale particles in water and food**
11:30-12:00	**F. Scandellari**	**The signal of water: stable isotopes from rain to table**
12:00-12:15	H. Heinzen	Chemical safety of freshwater for human consumption and aquatic life which also is food
12:15-12:30	K. Anagnostopoulou	HRMS strategies for the target analysis and suspect screening of persistent and mobile substances in drinking, ground and surface water of Greece
12:30-12:45	J. Moreno Rojas	Origin, implications, and management strategies for nitrate pollution in vulnerable zones in Andalusia
12:45-13:00	P. Vreča	SLONIP – A Slovenian web-based interactive research platform on the water isotopes in precipitation
13:00-14:00	Lunch	

FOOD AUTHENTICITY AND TRACEABILITY	**Moderators: L. Bontempo, R. Frew**	
14:00-14:30	**O. Donard**	**Novel analytical strategies using non-traditional isotopic and multi-isotopic dilution signatures for enhanced geographical food traceabilities**
14:30-14:45	M. Nikezić	Linking multi-elemental and Sr isotopic data of milk, cheese, water, soil and forage
14:45-15:00	T. Zuliani	Analytical challenges in determining the origin of olive oil using $^{87}Sr/^{86}Sr$ isotope ratios
15:00-15:15	V. Vacchina	Geographical origin discrimination of wines by a new multi-isotopic dilution method
15:15-15:30	H. A.J. Meijer	Age verification of 10-yr and 20-yr matured port wines using radiocarbon
15:30-15:45	M. Tuthorn	The next generation LC-IRMS for honey authenticity investigation
15:45-16:15	Coffee break	

FOODOMICS, NUTRITION & HEALTH	**Moderators: A. Cifuentes, U. Vrhovšek**	
14:00-14:30	**D. Lambropoulou**	**SMA®t-EVOO-tools: providing insights into Greek extra virgin olive oil authenticity and geographical origin**
14:30-14:45	B. Butinar	"Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" and the fate of EC Reg. 432/2012: are the basic terms and the figures any clearer now?
14:45-15:00	J. Masten Rutar	Fermented and non-fermented spirulina water and ethanol extracts antioxidant activity in vitro and at the cellular and proteome level
15:00-15:15	P. Starič	Cold plasma pre-treatment and grain priming: Zinc enhancement in buckwheat grains and sprouts
15:15-15:30	J. Andersen	Supplementing cheese with polyphenol rich fruits to increase content of beneficial bioactive polyphenols
15:30-15:45	N. Poklar Ulrih	The possibility of using olive leaves as a source of polyphenols in the light of circular economy
15:45-16:15	Coffee break	

FOOD DATABASE AND SEMANTICS	**Moderators: N. Ogrinc, D. Heath**	
16:15-16:45	**T. Eftimov**	**Towards AI-driven food and nutrition science and society: opportunities and challenges**
16:45-17:00	Đ. Čanadi Knežević	Fully automated official AOAC methods for determination of dietary fibers (IDF, SDF, TDF) by ANKOM technologies
17:00 - 18:30	Poster session	

ORAL PRESENTATIONS

Tuesday, 25.4.2023		
FOOD QUALITY AND SAFETY	Moderator: M. Horvat, L. Kourimska	
9:00-9:30	**Z. Pedrero Zayas**	**Hg And Se fate and interaction in fish: new insights and potential valorization of tuna by-products on sustainable aquafeeds**
9:30-9:45	S. Petromelidou	A preliminary survey of PFAS in farmed marine shellfish in Greece. Do PFAS pose a threat to marine biota and human health?
9:45-10:00	M. Malý	Transfer of phytocannabinoids from dried plant to aqueous infusions: Is cannabis 'tea' safe?
10:00-10:15	P. Pongrac	Biofortifying sprouts with zinc by seed priming
10:15-10:30	A. Vehar	Quality characteristics of treated wastewater irrigated tomatoes
10:30-14:00	Coffee break & Degustation	
14:00-15:30	MERFISH Science Café	
	M. Horvat	Mercury exposure and health effects: do we know enough about what is safe?
	J. Snoj Tratnik	Mercury exposure in prenatal life and neurodevelopment: findings from the Northern Adriatic birth cohort study
FOOD QUALITY AND SAFETY	Moderators: U. Blaznik, D. Lambropoulou	
15:30-15:45	M. Veronica Cesio	Decontamination studies on ready-to-eat- strawberries
15:45-16:00	A. Bogažalec Košir	Quantification goes digital – 10+ years of dPCR developments in GMO diagnostics
NEW ALTERNATIVE FOOD SOURCES	Moderators: U. Blaznik, D. Lambropoulou	
16:00-16:15	L. Kourimska	Selected non-protein nitrogen compounds in insects for food and feed purposes
16:15-16:30	M. Kulma	Descriptive sensory analysis of edible insects in Czechia: tasty or nasty? and does size really matter?
16:30-16:45	L. Koronaiou	Multiresidue determination of current-used pesticides in edible insects by orbitrap HRMS target, suspect and non-target approaches
16:45-17:00	A. Macan Schönleben	Stable Isotope Ratio Analysis of H, C, N and S in edible insect samples from Europe, Asia and Africa
17:00-17:30	**A. Lobnik**	**Optical sensors for food freshness and pesticides detection**
19:00	Dinner	

Wednesday, 26.4.2023		
FOOD QUALITY AND SAFETY	Moderator: M. Horvat, L. Kourimska	
9:00-9:45	**N. Pirrone**	**Future perspectives in using earth observation data technologies for a sustainable agriculture in Europe: the HE partnership on agriculture of data**
9:45-10:15	C. Zoani	Integrated Research Infrastructures' initiatives in support to food quality and safety and sustainability of agrifood systems
10:15-10:45	Coffee break	
RISK ASSESSMENT & MANAGEMENT	Moderators: C. Zoani, F. Scandellari	
10:45-11:15	**A. Schimmelmann**	**Can ionizing radiation cause isotopic fractionation of organic compounds?**
11:15-11:45	**U. Blaznik**	**National representative food consumption data (SI.Menu study) in dietary exposure and intake assessments**
11:45-12:00	N. Waegeneers	Unravelling the exposure and risk to free glutamate form food additive and non-additive sources in Belgium
12:00-12:15	A. Alilovič	Total mercury and methylmercury levels in hair, blood, and urine of individuals following controlled intake of tuna fish
12:15-13:00	Closing ceremony	

POSTER PRESENTATIONS

Session 1: FOOD AUTHENTICITY AND TRACEABILITY

PRESENTING AUTHOR	TITLE
A. Roncone	PROMEDLIFE: Novel food products for the PROmotion of a MEDiterranean LIFEstyle and healthy diet
B. Pavlovič	Authentication and traceability of fish and seafood using stable isotope and multi-elemental approach
C. Terro	Foodtrack – Stable Isotope tool for determination of authenticity and traceability of food
C. Paraskevi	Oxygen and hydrogen isotopes in milk and cheese casein from DPO cheese products in Greece
D. Kocman	An integrated toolbox for improved verification and prevention of adulterations and non-compliances in organic and geographical indications food supply chain – THEROS project
H. Abderrazak	Stable isotope ratios and multi-element analysis for food authenticity and traceability in the framework of the TUNTWIN project
J. Masten Rutar	Combined isotopic and elemental composition analysis for the assessment of Spirulina food supplements authenticity
J. Siira	Strontium isotope ratios of Finnish strawberries and agricultural soils
K. Babič	Characterization of Slovenian pork meat based on stable isotope ratio, multi-elemental analysis and multivariate modelling approaches
L. Jelenko	Effects of storage conditions on carbon isotopic composition of volatile organic compounds
M. Tuthorn	Isotope fingerprints: addressing authenticity of fish oils by GC-MS-IRMS
M. Perini	Authentication and geographical characterisation of Italian grape musts through glucose and fructose carbon isotopic ratios determined by LC-IRMS
M. Kolar	Differentiation of the mineral content of Slovenian honeys by botanical origin using principal component analysis
N. Šibanc	The potential of stable isotope technique and microbiome characterisation in determining truffle aroma formation and the authentication of truffles
R. Skipityte	Stable isotope and fatty acid composition of organically and conventionally produced Lithuanian milk
S. Berail	Isotopic signatures of «non-traditional» elements for authenticity and geographical assessments of food and beverages
T. Zuliani	Characterisation of Slovenian honey

Session 2: FOOD SAFETY AND QUALITY

PRESENTING AUTHOR	TITLE
D. Kočar	Determination of 5-hydroxymethylfurfural in honey by high-performance liquid chromatography
E. Heath	Chemicals migrating from beer cans: non-targeted and suspect analysis
H. Chakroun	Heavy metal content in crops grown near former metal mining sites and potential risk to the local population
L. Fras Zemljič	Functionalisation of PLA foils using chitosan and plant extracts as a bilayer coating
L. Martello	A newly synthetised chitosan derivative as a promising sorbent for fungicides enrichment from wine samples
M. Viltres Portales	Selenium biofortification of wheat plants by foliar application of liposomes: selenium speciation by synchrotron radiation techniques.
M. Sterniša	The importance of experimental design for the evaluation of polymer nanocomposites as antimicrobial coatings on food contact surfaces
M. Sterniša	Simultaneous detection of antimicrobial and anti-Biofilm activity of novel compounds using salmonella infantis – improving data analytics through machine learning
S. Smole Možina	Effects of lavandula essential oil and pure phenolic compounds on invasion mechanisms and protein profile of *Campylobacter jejuni*
S. Marković	Cr speciation in wine and beer using HPLC-ICP-MS and enriched stable isotopic tracers of 50Cr(VI) and 53Cr(III)
T. Haifa Tlil	The risk of plants contamination by potentially toxic elements near mining sites
U. Gradišar Centa	Thermal and rheological properties of nut butter as a tool for assessing butter quality
Z. Giannioti	Determination of regulated and emerging mycotoxins in organic and conventional gluten-free flours by LC-MS/MS
Ž. Kolenc	Microbial communities on microplastics from nearshore seawater and Mediterranean mussels *Mytilus galloprovincialis* in Slovenia
Ž. Kolenc	Evaluation of naturally adhered bacteria on lettuce *(Lactuca sativa)* leaves.

Session 3: NEW ALTERNATIVE FOOD SOURCES

PRESENTING AUTHOR	TITLE
Nayyer Rehman	Motivation and barriers to the adoption of new alternative foods among European consumers

Session 4: NANOMATERIALS & NANOTECHNOLOGIES

PRESENTING AUTHOR	TITLE
D. Lambropoulou	Design of novel molecularly imprinted polymers as effective extraction media for selective determination of triazole fungicides in juice samples
M. Dekleva	Optimisation of experimental parameters for electrochemical detection of neonicotinoids in honey with modified screen-printed carbon electrodes
M. Kolar	Bio-active nanofibrous nonwovens as in-situ producers of hexanal
P. Leban	Studying the uptake and distribution of metal-doped nanoplastics in hydroponically grown tomato plants
R. Singh Chouhan	Two-dimensional magnetic nanomaterial: versatility and detection performance capabilities for toxic metal ions
U Gradišar Centa	Antimicrobial active polymer nanocomposite PVDF-HFP/PVP/MoO3 as a nanostructured coating for potential application on food contact surfaces

Session 5: FOODOMICS

PRESENTING AUTHOR	TITLE
A. Kovačič	Characterisation of the neuroactive compound profile in organic, conventional and processed tomatoes
M. Škrjanc	NMR profiling of Slovenian honey and wine

Session 7: NUTRITION & HEALTH

PRESENTING AUTHOR	TITLE
U. Kastelic Kovačič	Cave bear diet revealed by stable isotopes analysis

Session 8: RISK ASSESSMENT & MANAGEMENT

PRESENTING AUTHOR	TITLE
P. Klemenčič	HUMAN BIOMONITORING: Dietary cadmium exposure in the Slovenian population of children and adolescents

Session 9: METROLOGY IN FOOD

PRESENTING AUTHOR	TITLE
D. Potočnik	Metrology for stable isotope ratio measurements
E. Heath	Aerobic degradation of bisphenol A alternatives with activated sludge: kinetics and biotransformation products
H. A.J. Meijer	Combining Optical Spectroscopy and IRMS for the Measurement of $\delta^{18}O$ and $\delta^{2}H$ of water and Ethanol directly in wine
S. Berail	Geographical origin of mineral waters by Sr isotope ratio MC-ICP-MS: long-term stability of the signatures obtained by different purification procedures

Session 10: QUALITY AND SOURCES OF WATER

PRESENTING AUTHOR	TITLE
K. Žagar	$\delta^{18}O$ and $\delta^{2}H$ fingerprinting of tap water
M. Sánchez-Parra	Incidence of nitrate contamination and origin in two water systems of southern Spain

MERFISH – Science Café

What is mercury, and how are people exposed to it? What health risks are associated with mercury exposure, especially for vulnerable groups?

These questions and many others will be addressed during the MERFISH Science Café, part of the 2nd ISO-FOOD Symposium, Portorož, Slovenia, on Tuesday, April 25th 2023.

Organised by Prof. Dr Milena Horvat and Dr Janja Snoj Tratnik from the Jožef Stefan Institute IJS, Department of Environmental Sciences, the Science Café will provide a platform for the general public to interact with scientists and find out about the research in a casual and accessible setting. The programme will involve talks on the study, followed by a question-and-answer session with the audience so that they can learn about and engage with this important topic addressed by the MERFISH project.

Funded under the H2020-MSCA-RISE program, MERFISH: Health-benefit understanding of mercury-selenium interactions from fish to human is a 48-month project coordinated by the Centre National de la Recherche Scientifique (CNRS) to tackle our understanding of mercury-selenium interactions from fish to humans. The MERFISH initiative aims to address this by bringing together international experts from a wide array of interdisciplinary research areas (analytical chemistry, fish nutrition, environmental science, communication, toxicology and aquaculture) from industry and academia, with a long-term goal of furthering our understanding of the source, transport, fate, and effects of mercury from fish to humans and the detoxification role of selenium. More information can be found at: https://www.merfish.eu.

The Science Café promises to be a unique event. Since it is taking place within the 2nd ISO-FOOD Symposium, it will allow Science Café participants to engage with an interdisciplinary array of researchers, scientists, policy makers, and stakeholders to present advances in isotopic and chemical methods to foster a lively and rewarding exchange of ideas and information.

Abstracts

Food Authentication using Synthetic Transformations coupled with Isotope Ratio Mass Spectrometry (FAST-IRMS)

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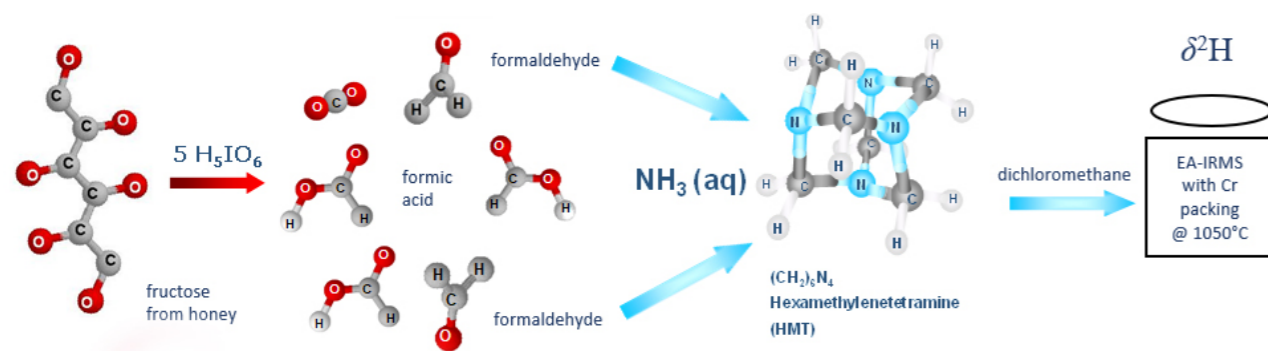
Figure 1: Periodate oxidation of fructose to formaldehyde, formic acid and CO2. Formaldehyde is converted to HMT (hexamethylenetetraamine) with ammonia and then extracted into dichloromethane before isolation and δ^2H by EA-IRMS.

The aim of this area of research is to develop relatively straight forward and rapid methods to permit measurement of the δ^2H value of the carbon-bound non-exchangeable (CBNE) fraction of hydrogen in organic molecules using an elemental analyser or gas chromatograph coupled to an isotope ratio mass spectrometer (EA- or GC-IRMS). The methods should increase accessibility to CBNE δ^2H measurements, which may be used for both detection of economically motivated adulteration and determination of the geographical origin of foods.

We have successfully developed a novel GC-IRMS procedure for the rapid isotope analysis of carbon-bound nonexchangeable (CBNE) hydrogen in mono and disaccharides to demonstrate the feasibility of detecting undeclared addition of exogenous sugar products in foods and beverages [1-2]. Procedures for the analysis of hydrogen stable isotopes in compounds that contain exchangeable hydrogen, such as carbohydrate 'hydroxyl-hydrogen' (-OH), are a contentious issue. Several approaches have been reported using equilibration techniques, with isotopically distinct water vapour, but there is still debate about the most reliable procedures to obtain reproducible results for intrinsic and extrinsic hydrogen between laboratories, taking into account considerations such as equilibration temperature/time, % of exchangeable hydrogen in different matrices, and drying to remove the contribution of adsorbed and absorbed water, etc. The new GC-IRMS method avoids these issues by derivatising exchangeable hydrogen atoms to remove their contribution to the hydrogen isotope signal in carbohydrates. Consequently, the new rapid GC-IRMS method using trifluro-acetate derivatives with a chromium reduction furnace has overcome the historical disadvantages that have hindered the uptake of this measurement by isotope ratio mass spectrometry (IRMS).

The utility of converting methoxy groups in food to methyl iodide for head-space GC-IRMS analysis is also discussed [3], with a view to speculating on potential applications in food authenticity and provenance determinations. However, we also acknowledge that not all laboratories have access to GC coupled to an IRMS. For many laboratories the stable isotope analysis 'entry point' is with EA-IRMS for bulk stable isotope analysis of foods. An alternative rapid method to permit measurement of the δ^2H value of CBNE hydrogen in organic molecules using an EA, containing a chromium filled reactor at 1050°C, coupled to an IRMS, is via the periodate oxidation of fructose and glucose to formaldehyde and formic acid (see Fig. 1). The formaldehyde may subsequently be converted with ammonia to the hexamethylenetetramine (HMT), which retains position-specific $\delta^{13}C$ and δ^2H isotopic information from the carbon and nonexchangeable hydrogen atoms in the parent molecule [4]. Here we present preliminary data on the use of HMT to detect sugar syrups in honey.

Acknowledgements

The authors would like to acknowledge provision of authentic pineapple juices by Dr Peter Rinke (SGF).

References

[1] A. Abrahim et al. (2020) *Food Chemistry*, 318, 126413.

[2] S. D. Kelly et al. (2021) *npj Science of Food,* 5, 10.

[3] M. Greule et al. (2008) *Rapid Communications in Mass Spectrometry*: 22, 3983-3988.

[4] S. D. Kelly et al. (2003) *Journal of Agricultural and Food Chemistry*, 51, 1801-1806.

Implementing Food Origin Verification Systems

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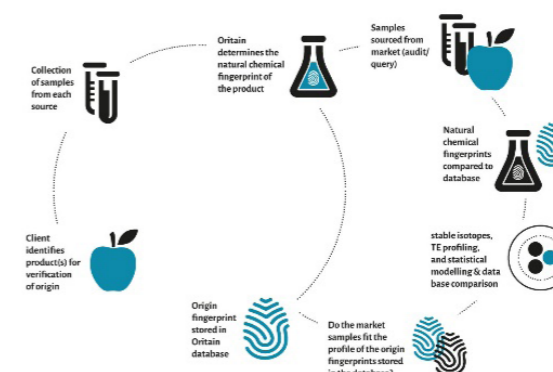
Food origin verification systems are used to verify the origin of food products at any point in the supply chain. These systems are becoming increasingly important as the global food supply chain grows and becomes more complex. Food origin verification systems use various technologies and techniques, including trace element and isotopic analysis [1]. There are many benefits of implementing a food origin verification system. These include marketing, detection of fraud, protection of the brand and increasing consumer confidence. There are several different types of food origin verification systems. Geochemical analysis, including trace metals [2] and stable isotopes [3], has been extensively used to verify a food product's origin by measuring the food's composition and comparing it to known sources2. This is an effective way to verify the origin of food products, but it is more expensive than other methods.

Implementing a food origin verification system can be a complex and time-consuming process. There are numerous steps from the concept demonstrated in a scientific publication to the implementation of a robust verification system. The steps involved in the process, including the design of the system, selecting the right technologies and techniques and making the chemical measurements, modelling the data and then verifying the outputs are valid.

The adequacy of the models is determined by extensive testing and validation. Once all that is achieved, it still remains to incorporate the system into the existing supply chain as an auditing programme and train staff to implement the system.

This talk will cover the steps from the scientific concept to the implementation of a working audit system for verification of the origin of natural products such as food and fibre. Examples from Oritain and clients will demonstrate the effectiveness of origin verification systems. The examples will include demonstrating the spatial discrimination achieved.

References

[1] J. Wakefield, K. McComb, E. Ehtesham, R. Van Hale, D. Barr, J. Hoogewerff, R. Frew (2019) Food Control, 106, 106699.

[2] J. Amit, S. Kumari, S. Kelly, A. Cannavan, D. Singh (2022) Current Research in Nutrition and Food Science, 5, 545-552.

[3] E. Ehtesham, A. Hayman, K. McComb, R. Van Hale, R. Frew (2013) Journal of Agricultural and Food Chemistry, 61 (37), 8914-8923.

Compound-Specific Analysis for the Verification of Food Authenticity

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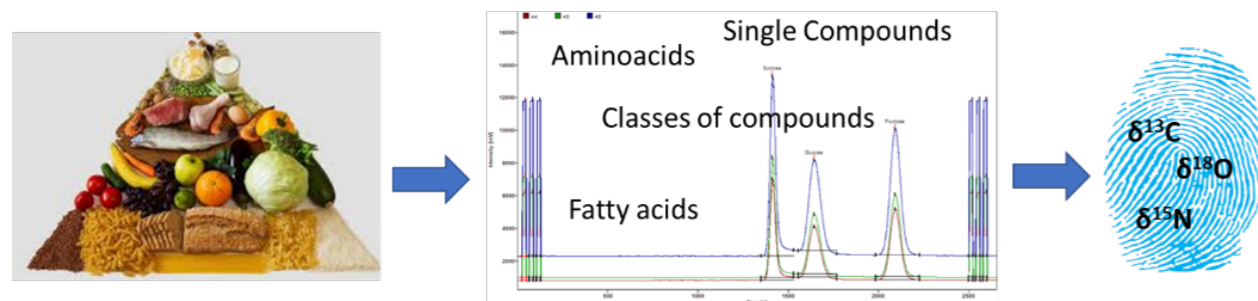
Stable isotope ratios analysis (SIRA) has been used for food authenticity testing for more than thirty years and is today being utilised on a routine basis, and in some cases also as official methods, for a wide variety of food commodities [1,2]. During the past decade major analytical method developments have been made and the fundamental understanding of fractionation processes resulting in isotopic signatures suitable for food authentication has been improved. However, the bulk stable isotope value represents an averaged value of all components in a food product, and may in some instances, not sufficiently distinguish isotopic variations between different samples. One of the very recent trends in SIRA based food authenticity testing is compound-specific isotope analysis (CSIA). It has been observed in several studies that compared to bulk samples, additional isotopic information can be obtained by focusing on isotopic signatures of specific food compounds. CSIA can be based on both off-line and online compound isolation prior to isotope analysis. In particular, the online systems are obtained through the combination of a gas chromatograph (GC) [3] or a liquid cromatograph (LC) [4] that separate out individual compounds in a complex mixture, linked to an isotope ratio mass spectrometer (IRMS), resulting in the stable isotopic ratios of specific compounds in samples.

The combination applications for food authentication based on GC- IRMS and LC-IRMS have grown considerably in the last few years. As a result, this presentation reports some CSIA principles and applications for food authentication cases used for the verification of geographical origin and authentication of agricultural or farming production processes for plants and animal products, as well as the authentication and origin of plant extracts and essential oils. A special focus will be in particular on the differentiation of organic and conventional food products.

References

[1] F. Camin, L. Bontempo, M. Perini, E. Piasentier (2016) *Comprehensive Reviews in Food Science and Food Safety*, 15, 868 – 877.

[2] F. Camin, M. Boner, L. Bontempo, C. Fauhl-Hassek, S. D. Kelly, J. Riedl, A. Rossamnn (2017) *Trends in Food Science & Technology*, 61, 176-187.

[3] H. Liu, J. Nie, Y. Liu, S. A. Wadood, K. M. Rogers, Y. Yuan, R.-Y. Gan (2023) *Food Chemistry*, 415, 135791.

[4] M. Perini, L. Bontempo (2022) *TrAC - Trends in Analytical Chemistry*, 147, 116515.

Session 1: FOOD AUTHENTICITY AND TRACEABILITY

Novel Analytical Strategies using Non-Traditional Isotopic and Multi-Isotopic Dilution Signatures for Enhanced Geographical Food Traceabilities

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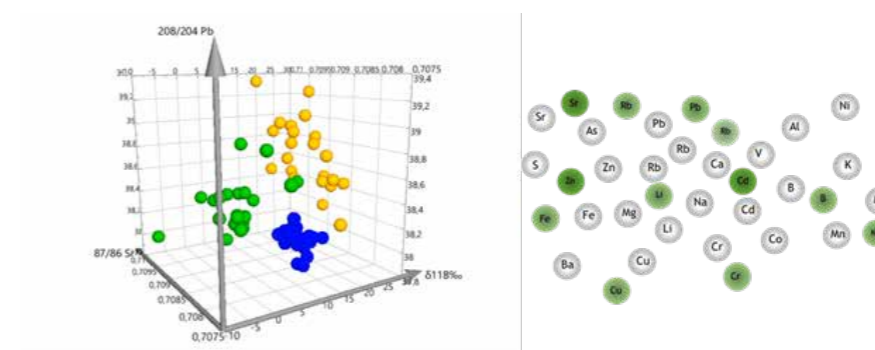
Tri-isotopic and multi-isotopic dilutions analytical strategies for high geographical identification

Major and trace elements are naturally up taken by plants from the soils on with they are growing for their developments. These bioavailable elements and isotopes represent a direct signature of the land on which there are growing. Inorganic constituents are then a direct signature of the geogenic of the land on which they have been developing. Further agricultural practices may also introduce additional elements to promote the growth of the plant. Both inorganic and isotopic content clearly contribute to directly sign the denomination of the "terroir": geographical origin and agricultural practice.

We have developed non-traditional isotopic signatures since they are direct constituents of the "terroir". The isotopes of Sr where first selected due to very strong discriminating potential. They are usually up taken from the soils to the plant with non-detectable fractionation. However, in some cases, limitations occur since similar geological sedimentary formations can yield similar Sr isotopic ratios. This is why we have added a second isotopic system with that of Pb isotopes also detected by MC/ICP/MS. Pb is mainly imported by the plant by atmospheric inputs and usually the atmospheric signatures override the geogenic signature since Pb is not an essential element for the plant. Finally, in order to increase the discrimination potential, we have added the power of Boron isotopes. Boron has a large isotopic span between the 2 isotopes and displays large fractionation process. This fact allowed us to used quadrupole ICP/MS to measure the precision of Boron isotopes samples on a variety of wines around the world. Combining the 3 isotopic signatures demonstrated an exceptional geographical discrimination power.

In order to promote a new, fast and precise methods to address geographical discrimination, we have recently developed a new multi-isotopic dilution based on a routine analytical triple Quadrupole ICP/MS (QQQ-ICP/MS). The multi-isotopic dilution method yielded routine high precision for most spiked elements and resulted in precision well below 2% for all the elements having their isotopic spike added. This new method, based on a novel analytical approach, also allows to unravel refined geographical origins.

We will present and compare the different analytical approaches used for high resolution geographical wine discrimination and critically assess their potential, benefits and pitfalls of both analytical strategies used for wine assessment. The results are here presented on wines samples but could be transferred to any food matrices where the link with the soil is essential.

2nd ISO-FOOD Symposium FROM FOOD SOURCE TO HEALTH

Nuts About Authentication: Using a Handful of Isotopic Tools to Crack the Hazelnut Origin Mystery

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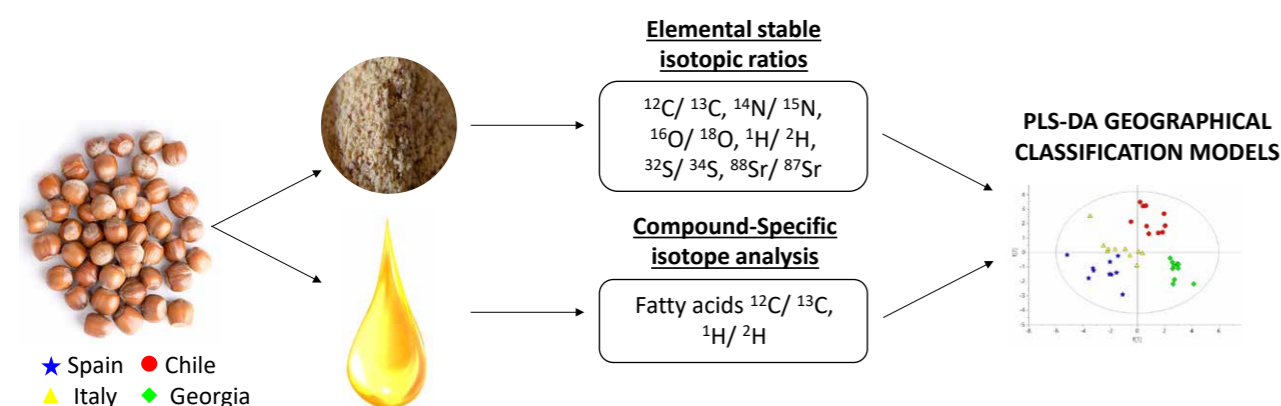
Hazelnuts are a widely used and prized ingredient in both sweet and savoury foods. Their sensory and qualitative characteristics are heavily influenced by the geographic region in which they are grown [1] and their market value fluctuates significantly depending on their geographic origin. The high value of hazelnuts makes them vulnerable to fraud. Reliable tools are necessary to verify their origin and ensure their authenticity, protecting consumers from economic gain-driven deception.

Isotopic analysis of light bio-elements ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$, and $^{34}S/^{32}S$) has been widely applied for the geographical verification of a range of foodstuffs with satisfactory results [2-4]. In addition, the isotope analysis of heavy geo-elements such as strontium ($^{87}Sr/^{86}Sr$) is thought to provide an even stronger link between soil and primary agricultural products [2-4]. However, only few studies based on this approach are available for nuts and no data are available on the application of isotopic markers for hazelnut geographical authentication.

A key element in authentication is the data treatment and chemometric analysis. As a large number of variables are studied, multivariate techniques such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) are crucial to extract all the relevant information.

The aim of this preliminary study is to assess the suitability of the main isotope ratios for hazelnut geographical authentication. To achieve this purpose, the elemental isotopic ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$, $^{34}S/^{32}S$ and $^{87}Sr/^{86}Sr$ of 40 samples of raw hazelnuts from 4 different origins and including 3 harvest years and the $^{12}C/^{13}C$ and $^{1}H/^{2}H$ of the fatty acids of the corresponding oil extracted from them were analysed.

The data was explored with PCA to see the natural distribution of the samples and their relation with the variables. Finally, PLS-DA classification models were built to identify the most promising variables for hazelnut authentication.

Results show that the most relevant variables to discriminate samples according to their origin are bulk $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{87}Sr/^{86}Sr$ (potential influence of fertilization is under discussion), and fatty acids $^{2}H/^{1}H$ and $^{13}C/^{12}C$ isotopic ratios, which allowed achieving a 92.5% of correct classification

Acknowledgements

References

[1] K. Król, M. Gantner (2020) *Agriculture*, 10, 375.
[2] K. Katerinopoulou, A. Kontogeorgos, C. E. Salmas, A. Patakas, A. Ladavos (2020) *Foods*, 9, 489.
[3] S. A. Drivelos, C. A. Georgiou (2012) *Trends in Analytical Chemistry*, 40, 38-51.
[4] F. Camin, M. Boner, L. Bontempo, C. Fauhl-Hassek, S. D. Kelly, J. Riedl, A. Rossmann (2017) *Trends in Food Science & Technology*, 61, 176-187.

The Authenticity of Food Flavourings and Geographical Traceability of Food Crops Using a Stable Isotope Methodology: Synthesis of Approach

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Authenticity of Food Flavourings — Methodology — Database

Geographical Origin of Fruits and Vegetables — Data analysis — Market testing

Food fraud or economically motivated adulteration is a growing concern for producers, consumers, regulatory agencies and scientific organisations. Food fraud prevention is paramount to protecting consumer trust and maintaining fair and sustainable business practices.

Grant Numbers P4-0432, focuses on two fraudulent acts, adulterating natural fruit, vanilla and truffle flavours with cheaper synthetic counterparts and mislabelling the country of origin of food crops (asparagus, garlic, strawberry, cherry, apple and kaki). In both cases, verification of the authenticity of flavourings and geographical traceability of products from the market was achieved through the following steps: (i) the development of suitable analytical methods; (ii) the establishment of databases; and (iii) data processing using chemometric approaches.

The first part of the study, devoted to developing, optimising, and validating robust analytical techniques for flavour authenticity, shows the advantages of coupling the HS-SPME extraction technique with the GC-IRMS method [1]

Data interpretation in the selected fraud cases requires an extensive reference data set of authentic food samples, i.e. a database or databank against which a sample under investigation can be compared. This study establishes ten dedicated databases of authentic, sufficiently representative samples that cover the natural variation of isotopic and elemental values [2].

Data analysis and the interpretation of results were also essential parts of this study. Comparing isotope ratios of VOCs collected in the flavour databases allowed the successful discrimination between synthetic and naturally produced VOCs. However, a comparative analysis alone could not provide a definitive answer concerning the geographical origin of fruits and vegetables. For this reason, different chemometric approaches were explored. DD-SIMCA was chosen as the most suitable method for determining whether the commercial product complies with its declaration (i.e. Slovenian origin) [3].

Finally, developed methods and databases were used to verify the naturalness of commercial flavourings and the geographical origin of selected fruits and vegetables from the market. Results of commercial flavourings and their flavoured products imply that the authenticity can be questioned, most often within natural flavoured vanilla and truffle samples. Mislabelling of truffle species has also been identified. Also, 37 % of investigated fruit and vegetable samples did not correspond to their stated declaration.

Acknowledgements

References

[1] L. Strojnik, F. Camin, N. Ogrinc (2020) *Talanta*, 219, 121264.
[2] L. Strojnik, J. Hladnik, N. C. Weber, D. Koron, M. Stopar, E. Zlatić, D. Kokalj, M. Strojnik, N. Ogrinc (2021) *Foods*, 10 (7), 1550.
[3] L. Strojnik, D. Potočnik, M. Jagodic Hudobivnik, D. Mazej, B. Japelj, N. Škrk, S. Marolt, D. Heath, N. Ogrinc (2022) *Food Chemistry*, 381, 132204.

The Next Generation LC-IRMS for Honey Authenticity Investigation

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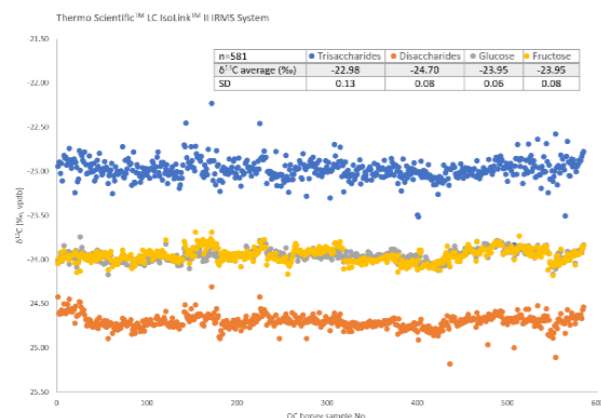
LC-IRMS is a state-of-the-art technique for the detection of low level adulteration of authentic honey with sugar syrups. [1] The new Thermo Scientific™ LC IsoLink II™ IRMS System delivers a reliable, robust and efficient solution for high precision honey fraud detection using compound specific carbon isotope signature of individual sugars.

The next generation LC-IRMS platform features patented technology and a unique design that simplifies routine maintenance, minimizes flow path blockage and significantly enhances system uptime and productivity. The fully integrated Thermo Scientific™ Vanquish™ Core HPLC and LC IsoLink™ II Conversion Interface design is space-saving and offers single vendor solution for lower cost of ownership.

Full LC IsoLink II IRMS System operation is driven by Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS)

Software which features complete integration with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software capabilities. Using a single software platform simplifies workflows, saves time and enables high efficiency.

To assess long-term stability, system robustness and data reproducibility, the LC IsoLink II IRMS System has been operated in an analytical food testing laboratory for over 2 years, allowing thorough system evaluation and optimization. We report data demonstrating excellent precision and reproducibility of $\delta^{13}C$ values for measurements of a laboratory honey standard and commercial honey samples.

References

[1] L. Elflein, K. P. Raezke (2008) *Apidologie*, 39, 574-587.

Control of Geographic Origin of Saffron Using Stable Isotope Analysis (IRMS)

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Saffron is the most expensive spice, due to the laborious harvest of the filaments (which are the saffron) of *Crocus sativus*. Only a few countries produce most of the world´s saffron production, with the Iran the by far biggest producer. Other relevant producing countries are Morocco, India, Greece and Spain, whereas in several other countries only small quantities of saffron are produced. However, most of the saffron gets higher prices on the local markets than saffron of foreign geographic origin. As saffron of local production is more highly esteemed by the consumers, there is the need to control the declare geographic origin of saffron.

In this study saffron samples of various origin are investigated by stable isotope analysis (SIA), because this method has already been successfully established to control the declared geographic origin of other food stuff. Additionally, further methods, as $^{87}Sr/^{86}Sr$ and molecular markers are applied.

The SIA results show that $\delta^{15}N$ is a very potent marker for the control of declared provenance. Furthermore, also $\delta^{13}C$, $\delta^{18}O$ and δ^2H are important parameters.

The variations in $\delta^{15}N$ can be explained by differing agricultural methods with respect to fertilization of the fields and with varying $\delta^{15}N$-values in the bio-available nitrogen in the soil. The differences in $\delta^{13}C$ are mainly due to differing levels of water availability and thus drought stress that the plants experience. The parameters $\delta^{18}O$ and δ^2H are mainly linked to the isotopic composition of the water to that the plants have access. We show that SIA enables the differentiation of many of the samples of different geographic origin, even though, not all of them. Due to the processes influencing and determining the respective isotope ratios, reference samples of identical vintage are required, to enable a reliable investigation and control of geographic origin. Nevertheless, we document that SIA can be a very potent tool for the verification of geographic origin.

GC-C-IRMS on Single Fatty Acids and EA-IRMS on Bulk Lipid to Study the Fractionation Processes in Bovine Organism and to Detect Differences in Four Matrices of Simmental Cows Fed on C3 and C4 Diets

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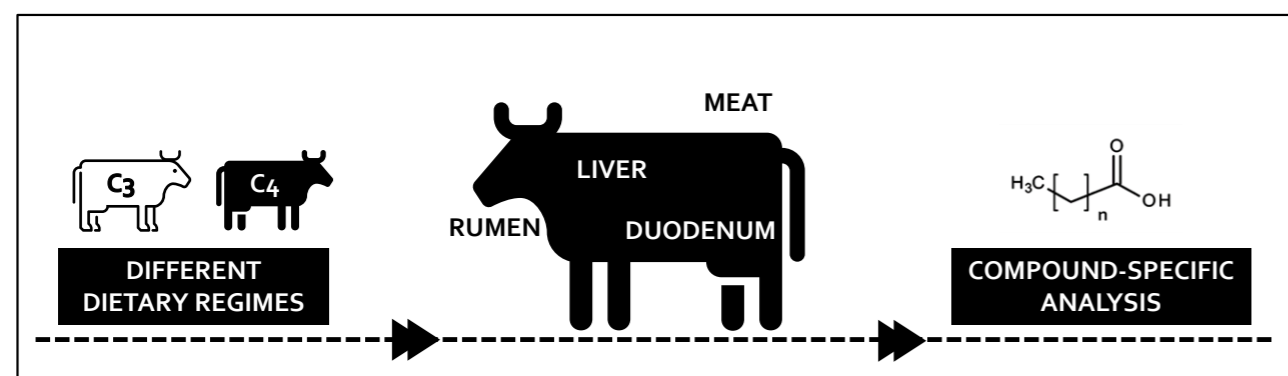
Fatty acids (FAs), carboxylic acids with a long aliphatic chain, detectable in both adipose tissue and muscle of animals, strongly contribute to different aspects of meat quality and are central to the nutritional value of this product [1].

Focusing of bovine meat, we must consider that the FAs may derive either from the animal diet only, as is the case with essential linoleic and linolenic acid, or from *de novo* endogenous synthesis, or both [2]. As for the biosynthetic pathway the FAs follow in cow organism, dietary FAs undergo substantial transformations into the digestive tract before depositing into the tissues. First, the hydrolyzation of complex lipids deriving from the diet, carried out by bacteria and protozoa in the rumen, produces long chain fatty acids (LCFAs) and other organic compounds [3]. Then, the free FAs released during hydrolysis are converted to saturated ones, primarily stearic and secondarily palmitic acid through biohydrogenation [3]. On exiting the rumen, the FAs flow into the duodenum, where the absorption takes place. Furthermore, the FAs reach the liver carried by the blood, whose flow, together with the FAs concentration, influences their supply to this organ [5].

In this work, two groups of multiparous cull cows fed according to two different dietary regimes (based on products deriving from plants characterized by either C3 or C4 photosynthetic cycle) were considered. The different paths C3 and C4 plants follow for CO_2 fixations result in discriminating carbon isotopic ratios ($\delta^{13}C$). Therefore, the ability to distinguish between animals directly comes from the isotopic differences in the feeding regimes. Different cow compartments (rumen, duodenum, liver and meat) led to the diet-based discrimination of the animals.

The presented results were obtained by analysing the $\delta^{13}C$ of both the bulk lipidic extract through EA-IRMS and six FAs through GC-IRMS in each compartment.

Furthermore, it is worth considering that several chemical reactions resulting in isotopic fractionation take place in the bovine organism. On this basis, the compound-specific analysis of the fatty acids in the different compartments of all cows gave the opportunity to compare the fractionation processes taking place in the bovine organism and to highlight differences depending on the dietary regime of the cows, whether C3- or C4- based.

Acknowledgements

References

[1] J. D. Wood, M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, F. M. Whittington (2008) *Meat Science*, 78 (4), 343-358.

[2] C. Galli, P. Risé (2006) *European Journal of Lipid Science and Technology*, 108 (6), 521-525.

[3] A. Buccioni, M. Decandia, S. Minieri, G. Molle, A. Cabiddu (2012) *Animal Feed Science and Technology*, 174 (1-2), 1-25.

[4] W. W. Christie, X. Han (2012) *Lipid Analysis*, 3-19.

[5] J. F Hocquette, D. Bauchart (1999) Reproduction, Nutrition, Development, 39 (1), 27-48.

Linking Multi-Elemental and Sr Isotopic Data of Milk, Cheese, Water, Soil and Forage

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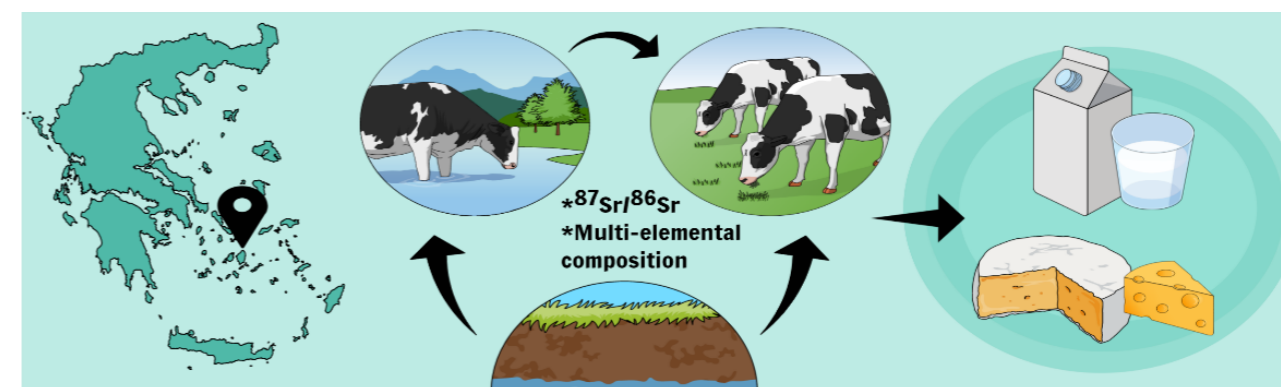
The strontium isotope ratio analysis is underused, relatively new approach that has shown to be a suitable tool for the designation of origin of many food commodities [1-3]. This potential stems from the fact that $^{87}Sr/^{86}Sr$ ratio is not modified during the uptake of the plant, but rather transferred unchanged to the living organisms in the food chain [4]. Furthermore, Sr isotope ratio is determined by the type of bedrock and soil, rather than human activities, climate, and changes in production season, thus precisely reflecting the geological composition of the soil where forage is grown. Milk and cheese composition depend primarily on the feeding of animals, and therefore on the surrounding environment [5].

In the presentation the results of a study on linking multi-elemental composition and Sr isotope ratios of soil, water, feed, milk and cheese from Naxos, Greece will be presented. Geologic setting of the island reflects the inorganic pattern of food produced there, such as widely-recognized PDO cheeses. The aim of the study was to determine the contributions of multi-elemental and Sr isotope compositions of water, soil and feed on milk and cheese, which would allow a more accurate characterisation of the milk and cheese provenance and provide an integrated overview on links between studied matrices and areas.

For the study purpose, multi-elemental composition and Sr isotope ratio were determined in samples of different types of milk and cheese, water, soil and feed. Samples were collected at different farms, during summer and winter season, through two years of production. The contribution of Sr isotope ratio from water and feed in the milk and cheese Sr isotope ratio was evaluated.

The elemental composition of the samples was determined by inductively coupled mass spectrometry (ICP-MS) after microwave digestion of samples, while Sr isotope ratio was determined by multi-collector ICP-MS, after performing Sr isolation from the matrix procedure.

Acknowledgements

References

[1] S. Hamzić Gregorčič et al. (2021) *Foods*, 10, 1729.

[2] E. Epova et al. (2019) *Food Chemistry*, 294, 35-45.

[3] M. Cardin et al. (2022), *Foods*, 11 (21), 3379.

[4] J. Griboff et al. (2019) *Food Chemistry*, 283, 549-558.

[5] D. Potočnik et al. (2020) *Food Chemistry*, 326 (3), 126958.

Age Verification of 10-yr and 20-yr Matured Port Wines using Radiocarbon

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The more exquisite port wines are those being 10, 20 or 30 years old. The labels of these products explain that these wines have matured on casks for this specific period of time. These statements convey to the consumer the idea of additional value, quality and taste of the product. EU-regulations require that claims about the content of food and beverages on product labels are correct and not misleading.

Port wines (like other fortified wines such as Sherry and Madeira) are basically produced as follows: first, the grape fermentation process starts just like in normal wine. Then, at an ethanol percentage of ≈ 6%, the fermentation process is stopped by adding highly concentrated wine-ethanol (called aguardente), up to ≈20%. In this way the remaining sugar is retained. Then, this fresh port wine is allowed to mature for a number of years to obtain these aged port wines. During this process, and/or at the end, some fresh aguardente is added to compensate for evaporation.

It is common practice to blend wines with different maturation ages to obtain wines that fulfil the defined and verified requirements regarding taste, smell, looks and several other aspects of matured port wines with a certain age. This is allowed as long as the average maturation age meets the claimed age on the product.

Of course, these matured products are expensive to produce, and prices for such aged port wines tend to be much higher than for young ones. However, some of these port wines are still sold in discounters for relatively low prices. This fact made a team of research journalists suspicious, and they approached us with the question if we could verify the acclaimed age.

We designed a method based on radiocarbon (^{14}C), in which we could put the so-called "bomb peak" to use. Thanks to this huge elevation of the atmospheric 14C concentration in the recent past, it is possible to determine the growth year of crops to almost a single year. This enabled us to verify the (average) maturation ages for these port wines by measuring radiocarbon in the ethanol and sugar fractions of these wines. The ethanol results are somewhat complicated to interpret, as the ethanol in the port wines originates from both the original grapes and from the aguardente additions. The sugars, on the other hand, originate entirely from the original grapes. Thanks to earlier work on wines and our atmospheric ^{14}C monitoring, we had proper knowledge of the radiocarbon content (and its variability) to be expected in Portuguese grapes for different harvest years.

Our results show that although the age claim of the majority of the investigated port wines is justified, quite a few have significantly younger maturation ages than stated on the bottle. We verified our method using so-called vintage port wines: matured port wines from just one single harvest year. Our results were in good agreement with the harvest years on the various bottles.

Our method, although firmly based on our knowledge of ^{14}C in crops and on our analysis methods, has still to be further verified. However, if our results stand, some of these port wine producers could be accused of fraudulent behavior.

Acknowledgements

Analytical Challenges in Determining the Origin of Olive Oil Using $^{87}Sr/^{86}Sr$ Isotope Ratios

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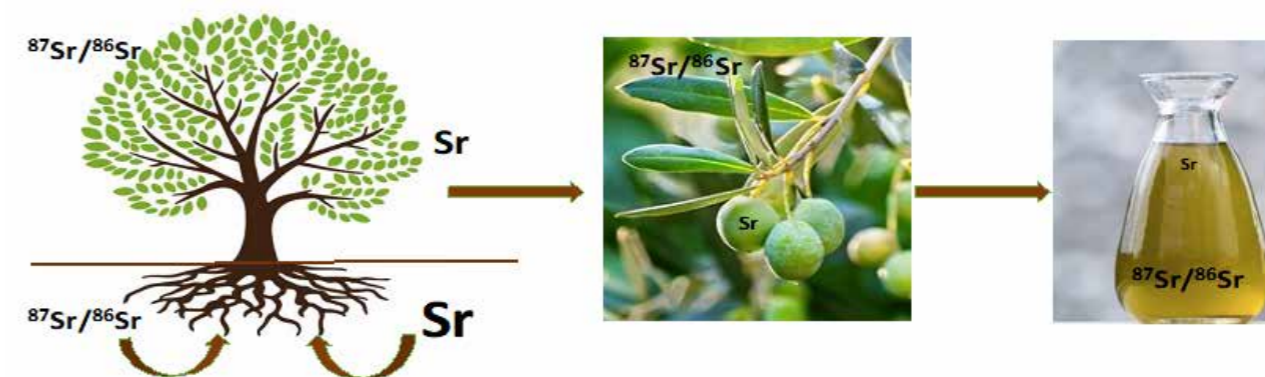
Olive oil is a very appreciated food with its beneficial effects to human health known for centuries [1]. Due to its great popularity and consecutively its high commercial value, olive oil is one of the most frequently counterfeited foods. There are mainly two types of falsification; one is adulteration and the other is mislabelling of the production area. Numerous analytical methods are in use for the discrimination of the olive oils' geographic origin, such as analysis of volatile compounds, fatty acids and triacylglycerol composition, trace elements and stable isotopes of light elements. Sr isotope composition could be also used to trace the geographical origin of olive oil, as it has already been successfully applied to other food matrices. The Sr isotopes do not fractionate during the uptake of Sr from the soil into the plant and during the relocation in the plant itself, thus reflecting the geological composition of the soil.

The olive oil has a complex matrix with high viscosity, organic load and very low content of trace elements, including Sr. Therefore, the first challenge is to obtain an accurate $^{87}Sr/^{86}Sr$ isotope ratio in the oil. The method involves numerous steps; extraction of Sr from the oil, digestion of the remaining organic matter, separation of Sr from the matrix (Rb), and measurement of isotopic ratios [2]. The Sr mass balance should be carefully followed throughout all analytical steps in order not to lose Sr and artificially cause its isotope to fractionate. In the presentation a method development for $^{87}Sr/^{86}Sr$ isotope ratios in olive oil using MC-ICPMS will be presented.

Most traceabilty studies focus on the differences between the $^{87}Sr/^{86}Sr$ isotope ratios of the products themselves. There are only few studies that examine the link between the olive oil and the corresponding soil, and a reliable protocol for geographical authentication of olive oil is yet to be adopted. An appropriate analytical approach should mimic the natural processes of Sr uptake from the soil. Usually, this is achieved by determining the $^{87}Sr/^{86}Sr$ isotope ratios in the bioavailable fraction of the soil. But does this really work for all types of soils? In the presentation the effects of different extractants used for Sr extraction from the soil on the $^{87}Sr/^{86}Sr$ isotope ratio and how are they linked to the oil will be discussed.

Acknowledgements

References

[1] N. P. Kalogiouri et al. (2020) *Analytica Chimica Acta*, 1134, 150.

[2] M. Furdek Turk et al. (2022) *Journal of food composition and analysis*, 112, 104675-1.

Geographical Origin Discrimination of Wines by a New Multi-Isotopic Dilution Method

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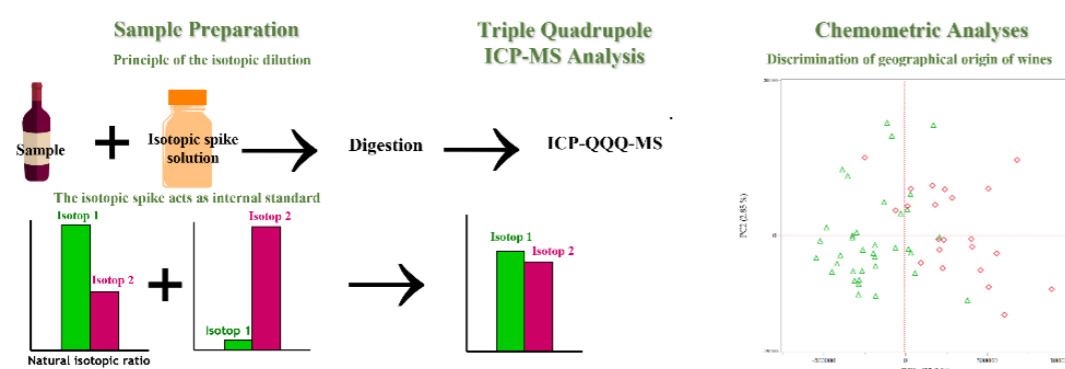
Wine is one of the oldest alcoholic beverages consumed worldwide. Since beverages are among the foods most likely subject to fraudulent activities, wine traceability and authenticity represent key factors for consumers' and producers' protection [1].

It has been shown that chemistry is able to link wines to their place of origin since the wine composition is strongly affected by the geochemistry of the soil in which the vine has been cultivated, as well as by potential anthropogenic contaminations and agricultural practices [2].

ICP-MS methods are particularly suitable to evaluate trace elements composition in foodstuffs, and their application for food traceability has rapidly increased through the years [3]. In this study, a mix of 22 major and trace elements measurable by ICP-MS and proved to be relevant for geographical discrimination purpose have been selected.

A new approach has been developed to measure simultaneously these 22 elements. It involves a quantification by isotopic dilution to improve the precision of the measurements whenever possible (one of the key factors achieve a good

discrimination) and the use of a triple quadrupole ICP-MS to eliminate potential interferents.

The presentation will introduce the development of this new method, its analytical performances, and its application to discriminate the geographical origin of wines at the country or even at the region level with examples from Spain, Italy and Tunisia.

Acknowledgements

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References

[1] E. N. Epova, S. Bérail, F. Seby, V. Vacchina, G. Bareille, B. Medina, L. Sarthou, O. F. X. Donard (2019) *Food Chemistry*, 294, 35-45.

[2] M. Suhaj, M. Korenovská (2005) *Acta Alimentaria*, 34, 393-401.

[3] M. Aceto (2016) *Advances in Food Traceability Techniques and Technologies*, 137-164.

PROMEDLIFE: Novel Food Products for the Promotion of Mediterranean Lifestyle and Healthy Diet

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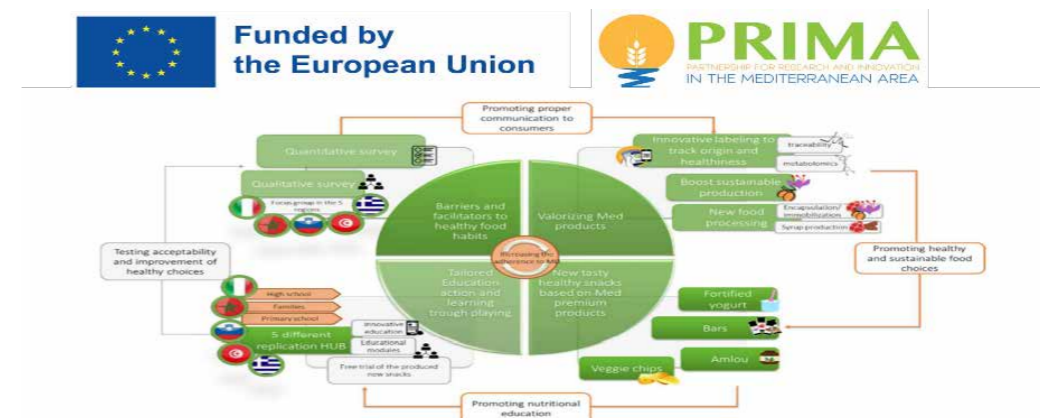
Mediterranean diet (MD) has long been considered a landmark in healthy nutrition, but the younger population lost the connection with traditional foods while older people experience life changes that result in significant swaps in their diet. Both groups could benefit from a diet rich in antioxidants that has been shown to help prevent disease over the long term. PROMEDLIFE aims to reverse, through a multi-actor approach, the decline in adherence to the MD pattern regional considering cultural specificities and applying validated approaches in different cultural environments. Four lines of intervention will be adopted: 1) The project will analyze socio-economic, cultural and personal factors driving consumers in adopting a healthy lifestyle. 2) PROMEDLIFE will promote tailored and country-specific actions using "learning through play and living labs approaches" targeting primary and high school students and their families/carers. 3) Part of the work will be devoted to creating new healthy snacks targeted at young and older adults, based on traditional Mediterranean premium ingredients (saffron, dates, argan oil, selected vegetables). The products will be tested in each country in terms of acceptability and ability to promote healthy choices as well as characterized chemically and according to their bioactivity. New cultivation technologies and agronomical practices (i.e. soilless culture approaches and/or selection of local varieties for saffron and Argan trees) and innovative food processing technologies (e.g., encapsulation/immobilization) will be applied and tested. 4) PROMEDLIFE will codify and valorize local Mediterranean products through the development of food labelling using innovative tools to increase people's connection with their cultural and local heritage and improve awareness of food healthy choices. Revisiting the MD proposed in a more modern and appealing

way through a transversal approach will guarantee the link between the innovativeness of PROMEDLIFE and the productive sector. PROMEDLIFE will aspire to understand the factors affecting consumers' healthy choices and turn to education to promote adherence to Mediterranean lifestyle in the young generation and their families. PROMEDLIFE will conserve local biodiversity and exploit its uniqueness for both cultural and regional economic development while improving the value chain and market competitiveness of locally produced sustainable Mediterranean food.

Authentication and Traceability of Fish and Seafood Using Stable Isotope and Multi-Elemental Approach

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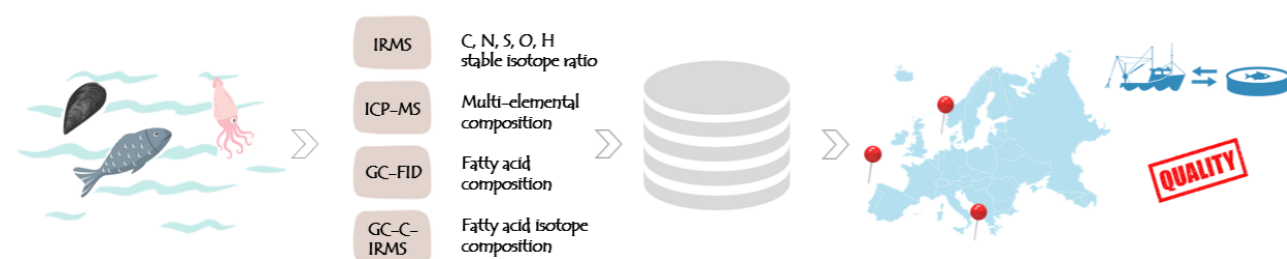
Aquatic products are highly desirable since they provide many important components in the daily diet, such as essential omega-3 fatty acids, high-value proteins, vitamins and minerals [1]. The expanding commercialisation of products derived from fisheries and aquaculture provides many opportunities for mistreatment and fraud. To avoid and reduce the risks of adulteration and counterfeiting of fish and seafood, the development of analytical techniques, databases, and chemometric approaches is needed.

This study demonstrates how stable isotopes of light elements, fatty acid composition and elemental profiles can be used to verify the declared geographical origin of fish and seafood, differentiate between organic and conventional fish production and assess the quality of product and its environment to prevent or reduce fish and seafood fraud. We expect that the farmed fish will have significantly different isotopic signatures compared to wild animals.

Representative samples from three different areas (Mediterranean Sea, Atlantic Ocean, and North Sea) will be selected to establish a fish and seafood authentic database. The database will include stable isotope data of light elements (δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O, δ^{2}H), carbon stable isotope values of individual fatty acids and fatty acids composition as well as elemental composition.

From this data, we will construct an appropriate statistical model to identify the biomarkers that differentiate between organic and conventional fish production (aquaculture) and to discriminate between samples' origins. Moreover, the nutritional value and quality of selected fish and seafood samples will be evaluated, and their impact on environmental and human health will be assessed. The final part of this study is dedicated to verifying possible mislabelling (production type and geographical origin) of fish and seafood samples from the market.

Overall, the developed methodology in this study will provide a sound basis for establishing an adequate traceability system for fish and seafood.

Acknowledgements

References
[1] J. Oehlenschläger (2013) *International Journal for Vitamin and Nutrition Research*, 82, 168-176.

Foodtrack – Stable Isotope Tool for Determination of Authenticity and Traceability of Food

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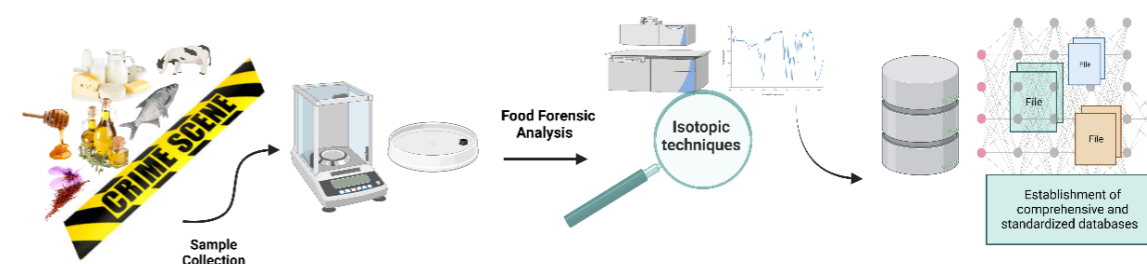
Food fraud practices for economic gain significantly decrease the value of food products, often mislead the consumer causing them to lose their trust and can lead to severe economic and public health risks [1]. Food authenticity testing requires robust and sophisticated analytical techniques such as the stable isotopic analysis.

Stable isotope-ratio signatures (^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, and ^{34}S/^{32}S) play an increasingly important role in food forensics in three main areas of application: (i) detection of adulteration; (ii) assignment of geographical origin; and (iii) identification of the mode of production. However, a common requirement in food authenticity and traceability studies is the need for a standardized and regularly updated stable isotope reference database.

This presentation deals with developing the FoodTrack database, which includes data about products that are most susceptible to fraud (milk, saffron, olive oil, honey, fish and seafood). In this study, stable isotopes and elemental composition are applied to investigate the following parameters: annual effects, seasonal changes, and region of production. The database will be populated with verified data from the literature and authentic samples data to secure food chain traceability and verify the products' claims and labelling.

The FoodTrack database has been structured to enable further multivariate data analysis, Geographic Information Systems (GIS) applications, and modelling. Four different ways of evaluating the results will be presented in the FoodTrack web-based tool covering:

(i) FoodTrack data visualization in a graphical mode (maps) that allow the users to see and understand trends, outliers, and patterns in the dataset; (ii) clustering - an unsupervised data mining approach to group samples according to the year and season of production; (iii) discriminant analysis to differentiate food products according to the year, season, and region of production; and (iv) driven soft independent modelling of class analogy (DD-SIMCA) to verify the declaration of samples. These methodologies trace the food supply chain, but do not provide information on some of the decisions that are made based on the data. Thus, a new approach by involving Explainable Machine Learning will be used to classify a given food product and provide an explanation about the prediction that is made.

This approach will serve as the basis for further development of isotopic mapping (isoscapes), providing a cost-effective extension to the isotopic dataset approach.

Acknowledgements

References
[1] P. Visciano, M. Schirone (2021) *Trends in Food Science & Technology*, 114, 424-442.

Oxygen and Hydrogen Isotopes in Milk and Cheese Casein from DPO Cheese Products in Greece

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Hydrogen ($^2H/^1H$) and oxygen ($^{18}O/^{16}O$) isotopic ratios in milk and cheese casein has been used to check the authenticity of investigated samples from selected sites in Greece. Eight (8) bulk samples of Holstein cow's milk, nine (9) bulk samples of sheep and goat's milk, and one (1) cheese sample were collected in January 2021 from livestock farms in Naxos Island, South Aegean, Greece. Moreover, two (2) DPO graviera cheese samples were sampled from different geographic areas in Greece. Finally, two (2) mixed milk samples intended for cheese-making were subjected to isotopic analysis before and after the pasteurization. Three milk samples were taken from milk coolers in 25 ml falcon bottles from each station and subjected to isotopic analysis to determine their hydrogen ($^2H/^1H$) and oxygen ($^{18}O/^{16}O$) isotopic ratios in milk casein using the procedure described by Potočnik et al. [1]. All data are presented in Figure 1 where it appears that DPO Graviera Naxou has a different isotopic signature from the other DPO cheeses. Naxos mixed cow/goat/sheep milk samples present a shift to more positive $\delta^{18}O$ values in Graviera Naxou about $\Delta^{18}O_{milk-cheese}$= 1.9‰. This is close to the fractionation

effect presented in Table 1 originating from the pasteurization processes which is about $\Delta^{18}O_{pasteurization}$ = 1.3‰.

		MEAN ‰ V-SMOW		‰ V-SMOW
		δ^4H	$\delta^{18}O$	
JAN-21	Naxos mixed cow/goat/sheep milk samples (field sampling)	-93.2	12.9	
JAN-21	Naxos mixed cow/goat/sheep milk samples (intended for cheese-making before pasteurization)	-111	12.8	$\Delta^{18}O_{pasteurization}$=1.3
JAN-21	Naxos mixed cow/goat/sheep milk samples (intended for cheese-making after pasteurization)	-100.6	14.1	

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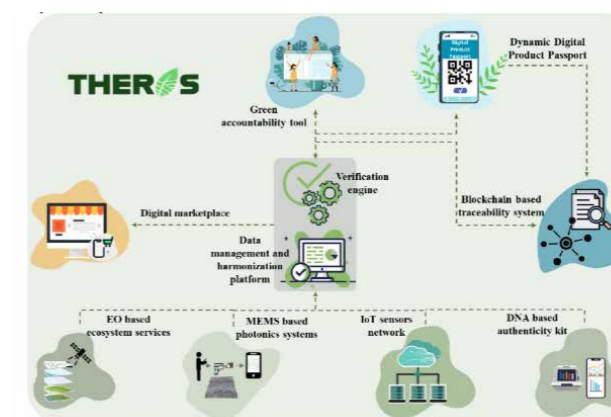
References

[1] D. Potočnik et al. (2020) *Food Chemistry*, 326, 3, 126958.

An Integrated Toolbox for Improved Verification and Prevention of Adulterations and Non-Compliances in Organic and Geographical Indications Food Supply Chain – THEROS Project

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Food fraud has increased on a global scale due to the growing demand for food along with economically driven motives. [1] The deliberate addition of inferior materials or substitution of vital food components in a foodstuff, to heighten appearance qualities and to gain greater profits, comprises food adulteration. [2] Additionally, with the expansion of the range of food products (i.e., organic and protected geographical indication), adulteration is becoming more intentional in the form of mislabelling. The ability to apply large scale inspections comes with high administrative burden, while at the same time conventional laboratory methods for detecting such frauds have a high efficacy rate, but their cost and time demands raise barriers, therefore alternatives should be explored towards the development of a functional system. New remote and field deployable technologies could address these challenges and contribute to this goal.

To this end, in this contribution we present the THEROS project that aims to develop and implement an integrated toolbox being capable to modernise the process of verifying organic and geographical indications food products and preventing adulterations and non-compliances, while demonstrating enhanced traceability, security and transparency in the supply chain, through the use of various technologies and innovations that leverage Earth Observation, photonics, internet of things(IoT), DNA metabarcoding, blockchain, digital interfaces and product passport, advance analytics, machine learning, artificial intelligence and business models. At the same time,

efficient mechanisms will be employed in order to ensure interoperability with existing control systems, as well as improved accessibility and sharing of data through harmonized and standardized means, whilst also demonstrating their uptake by relevant stakeholders for improved decision-making.

Acknowledgements

References

[1] H. Onyeaka, M. Ukwuru, C. Anumudu, A. Anyogu (2022) *Trends in Food Science and Technology*, 125, 26-32.

[2] M. Momtaz, S. Y. Bubli, M. S. Khan (2023) *Foods*, 12 (1), 199.

[3] P. Visciano, M. Schirone (2021) *Trends in Food Science and Technology*, 114, 424-442.

[4] [4] X. Wu, S. Shin, C. Gondhalekar, V. Patsekin, E. Bae, J. P. Robinson, B. Rajwa (2023) *Foods*, 12, 402.

[5] [5] A. E. Sharrad, P. Reis-Santos, J. Austi, B. M. Gillanders (2023) *Food Control*, 148, 109606.

Stable Isotope Ratios and Multi-Element Analysis for Food Authenticity and Traceability in the Framework of the TUNTWIN Project

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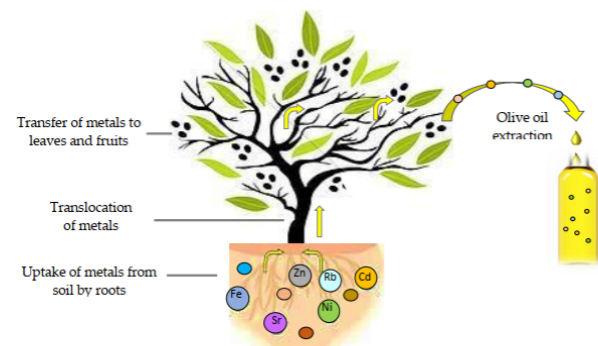
Figure I.2 : Schematic pathways of some trace elements from the soil to the olive tree and olive oil

The analysis of stable isotopes has proven ist efficiency in determining the geographical origin of food. In addition, isotope ratios can contribute, irrefutably, to the determination of the geographical and geological origin of the element. These key societal needs are highlighted in the current application of the TUNTWIN project notably for olive oil and wine, the two products of major economic interest for Tunisia.

The globalization of the food industry has raised consumer interest in the geographical origin and the quality of food products. The global increase in food production and consumption, however, has led to fraudulent practices spreading. It threatens both the health of consumers and the economic balance of the food industry, which suffers huge financial loss every year. Olive oil is one of the most adulterated food products. As a result, a large array of analytical strategies was proposed for the geographical authentication of olive oil. The most reliable approaches that have demonstrated promising results for the geographical traceability of food products were based on the multielemental and isotopic fingerprinting. Nevertheless, trace elements, initially found at low to critically low concentrations in olive oil, are dissolved in a complex lipid matrix and thus the samples introduction in plasma-based instruments and the precise measurements of chemical components are challenging.

The present work presents a reliable analytical approach based on the combination of three dimensional geographic informations in order to establish an advanced geographical authentication tool able to overcome the most sophisticated fraudulent practices: (1) the mineral composition of the soil through the analysis of trace elements; (2) the geological background through the analysis of Sr isotopic composition; and (3) the pedo-climatic context through the determination of stable isotopes of carbon in olive oils.

The trace elements were quantified in olive oils from Tunisia, Spain and France with high precision and accuracy by quadrupole ICP-MS following an optimized analytical procedure. The elemental concentrations combined with chemometrics allowed to classify olive oils according to their geographical provenance.

An innovative method was developed and successfully applied for the quantitative extraction of Sr from olive oil matrix and accurate measurement of $^{87}Sr/^{86}Sr$ isotopic ratio by MC-ICP-MS. The conservation of $^{87}Sr/^{86}Sr$ isotopic ratios during the transfer of Sr from the soil to the plant and during olive oil extraction was demonstrated.

The results were correlated with the geological characteristics of the bedrocks and thus highlighted that Sr isotopic composition of olive oil can be used as a reliable tool for fingerprinting olive oil geographic provenance.

The stable isotopes of carbon were determined in olive oils by IRMS and allowed to trace the physiological processes of the olive tree to specific environmental characteristics.

Acknowledgements

Combined Isotopic and Elemental Composition Analysis for the Assessment of *Spirulina* Food Supplements Authenticity

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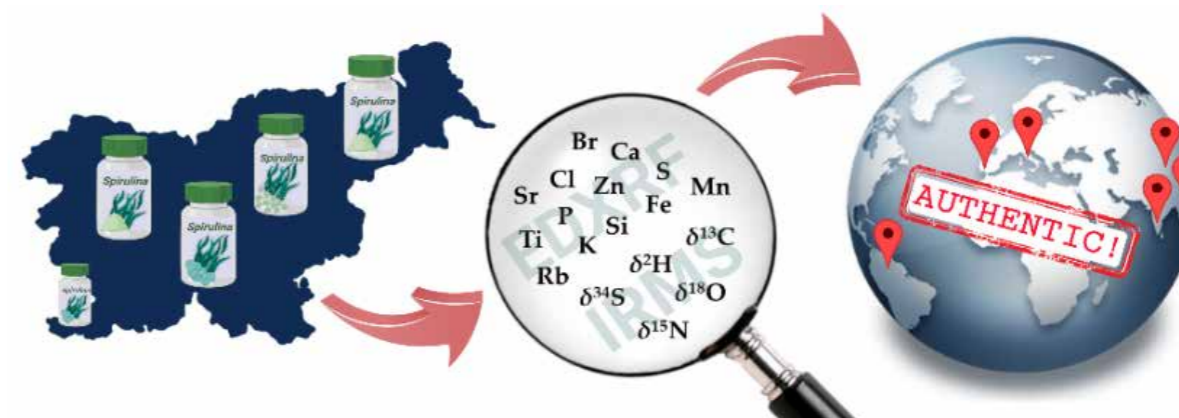
Different pedoclimatic conditions and agricultural measures influence algal growth and affect elemental and isotopic composition of final products. Combined, these parameters could be used as a tool for verifying the origin and authenticity of microalgal products and, in this way, provide a method for quality control [1,2]. This study characterizes the elemental and isotopic composition of light elements (C, N, S, H and O) of *Spirulina* food supplements in order to provide a quality overview of these products sold commercially in Slovenia.

Forty-six commercially available *Spirulina* dietary supplements were gathered from the Slovenian market. The samples were declared to originate from Hawaii, Italy, Japan, Portugal, Taiwan, India, European Union (EU), non-EU, China and were dried in powder, tablet or capsule form or fresh. C, N and S isotopic composition in the samples were measured by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) and O and H stable isotope ratio was determined by high temperature TC/EA IRMS. *Spirulina* elemental composition was analyzed using Energy Dispersive X-Ray Fluorescence Spectrometry (ED-XRF). Multivariate statistical analysis (Discriminant Analysis, Principal Component Analysis, and Orthogonal Partial Least Squares Discriminant Analysis) was applied for identification of characteristic parameters for discrimination of samples according to their isotopic and elemental composition.

The $\delta^{13}C$ values in analyzed *Spirulina* products ranged from -32.3 to $-16.7‰$, $\delta^{15}N$ from -5.35 to $13.8‰$, $\delta^{34}S$ from -1.75 to $13.8‰$, δ^2H from -207 to $-97.4‰$ and $\delta^{18}O$ from 12.8 to $27.2‰$. The content of micro-elements in descending order was: Fe > Mn > Sr > Zn > Ti > Br > Rb and of macro-elements: K > P > S > Si > Cl > Ca. Results showed 82.8% correct classification of the samples. A reliable differentiation (100%) of Italian, Portuguese and Hawaiian samples was achieved, followed by Chinese and Indian samples. Separation of Taiwanese samples was less successful (66.7%), but still notable. Different geographical location of production site, but also culturing and processing methods and different environmental conditions seem to be the parameters responsible for *Spirulina* product discrimination, regarding their elemental C, N and S isotopic composition. Conversely, oxygen and hydrogen isotopic composition of the analyzed products showed a correlation, similar to that in water, suggesting their origin to be mainly from local meteoric water, while other parameters' influence on their values is negligible.

This study shows that combination of macro- and micro-elemental and stable isotopic composition of elements C, N, S, O and H analyses represents a promising technique for commercial *Spirulina* products' authenticity assessment, relating to their composition and country of origin.

Acknowledgements

References

[1] S. A. Drivelos, C. A. Georgiou (2012) *Trends in Analytical Chemistry*, 40, 38-51.
[2] R. N. Singh, S. Sharma (2012) *Renewable and Sustainable Energy Reviews*, 16, 2347-2353.
[3] M. Cuaresma, M. Janssen, C. Vílchez, R. H. Wijffels (2011) *Bioresource Technology*, 102, 5129-5137.

Strontium Isotope Ratios of Finnish Strawberries and Agricultural Soils

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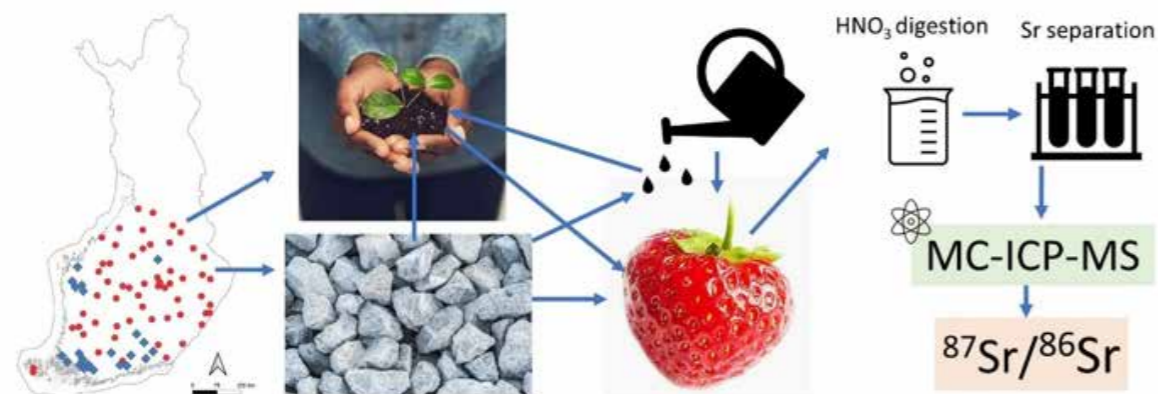
Discovering new methods to detect food frauds is crucial to maintain food security, support the local production and providing novel tools for the food authorities in the EU. Food frauds are an increasingly observed world-wide problem including both the forging of origin and consistency of food. *The development of strontium isotope method in identification of food forgeries* (SIKRUT) is a research project of the Finnish Food Authority aiming to develop a reliable method based on strontium isotopes ($^{87}Sr/^{86}Sr$) for identifying the frauds of food origin.

Strontium isotope ratios provide a well-known tool for tracing the origin of different food materials based on the local $^{87}Sr/^{86}Sr$ variation in the bedrock and soil [1]. Previously the method has not been widely applied in Finland due to the lack of local baseline. Finland is geologically very heterogeneous but well mapped and studied area, which makes it an interesting target to study $^{87}Sr/^{86}Sr$ variation in agricultural environments. This study presents the $^{87}Sr/^{86}Sr$ ratios and elemental concentrations from 120 Finnish strawberry samples and 47 soil samples from strawberry fields. The study covers a large part of the cultivated areas of Finland. Elemental concentrations were analysed using a sector-field inductively coupled plasma mass spectrometer (ICP-SFMS), and $^{87}Sr/^{86}Sr$ ratios with a multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) [2].

The study shows that both rock and soil type are connected to the variation of the $^{87}Sr/^{86}Sr$ ratios of the strawberries. In addition, a considerable spatial correlation with the $^{87}Sr/^{86}Sr$ ratios of the strawberries can be observed.

A comparison between the $^{87}Sr/^{86}Sr$ ratios of the strawberries, soils from the strawberry fields and the bedrock shows considerable differences between different proxies. The $^{87}Sr/^{86}Sr$ ratios of the strawberries are significantly lower than in the soils and the bedrock, and they do not show a clear linear correlation. Moreover, we found clear indications that irrigation practice (watering or no watering) and irrigation source (groundwater or surface water) effect on the $^{87}Sr/^{86}Sr$ ratios of the strawberries and the soils which might explain the differences in the isotope ratios of different proxies from the same geospatial locations.

References

[1] G. Åberg (1995) *Water, Air, Soil, and Pollution*, 79, 309-322.

[2] I. Rodushkin et al. (2016) *Journal of Analytical Atomic Spectrometry*, 31, 220-233.

Characterization of Slovenian Pork Meat Based on Stable Isotope Ratio, Multi-Elemental Analysis and Multivariate Modelling Approaches

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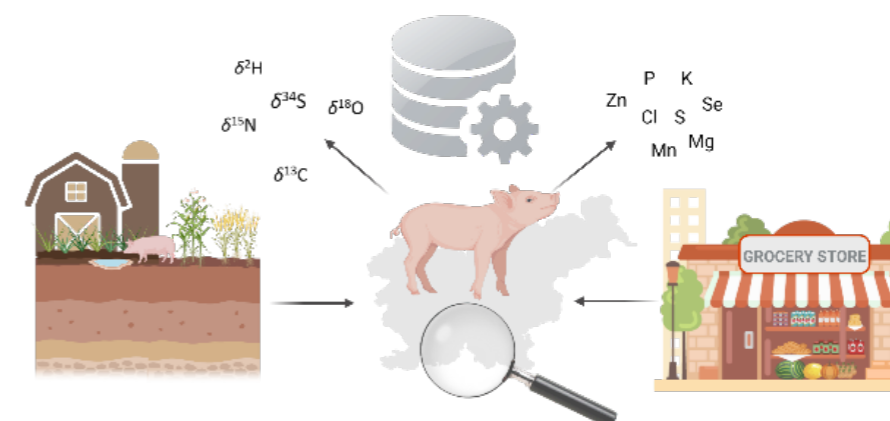
Food scandals and incidents, concern for health and improving socioeconomic circumstances have led to an increase in consumer demand for higher quality and safer food, which, in turn, has led to an increase in demand for sensitive and reliable methods for detecting food adulteration [1]. In Slovenia, pig farming has a long-standing tradition that ensures high-quality pork and pork-based products that consumers highly appreciate. However, the level of self-sufficiency in 2021 was 42.8%, meaning that a large proportion of pork is imported and subjected to false labelling.

This study examined the applicability of stable isotopes of light elements and multi-element data for determining the geographical origin, breed and feed type. Furthermore, a verification of whether pork on the Slovenian market conformed to its declaration was also performed, which requires comparing the samples to be checked against authentic samples. We, therefore, established a database of authentic meat samples: 70 Slovenian samples (28 Krškopolje breeds and 42 modern breeds of Slovenian pigs) collected directly from farms.

Linear discriminant analysis (LDA) and Orthogonal projections to latent structures Discriminant Analysis (OPLS-DA) were used to distinguish between samples from different geographical regions: Mediterranean, Pannonian, Alpine and Dinaric, breeds and types of feed, while Data-driven soft independent modelling of class analogy (DD-SIMCA) was used to verify the correct labelling of Slovenian pork. We found that it was possible to discriminate pork from four different geographical regions of Slovenia, although Alpine samples overlap with Pannonian ones. The first evaluation of results indicates a good separation of pork meat according to breed, using the OPLS-DA model. The overall prediction ability was 98% for breed differences.

Based on the analysis of stable isotopes and elemental composition, it was possible to distinguish diet type between Krškopolje and Modern Slovenian pigs. A model based on DD-SIMCA was also developed and applied to the origin control of Slovenian meat. All tested commercial samples were correctly labelled as Slovenian origin.

Further research with randomly selected samples from the market is needed to compare their geographical origin with authentic samples in the database.

References

[1] S. Zhao, H. Liu, M. Qie, J. Zhang, L. Tan, Y. Zhao (2021) *Food Reviews International*, 1-21.

Effects of Storage Conditions on Carbon Isotopic Composition of Volatile Organic Compounds

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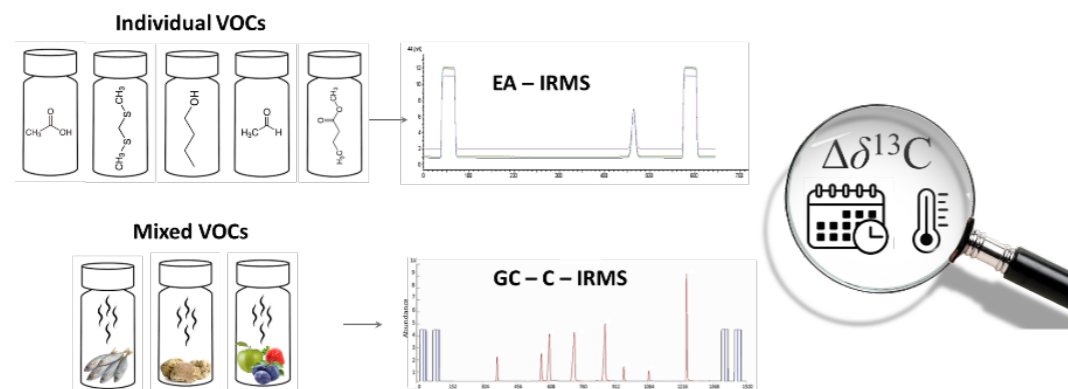
Compound-specific isotopic analysis (CSIA) is perhaps the most specific and sophisticated method for determining food authenticity. Studies have shown that it is possible to distinguish between natural and synthetic aromas based on the isotopic values of individual volatile organic compounds (VOCs) [1].

Reference materials (RMs) are vital for stable isotope ratio determination of light elements such as $^{13}C/^{12}C$. In-house RMs provide traceability to measurement results when used for normalisation or quality control and assurance purposes [2]. Guidance available for the use of isotope ratio RMs emphasises the need that RMs should be pure compounds, easy to use, stable and constant in isotopic content over a long period, chemically identical or close to the samples to be measured and at the same time their isotopic composition should be in the range of the samples to be measured [3].

However, the analytical procedures for organic stable isotope analysis are often non-standardised, and limited certified reference materials (CRMs) are available. The lack of reference materials makes data normalisation challenging since most CRMs are certified using EA-IRMS and generally cover non-volatiles.

This study was directed towards investigating suitable compounds to be used as stable isotope reference materials for determining the carbon isotopic composition of volatile organic compounds in fruits, truffles, and fish. We evaluate how the isotopic composition changes under different temperature conditions and storage times and which compounds meet the requirements for RM. We focus on various VOCs, such as alcohols, aldehydes, ketones, esters, and acids, which occur in food and are important for determining the authenticity and origin of products.

The study was performed in two parts. In the first part, selected standards of VOCs of natural and synthetic origin were stored at 2-8 °C and in a dark, well-ventilated place at room temperature. Measurements will be carried out within six months using an Elemental Analyzer – Isotope Ratio Mass Spectrometer (EA-IRMS). Changes in isotopic composition depending on the method and storage time will be assessed.

In the second part, laboratory mixtures of VOCs that best describe the matrix of fruits, truffles, and fish were prepared. The mixtures will be analysed using a Gas Chromatography – Combustion Isotope Ratio Mass Spectrometer (GC-C-IRMS). Changes in the isotopic composition of prepared mixtures of laboratory standards within six months will be monitored.

Acknowledgements

References

[1] L. Strojnik, J. Hladnik, N. C. Weber, D. Koron, M. Stopar, E. Zlatić, D. Kokalj, M. Strojnik, N. Ogrinc (2021) *Foods*, 10 (7), 1550.

[2] P. J. H. Dunn, D. Malinovsky, G. Holcombe, S. Cowen, H. Goenaga-Infante (2021) *Rapid Communications in Mass Spectrometry*, 35 (20), e9177.

[3] R. A. Werner, W. A. Brand (2021) *Rapid Communications in Mass Spectrometry*, 15 (7), 501–519.

Isotope Fingerprints: Addressing Authenticity of Fish Oils by GC-MS-IRMS

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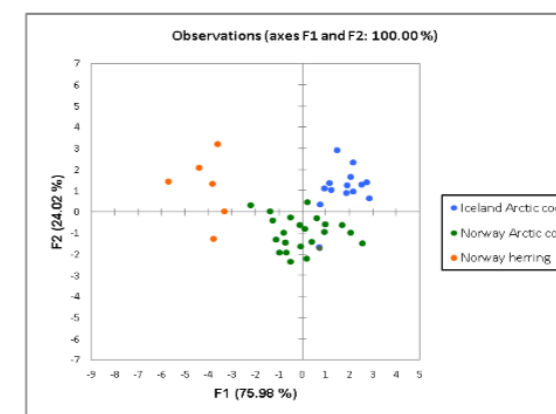
As fish oils become a popular and precious source of omega-3 fatty acids, the risk of mislabelling and adulteration has risen significantly. The fatty acid profiles of different fish oils do not often allow the discrimination between different sources and geographical origins. In this study, the compound specific multi-isotope analysis of fatty acids in 30 salmon oils and 43 cod liver oils were performed allowing the discrimination of fish oils from different provenance, following risk-based comparisons from market experience.

In the light of emerging cases of food fraud, we present how Thermo Scientific™ GC-MS-IRMS advanced technology can tackle these problems for addressing authenticity of fish oils. By coupling GC-IRMS with an organic mass spectrometer (MS), the isotopic compositions and the comprehensive qualitative and quantitative sample information with high levels of selectivity, sensitivity, and confidence are accessible simultaneously from a single injection.

Authentication and Geographical Characterisation of Italian Grape Musts through Glucose and Fructose Carbon Isotopic Ratios Determined by LC-IRMS

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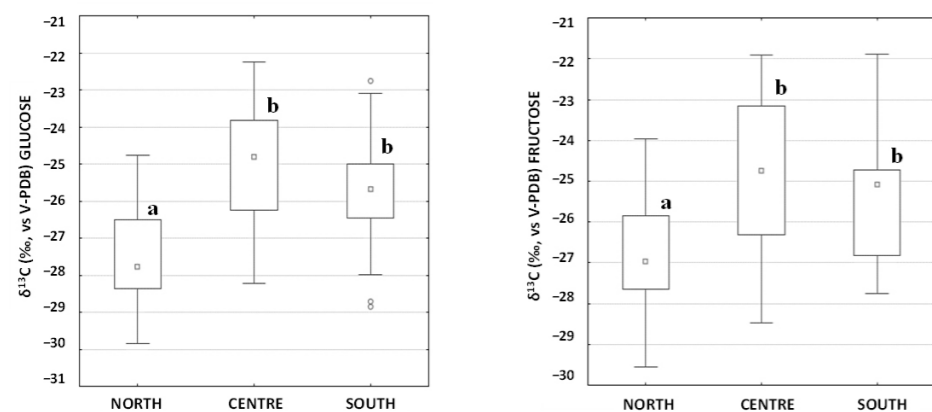
Figure 1. Box-Whisker plot of the glucose and fructose LC-IRMS analysis divided by the origin of the sample (north, centre or south Italy). Different letters identify statistically different groups according to the ANOVA ($p < 0.01$).

The authenticity of grape musts is normally checked through the stable isotopic analysis of carbon ($\delta^{13}C$) after fermentation and distillation by following the official OIV MA AS-312-06 method. Unfortunately, it presents some issues that are difficult to overcome. Grape must samples can only be analysed after they have been fermented to obtain ethanol. The process must be carried out under careful control of the fermentation to avoid the presence of unwanted by-products arising from a premature fermentation interruption. Moreover, if the musts have been preserved by the addition of sulphur dioxide (SO_2), they must undergo an additional step to eliminate the SO_2, which would affect the fermentation. Once the product has been fermented, the ethanol must be separated using specific distillation columns (such as the Cadiot ones) making it possible to obtain ethanol free of isotopic fractionation with a minimum alcohol degree of 95% vol.

In this study, the alternative use of a technique based on $\delta^{13}C$ isotopic analysis of the major sugars of the grape must by liquid chromatography coupled with isotope ratio mass spectrometry (LC-IRMS) is provided. In LC–IRMS, analytes are separated on an LC system and consecutively oxidized in an online reactor to CO_2, which is required for the determination of compound-specific carbon isotopic ratios. This technique has been already used in the study of matrices such as wine [1], ethanol [1,2], glycerol [2], and honey [3] to detect fraudulent alterations of their natural composition such as the addition of exogenous sugars to the products. The LC-IRMS allows a single separation of the individual

components of a sample and makes it possible to determine their $\delta^{13}C$ values online, avoiding both the disadvantages of off-line methods and the disadvantages of methods requiring a derivatization step (such as GC-C-IRMS), causing the addition of extra carbons.

In order to discriminate between musts from different areas of Italy, a preliminary dataset was considered; the $\delta^{13}C$ isotopic ratios of glucose and fructose of around 100 authentic Italian must samples from 16 different sampling regions were analysed. In addition, the $\delta^{13}C$ variability in authentic and fake must (added with increasing percentages of exogenous sugars) has been explored and tested to verify their validity as fraud detectors. The two analysed parameters, ranging from −29.8‰ to −21.9‰, are well correlated ($R^2 = 0.7802$) and the northern Italian regions showed significantly more negative $\delta^{13}C$ values for both sugars than the rest of the dataset (Figure 1). By using the LC-IRMS technique, the addition of exogenous sugars, such as fructose and glucose from C_4 photosynthetic cycle plants, is easily detectable as it modifies the $\delta^{13}C$ of the individual sugars.

References

[1] F. Guyon, L. Gaillard, M.-H. Salagoïty, B. Médina (2011) *Analytical and Bioanalytical Chemistry*, 401 (5), 1551-1558.

[2] A. I. Cabañero, J. L. Recio, M. Rupérez (2008) *Rapid Communications in Mass Spectrometry*, 22 (20), 3111-3118.

[3] L. Elflein, K.-P. Raezke (2008) *Apidologie*, 39 (5), 574-587.

Differentiation of the Mineral Content of Slovenian Honeys by Botanical Origin Using Principal Component Analysis

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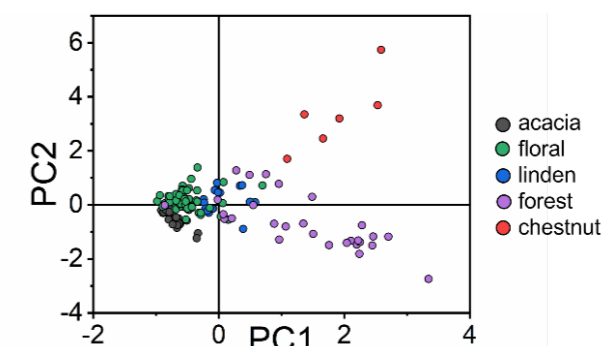
Honey is a substance derived from nectar and secretions from different plant species and is produced by honeybees. It is mixture of various types of sugars and water, which combined represent up to 95 % of the total weight. The remainder is made up from proteins, amino acids, vitamins, and minerals [1].

The composition of honey, presence and ratios of its components, is indicative of the botanical (plant species) and geographical origin (geology, climate) [2,3].

Mineral content in honey is very low (below 0.2 %) and is somewhat linked to its colour – lighter honeys have lower metal content, while darker honeys contain higher levels of minerals. Despite this it has been shown that the mineral content is dependent on the nectar or secretion source of the honey and thus it is possible to differentiate honeys by their botanical origin [2,3].

To achieve this an elemental analysis of many honey samples is done and the results are statistically evaluated (e.g., Principal Component Analysis) [1-4].

The present study focused on Slovenian honeys of many types and the possibility to differentiate them by their botanical origin based on the mineral content. For this purpose, 142 honey samples of five varieties (floral, forest, acacia, linden, and chestnut) were collected and digested in a microwave digestion system using a mixture of concentrated nitric(V) acid and hydrogen peroxide. Concentrations of elements in the sample solutions were measured by ICP-MS.

The mass percentages of 24 elements were determined, some were found in expectedly significant quantities (Na, Mg, K, Ca, and Fe), while most in low levels or below the detection limit.

A selection of 12 elemental profiles (B, Mg, Al, K, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Ba) were used for PCA analysis to distinguish between the honey varieties. The first three principal components were calculated, covering 42.6 %, 18.3 %, and 10.2 % of the variance, respectively. A 2D score and loading plot and a 3D score plot were graphed.

Successful clustering of honey types was accomplished. Floral, acacia and linden honeys are close to the origin and are discernible from each other. Floral and acacia honeys are in compact clusters, while linden honeys are spread out. Forest and chestnut honeys are grouped in a wider, oval shape and reach away from the origin at different angles.

References

[1] L. Strojnik, J. Hladnik, N. C. Weber, D. Koron, M. Stopar, E. Zlatić, D. Kokalj, M. Strojnik, N. Ogrinc (2021) *Foods*, 10 (7), 1550.

[2] P. J. H. Dunn, D. Malinovsky, G. Holcombe, S. Cowen, H. Goenaga-Infante (2021) *Rapid Communications in Mass Spectrometry*, 35 (20), e9177.

[3] R. A. Werner, W. A. Brand (2021) *Rapid Communications in Mass Spectrometry*, 15 (7), 501-519.

The Potential of Stable Isotope Technique and Microbiome Characterisation in Determining Truffle Aroma Formation and the Authentication of Truffles

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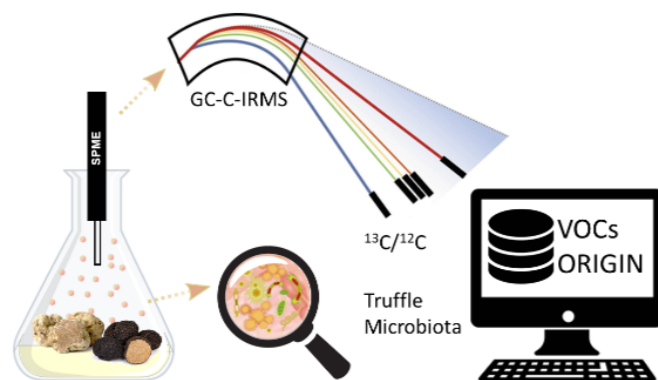
Volatile organic compounds (VOC) released by truffles enable the truffle to communicate with plants (symbiotic hosts), animals (vectors) and other microorganisms (decomposers). In addition to their biological function, VOCs produce the unique truffle aroma, which is highly appreciated by people, is of great gastronomic importance, and is very susceptible to food fraud (1).

This study describes the development of a procedure including $\delta^{13}C$ measurements using HS-SPME GC-C-IRMS methodology, microbiome characterisation, data processing and database creation of authentic truffle VOCs.

In this work, the $\delta^{13}C$ values of 21 aroma compounds were determined in 87 fresh ascocarps from six truffle species (*Tuber aestivum, Tuber magnatum, Tuber mesentericum, Tuber brumale, Tuber excavatum,* and *Tuber macrosporum*) harvested in 2018/19 in 11 countries in S, SE, and central Europe. The $\delta^{13}C$ values of the 20 truffle aroma compounds were reported for the first time, and 13 synthetically derived VOCs were also characterised. Only one compound, benzene, 1-methoxy-3-methyl-, was identified in all studied truffle species with minimal variability. Compounds such as 1-butanol, 2-methyl, butanal, 2-methyl-, 3-octanone, and dimethyl sulphide were also present in almost all truffle species showing the greatest natural isotope variation among truffle species. In addition to truffle species, harvest location and sample quality were also found to influence $\delta^{13}C$ values. The role of general fungal and bacterial communities analysed using high throughput sequencing (Illumina MiSeq) in aroma formation was also observed for certain VOCs and their effect on $\delta^{13}C$ values. Compounds with more negative $\delta^{13}C$ values, 1-hexanol, 2-hexen-1-ol, (E), ethyl acetate, benzene, 1,4-dimethoxy- and 2,4-dithiapentane, are also likely associated with microbial activity.

Moreover, the aroma compounds could be derived from truffles and microbes, resulting in a broad authentic range found in 1-butanol, 2-methyl-, 3-octanone, butanal, 2-methyl-, butanal, 3-methyl- and dimethyl sulphide. Therefore, it is not surprising that the $\delta^{13}C$ values of natural and synthetic compounds overlap. Nine of the 15 VOCs for which there are values for synthetic samples were successfully separated by used method. Eleven aromatised commercial truffle products were also analysed to evaluate the database's usefulness. The results indicated possible falsifications.

Acknowledgements

References

[1] [1] Strojnik, L., Grebenc, T., & Ogrinc, N. (2020). Species and geographic variability in truffle aromas. *Food and Chemical Toxicology*, 142, 111434.

Stable Isotope and Fatty Acid Composition of Organically and Conventionally Produced Lithuanian Milk

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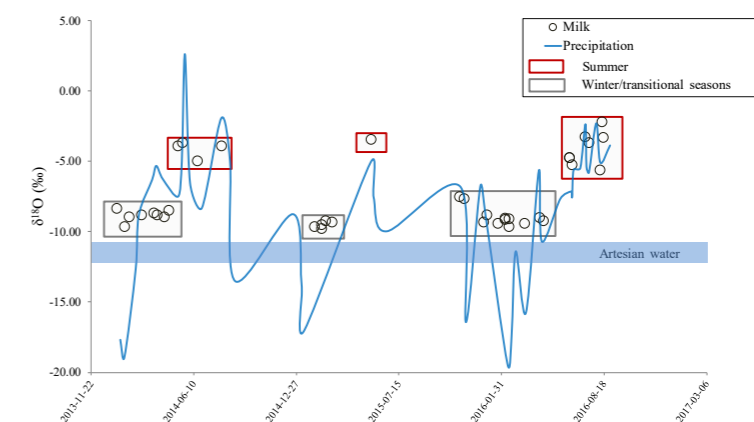
Increasing sales of organic milk mean intensified tests for authenticity are required. In addition to comprehensive documentation, analytical methods to identify organic milk, and thus to differentiate it from conventional milk, are needed for consumer protection [1,2].

We measured oxygen stable isotope ratios as well as fatty acid compositions in raw cow milk, artesian water and precipitation to better understand the impact of drinking water sources to cow milk as well as to observe possible isotopic fractionation in the end product – milk water. Conventional milk from local supermarkets samples were measured for comparison.

Organic milk samples were collected periodically to reflect seasonality when different water sources are available during the winter (artesian water) and summer (precipitation), spring and autumn seasons reflected transitional periods. Oxygen stable isotope ratios in milk water were lower in winter and transitional seasons and higher in summer seasons showing the dependence on the main water source [3]. The fatty acid C16:0 and C18:0 ratio decreased during the transition from winter season to summer season in organic milk with the same trend in different years.

Overall, combined $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ analysis of cow milk provides valuable information about the origin of milk and the dietary regime of cows and can be helpful for milk products authentication. However, our analysis showed that seasonal variations in isotopic ratios are typical in natural samples. Thus, databases and comparative material are needed to determine the origin of food products.

Overall, this study shows the applicability of the stable isotope ratio method in linking raw cow milk directly to the water sources. Despite these results represent local data, they can be valuable for other studies on geographical origin determination of various food and drink samples.

References

[1] 1. Molkentin, A. Giesemann (2007) *Analytical and Bioanalytical Chemistry*, 388, 297-305.
[2] S. Hemmerling, U. Hamm, A. Spiller (2015) *Organic Agriculture*, 5, 277-313.
[3] A. Garbaras et al. (2019) *Animals*, 9 (3), 69.

Isotopic Signatures of «Non-Traditional» Elements for Authenticity and Geographical Assessments of Food and Beverages

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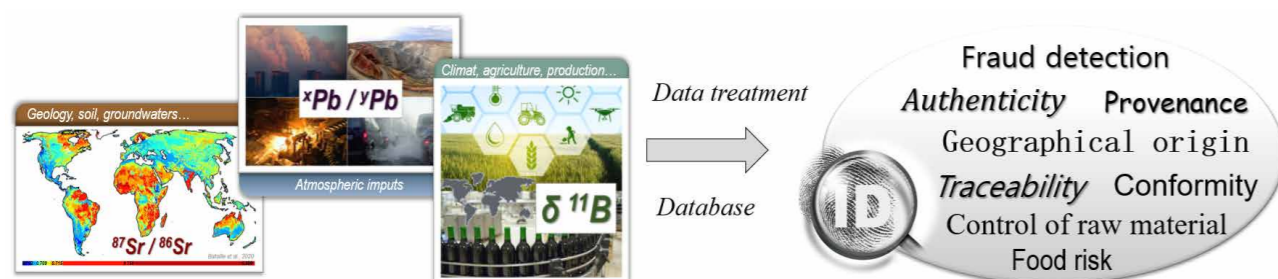
Authenticity and traceability of food are an area of high priority since they link directly the quality. However, more and more food and raw materials are particularly susceptible to misdescription or adulteration and therefore, it poses an increased risk for customers and producers. Specifically, high-quality food products (labelled PGI, PDO, TSG, brand house beverages, etc.) are vulnerable to such fraud.

The new advanced analytical approaches, notably, stable isotope analysis of non-traditional elements such as strontium (Sr), lead (Pb), and boron (B) combined to trace element profiles make a decisive contribution to geographical provenance assessment. These isotopic signatures are indeed influenced by geological scene, anthropogenic, agricultural, and environmental impact or even production processes. Thus, the product acquires a unique multi-isotopic signature, which is an integral characteristic persisted for a long time.

Sample preparation methods were developed and optimized regarding the matrix specification of each sample, notably, an appropriate ion-exchanging resin needs to be applied for a reliable analyte purification (>80%) prior to MC-ICP-MS analysis. Isotope measurement was performed by MC-ICP-MS with a precision down to 0.003% (2RSD, Sr), and 0.02% (2RSD, Pb) in the conditions of wet or dry plasma respectively.

Boron isotopes in wine were determined using quadrupole ICP-MS with a precision down to 0.5% (2RSD).

An integral review of these isotopic and elemental markers shows a great potential in reliable authenticity assessment of foods and beverages, especially for those within a limited geographical area of production and standardized processes. The efficiency of geographical provenance determination using this approach was confirmed by its successful application to different food and beverages such as wines, products labelled PGI from France (rice "Camargue" and dry-cured ham "Bayonne"), mineral bottled waters, vanilla and tea from wide-worlds origins.

Comparing an original sample for reference can confirm or disprove a suspected case and ensure the originality of a product. The performance of this approach will be demonstrated using an original authenticity database of champagne, treated through an open-source machine learning and data visualization software, which allowed perform reliable counterfeit detecting and even identify the most suspects.

This work is the result of an extensive collaboration and aimed to promote a valid indication approach to dealing with fraudulent practices in the food sector.

Characterisation of Slovenian Honey

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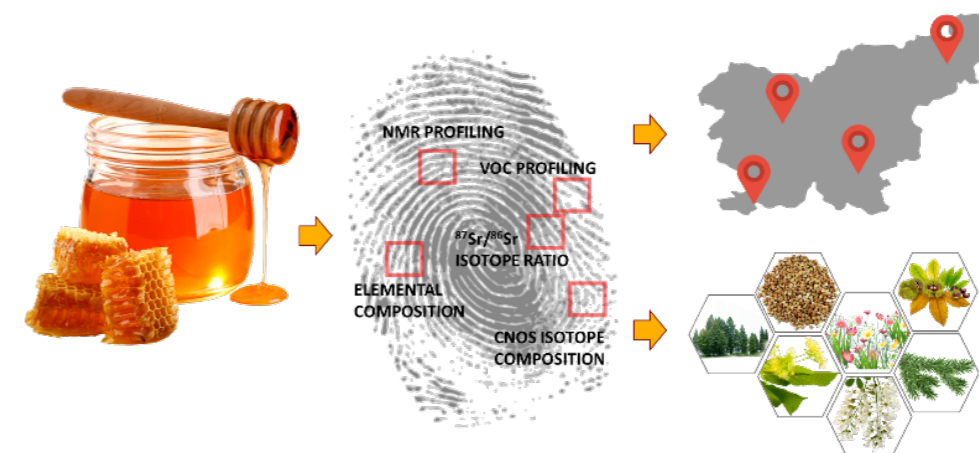
Honey is a natural sweet substance produced by *Apis mellifera* bees from the nectar of flowers or secretions from living parts of plants or secretions of insects that suck plant sap on living parts of plants, which bees collect, process with certain substances of their own, store, dry and leave ripen in the honeycomb [1]. The colour, taste, smell and aroma of honey vary depending on the plant's origin. The biochemical properties and quality of honey depend on the origin of the nectar, the maturity of the honey, climatic conditions, the season, the composition of the soil, the method and conditions of production and storage, as well as the skills of the beekeeper [2].

For the recognition of the floral and geographical origin of honey, analytical and/or physicochemical markers are needed. For honey discrimination, analyses of minerals and trace elements, volatile organic compounds, the protein pattern, flavonoids, and physicochemical parameters like electrical conductivity, pH, total acidity and water activity have been extensively examined [2].

Slovenia is a geographically, floristically, faunal and vegetational diverse country. The vegetational and floristic variety of Slovenia results in a wide variety of Slovenian honey. As data about the physicochemical properties of Slovenian honey are scarce, the aim of the present study was to establish a record of data with parameters that are important for determining the geographical origin of honey. In total, 40 samples of honey from different locations and varieties from two production years (2020 and 2021) were analysed. The parameters determined were $^{13}C/^{12}C$ isotope ratio in honey ($\delta^{13}C_{honey}$), isotope ration of $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$ in honey proteins, multi-elemental composition, 87Sr/86Sr isotope ratio, volatile organic compound and NMR profiling.

Considering the results of the elemental composition of honey and stable isotope analysis, we separated honey samples according to type and region. Based on volatile organic compounds, we determined the compounds characteristic of each type of honey and its geographical region. With NMR profiling, we were able to determine compounds that are characteristic of different types of honey. We were able to generally follow the chemical profile of individual types of honey regardless of the production year. With the analytical techniques applied, we could recognise if there has been a mixing of species, the presence of unauthorised additives, and mislabelling in terms of plant or geographic origin. Last but not least, the study has significantly enriched the database of Slovenian honey.

Acknowledgements

References
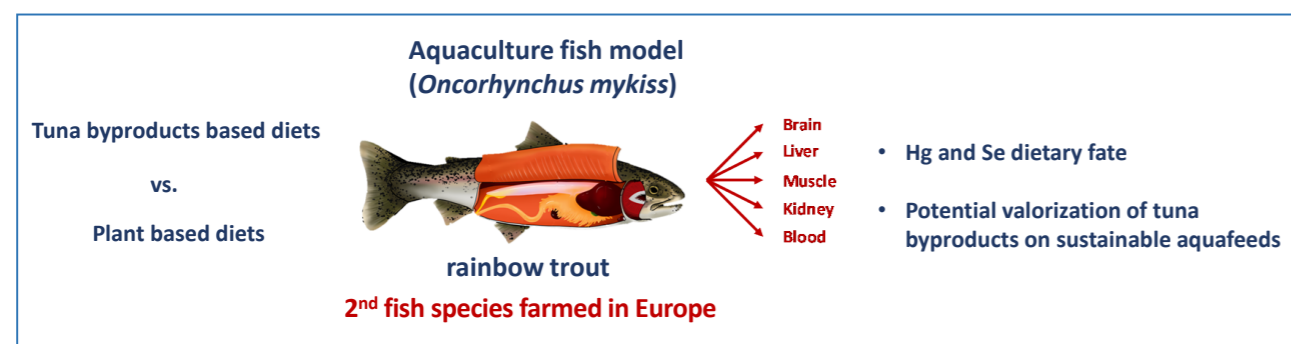
[1] M. Oroian et al. (2016) *International Journal of Food Properties*, 19 (8), 1825.

[2] M. V. Baroni (2015) *Journal Agricultural and Food Chemistry*, 63, 4638.

Hg and Se Fate and Interaction in Fish: New Insights and Potential Valorization of Tuna Byproducts on Sustainable Aquafeeds

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Aquaculture fish model (*Oncorhynchus mykiss*)

Tuna byproducts based diets
vs.
Plant based diets

Brain
Liver
Muscle
Kidney
Blood

• Hg and Se dietary fate
• Potential valorization of tuna byproducts on sustainable aquafeeds

rainbow trout
2nd fish species farmed in Europe

Aquaculture is unquestionably one of the fastest growing industry in the food sector (8% yearly increases in the last 10 years) [1]. The rapid production growth of fed-species leads to an increased demand for aquafeeds and consequently, seeking for new alternatives to developing sustainable fishmeal. Tuna can industry discard around 65% of byproducts, which represent a precious protein source for sustainable aquafeeds production. Nevertheless, the principal disadvantage of the use of tuna byproduct-based diets is their potential high content of heavy metals, such as mercury (Hg).

Due to its extreme toxicity, Hg has been recently included in the top ten chemicals of major public health concern by the World Health Organization (WHO). Methylmercury (MeHg) hazardous effects are biomagnified through the trophic chain, resulting in serious social and environmental and human health effects. In general, human exposure to MeHg is mainly associated with fish consumption. Several food safety agency have fixed a threshold of MeHg content and clearly advise about the non-consumption of certain fish species by vulnerable populations (including pregnant women and young children) depending on their MeHg concentration. Interestingly, tuna byproducts contain relatively high selenium (Se) concentrations levels, which could play a role on Hg bioaccumulation. The role of Se against Hg biopaccumulation/toxicity has been evoked but still remain poorly understood. The main objective of this work is contributing to the understanding of the dietary fate of Hg and Se paying special attention to the potential valorization of tuna byproducts on sustainable aquafeeds.

A model aquaculture fish species, rainbow trout (*Oncorhynchus mykiss*), was dietary exposed to Hg and Se species under controlled conditions. The dietary Se effect on Hg bioaccumulation in flesh, but also in key organs like brain and liver, was kinetically tracked during a 6-month feeding trial, where animals were exposed to plant and tuna byproducts-based diets. A battery of analytical approaches comprising total Hg and Se content, biomolecular speciation and (Hg) isotopic analyses was successfully exploited providing new insights on the Hg and Se pathways in trout. Despite the relatievely high Hg content on tuna byproducts aqufeeds, the resulting Hg levels in trout muscle was lower than the maximum recommended by food safety agencies (0.3 μgHg/g ww). In addition, the trophic transfer of selenoneine, strong antioxidant, from tuna byproducts diets to farmed trout was demonstrated. In summary, the described approach could help in setting new guidelines for aquaculture feeding practices to avoid fish contamination with Hg toxic forms and to insure proper bioassimilation of Se. This study constitutes a solid basis on the evaluation of tuna byproducts for their potential use as a sustainable alternative to wild fish-based aquafeeds.

References

[1] FAO, T*he State of World Fisheries and Aquaculture 2020. Contributing to Food Security and Nutrition for All*, Rome, Italy, 2020, 225.

Quantification Goes Digital – 10+ Years of Dpcr Developments in GMO Diagnostics

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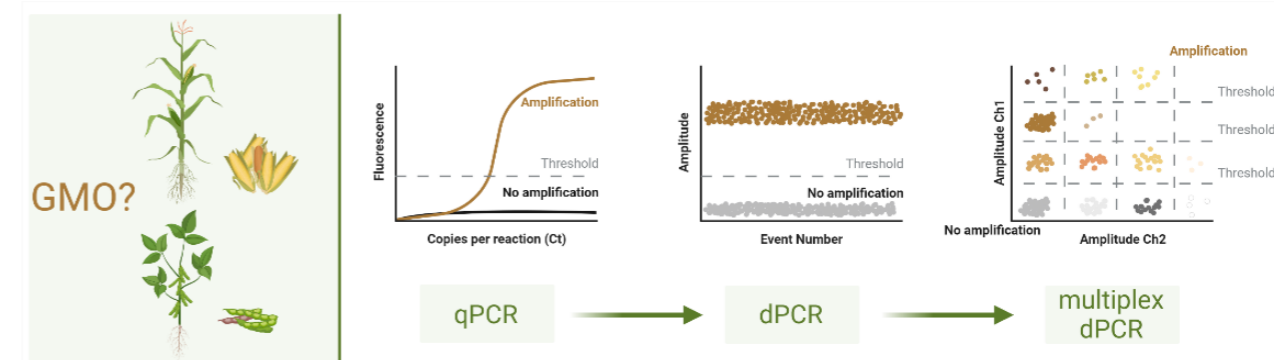
The first commercial digital PCR (dPCR) system, BioMark (Fluidigm) was launched in 2006, kicking off the next generation of nucleic acid quantification. Three years later the first paper was published on the use of dPCR for GMO quantification [1]. However, a major drawback of the instrument was the low throughput, which made the methodology unpractical for diagnostics. This changed with the introduction of high(er) throughput platforms, from which the first was BioRad's QX100 in 2012. At that time, dPCR has been introduced in our laboratory.

One of the first applications of dPCR suitable for diagnostics in our laboratory was shown with the development of dPCR methods for detection and quantification of GM-maze line MON810 [2]. The methods showed comparable accuracy, dynamic range, sensitivity and limit of quantification to the golden standard quantitative real-time PCR (qPCR). It also showed the transferability of qPCR assays to dPCR.

With proven transferability, another advantage of dPCR was explored, the power of multiplexing. Two multiplex assays were developed in 2015 [3] and 2017 [4], focused on detection and quantification of all at that time in EU approved GM-maize and GM-soybean lines. These methods enabled group multiplexing; all transgenic target assays marked with one fluorescent label (12 maize and 15 soybean lines) and the corresponding reference gene assay with the second fluorescent label. In parallel a different approach was in development. Here a true tetraplex was possible using different concentrations of fluorescent labels, enabling detection and quantification of seven GM-maize lines and maize reference gene in two reactions [5].

Besides exploring the limits of the method, a better support for diagnostics was established. Food presents a complex matrix for molecular biology methods, limiting qPCR methods especially in terms of quantification. A duplex dPCR method targeting the most widely spread GM-soybean line MON40-3-2 and soy reference gene [6], enabled quantification in routine samples where qPCR fell behind.

Despite the rocky start, almost 15 years down the line, the advantages of dPCR in GMO diagnostics have been accepted by the community and the technology has made it into routine laboratories. And with the development of new platforms nucleic acid quantification in food safety and sustainability is bound to remain digital.

References

[1] S. Bhat *et al.* (2009) *Analytical and Bioanalytical Chemistry*, 394, 457–467.
[2] D. Morisset et al. (2013) *PloS one*, 8 (5), e62583.
[3] D. Dobnik et al. (2015) *Analytical chemistry*, 87 (16), 8218-8226.
[4] A. Bogožalec Košir *et al.* (2017) *Scientific Reports*, 7 (1), 8601.
[5] D. Dobnik *et al.* (2006) *Scientific reports*, 6 (1), 35451.
[6] A. Bogožalec Košir et al. (2019) *Food Chemistry*, 294, 73-78.

Quality Characteristics of Treated Wastewater Irrigated Tomatoes

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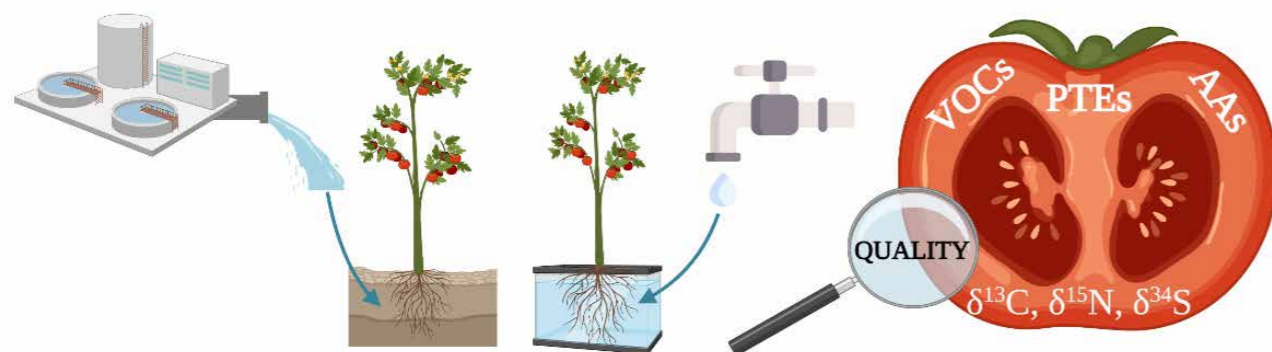
Population growth and climate change contribute significantly to the problem of water scarcity in many regions. A possible solution is to use treated wastewater to irrigate crops, thereby reducing the amount of freshwater extraction from rivers and groundwater. However, although treated wastewater is a source of nutrients, it also contains many anthropogenic chemicals and elements, which can be potentially harmful to ecosystems and human health and could also affect the quality of the crop. In our study, we chose to investigate the tomato plant since tomatoes are one of the most widely cultivated and consumed vegetables globally, both fresh and processed, and are considered a good source of minerals, vitamins, proteins, essential amino acids, monosaturated fatty acids, carotenoids and phytosterols. Also, its roots, stems, leaves, and edible fruits make the tomato an ideal research plant [1].

Our study aimed to assess the effects on tomato fruit quality by comparing tomatoes (*Lycopersicum esculentum* L. cv. Rally) irrigated with potable and treated wastewater grown in soil (lysimeters) and soil-less (hydroponically). Soil-grown tomatoes were irrigated with a) potable water b) treated wastewater, and c) treated wastewater spiked with 14 model contaminants of emerging concern (CEC) (0.1 mg L-1). Hydroponically-grown tomatoes were irrigated with: a) nutrient solution and b) nutrient solution spiked with CEC (0.1 mg L-1). The model CEC included industrial chemicals, pharmaceuticals and common stimulants.

The quality of the tomato fruits was assessed regarding elemental composition, volatile organic compounds, and the amino acids using validated methods based on ICP-MS, HS-SPME GC-MS, and GC-MS. In addition, the stable isotopic composition of light elements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) was performed to study the influence of different treatments on the final product. The quality parameters were chosen since they influence flavour, texture, structure, mouthfeel, the colour of tomatoes, the content of bioactive compounds and specific elements that promote good health.

Based on elemental composition, no health risk from consuming hydroponically or soil-grown tomatoes was identified. Results showed that elemental composition was affected by the addition of CEC and there were also significant differences between hydroponically- or soil- grown tomatoes and those irrigated with treated wastewater and potable water. The aroma profile differentiated only between hydroponically or soil-grown tomatoes. These two groups had also significantly different stable isotopic composition of $\delta^{13}C$, $\delta^{15}N$. Other findings, including effects on amino acid composition will also be presented.

Acknowledgements

References

[1] Kovačič et al. (2023) J*ournal of Hazardous Materials*, 448, 130964.

Uptake of Chemicals of Emerging Concerns in Tomatoes Irrigated With Treated Wastewater

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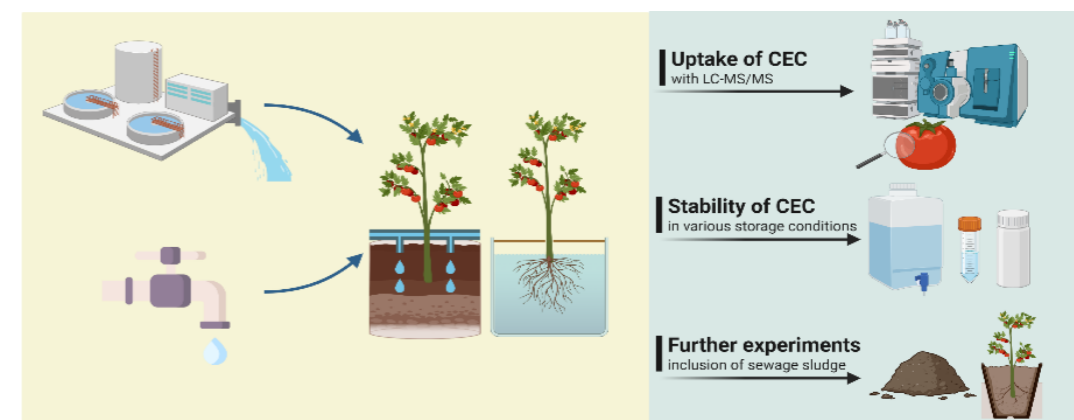
Water scarcity is a major environmental issue strongly correlated to climate change and population growth. A cost-effective and sustainable solution to water shortages in agriculture is the utilisation of treated wastewater. However, there might be effects on plant development, quality attributes and uptake of chemicals of emerging concern (CEC), which can pose health risks. This study examines the safety of treated municipal wastewater reuse by conducting uptake experiments using the tomato (*Solanum lycopersicum L.* cv. Rally) as the model plant. Specifically, we evaluated the uptake of 14 CEC, including pharmaceuticals, hormones and industrial chemicals, in plants grown in soil-less (hydroponic) and soil (lysimeter) media to measure the impact of different growth regimes. Soil-grown tomatoes were irrigated with: potable water, treated wastewater and treated wastewater spiked with CEC (0.1 mg/L), while tomatoes produced hydroponically were grown in nutrient solution and nutrient solution/CEC (0.1 mg/L). Samples were extracted ultrasonically and analysed by LC-MS/MS. Only BPS, 2,4 BPF, and naproxen were detected in tomatoes grown in spiked media, with higher uptake in the case of hydroponically grown tomatoes [1]. For the detected CEC, average chronic diet exposure from consuming the analysed tomatoes was calculated and showed no health concern for any age group [1].

Based on the results, experiments were repeated with an expanded list of CEC (28: pharmaceuticals, personal care products, hormones, pesticides and industrial chemicals) at ten times higher concentrations (1 mg/L). After developing and validating an analytical method for determining CEC in wastewater, a stability experiment was performed in potable water and treated wastewater. Most CEC were stable for three weeks at 20°C and light exposure, which led to a reformed protocol for the preparation of irrigation media. In the second field experiment, growing regimes were expanded, including potable water and wastewater, with and without the addition of compounds in all cases. We also included a third experiment to evaluate the safety of using stabilised sludge, a by-product of wastewater treatment, as a potential soil amendment. In this case, the tomatoes were grown in pots with and without the addition of sludge. Following the experiments, we are currently developing methods for identifying CEC in tomatoes, leaves, roots and soil, which will serve to evaluate CEC uptake and translocation. This study argues that although treated wastewater reuse can be an effective strategy for dealing with water shortages, its use must be approached with caution and careful monitoring of potential risks, such as introducing CEC and their transformation products into the food chain.

Acknowledgements

References

[1] A. Kovačič et al. (2023) *Journal of Hazardous Materials*, 448, 130964.

Decontamination Studies on Ready-to-Eat Strawberries

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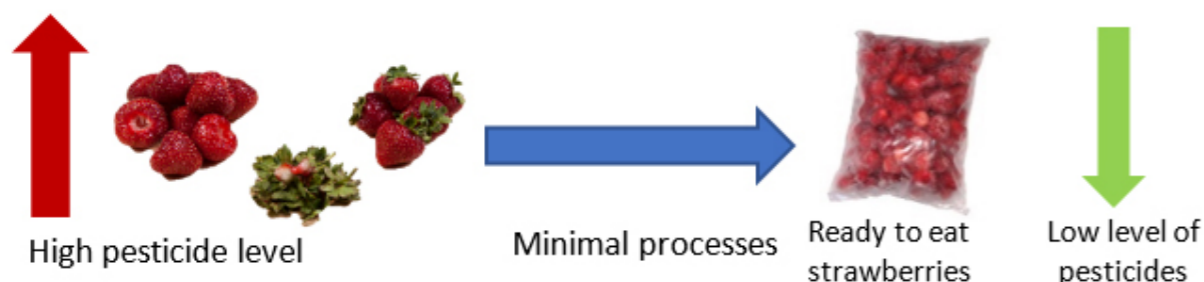
Ready-to-eat (RTE) fresh fruit and vegetables are valued by consumers for their fresh-like characteristics and high nutritional value. They are obtained by minimal processes such as washing, cutting and disinfection. To improve quality and increase shelf-life, RTE products are usually packaged under modified atmosphere and stored under refrigeration. Nevertheless, those fruit and vegetables should be also safe foods, no matter the minimal processing steps before consumption, avoiding chemical or biological contamination. Particularly, chemical contamination can be due to pesticide application during cropping and postharvest and their occurrence could be risky for consumers health. In this study, the effects of some common minimal processes were evaluated on fresh strawberries. Strawberries were taken as a model of the dynamics of pesticide residues in RTE products, because they have been steadily in the top-ten position of fruits with the greatest number of residues in the USDA Surveys.

The effect on pesticide residue levels of each step, and the overall sequence of calix removal, washing, disinfection with peracetic acid, sodium hypochlorite. Moreover, the use of ozone and ultrasound followed by modified atmosphere packaging was studied. Also, the microbiological loads at the end of the process throughout 9 days storage in passive modified atmosphere at 5 C. were measured.

A QuEChERS CEN 15662 protocol was validated for 27 pesticides with LOQ ranging from 5 µg kg-1 to 10 µg kg-1. A monitoring survey during two years was performed on commercial strawberries, in order to map the commonly occurring pesticides. Residues of azoxystrobin, carbendazim, difenoconazole, propamocarb and spinosad (sum of A and D) were found in concentration ranges of 0.005 to 2.2 mg kg-1. Carbendazim and propamocarb exceeded the established MRLs. Strawberry calix concentrated the greatest number of pesticides. Pesticide level dropped 67 to 80% after its removal. Washing the intact fruit before calix removal, washed 12 to 40% of residues whereas peracetic acid disinfection cleaned up to 80% of pesticides. Sodium hypochlorite took out up to 84% pesticides. No significant differences in pesticide dissipation in the modified atmosphere experiments were detected. Washing and disinfection with different chemical agents proved to be useful minimal processes that do not alter food quality and contribute to its safety, reducing the pesticide residues concentration while keeping the microbiological count under MAP in safe levels.

Acknowledgements

References

[1] USDA (2018) National Agricultural Statistics Service. *United States Department of Agriculture, https://quickstats.nass.usda.gov/.*

[2] M. Anastassiades, E. Scherbaum, B. Taşdelen, D. Štajnbaher, in *Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety*, H. Ohkawa, H. Miyagawa, P. W. Lee (Eds.), Wiley, 2007, 439-458.

Transfer of Phytocannabinoids From Dried Plant to Aqueous Infusions: Is Cannabis 'Tea' Safe?

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Transfer of phytocannabinoids?

Cannabis sativa L. is one of the oldest medicinal plants, cultivated mainly in Central Asia since ancient times. Research on this plant has made great progress in recent years, due to the renewed interest of both public institutions and private companies [1]. Moreover, the demand for cannabis-based products is growing significantly among the general public [2]. One of the relatively new trends in western countries is cannabis 'tea', which has gained popularity due to its potential health benefits. However, the cannabis plant, depending on the chemotype, contains the psychotropic phytocannabinoid delta-9-tetrahydrocannabinol (Δ^9-THC) and its precursor delta-9-tetrahydrocannabinolic acid (Δ^9-THCA), both of which can pose a threat to health. To evaluate the risks of drinking cannabis tea, we investigated the transfer of these and other phytocannabinoids from dried cannabis into aqueous infusions under various conditions. The data obtained by UHPLC-HRMS/MS method used to characterize both cannabis samples and aqueous infusions showed that the soaking time (3, 5, 10 min) and the amount of cannabis used (0.2, 1.0, 5.0 g / 250 ml of water) had minimal impact on phytocannabinoids transfer. On the other hand, when the tea was boiled (10 min), the concentrations of all 17 targeted phytocannabinoids increased, for some analytes quite dramatically (up to 40 times), while those of phytocannabinoid acids were 2-8 times higher than their neutral analogues. Despite this, even under these most critical conditions, only 2.8% of Δ^9-THC was transferred from the dry plant into aqueous infusion (4.48 µg into 1 L), which in the case of a 70 kg person who consumes 1 L of cannabis tea per day, represented only 6.4% of the ARfD (1 µg/kg b.w.) defined by the EFSA [3]. Consequently, our research suggests that the potential benefits of drinking hemp tea far outweigh the risks associated with the ingesting Δ^9-THC and Δ^9-THCA in the standard herbal tea preparation procedure. However, some cannabis tea producers recommend adding cream, milk or a small amount of cannabis oil to the infusion when the herb is soaked. Due to the lipophilic nature of phytocannabinoids, their concentration in the infusion can be increased several times by this procedure. Therefore, the risk assessment of the potential intake of psychotropic phytocannabinoids due to the consumption of cannabis tea should not only be based on the measurement of its content in the dry material, but also consider the method used for the preparation of tea.

Acknowledgements

References

[1] S. A. Bonini, M. Premoli, S. Tambaro, A. Kumar, G. Maccarinelli, M. Memo, A. Mastinu (2018) *The Journal of Ethnopharmacology,* 227, 300-315.

[2] J. Manthey (2019) *International Journal of Drug Policy,* 68, 93-96.

[3] EFSA (2015) *EFSA Journal,* 13 (6), 4141.

Biofortifying Sprouts with Zinc by Seed Priming

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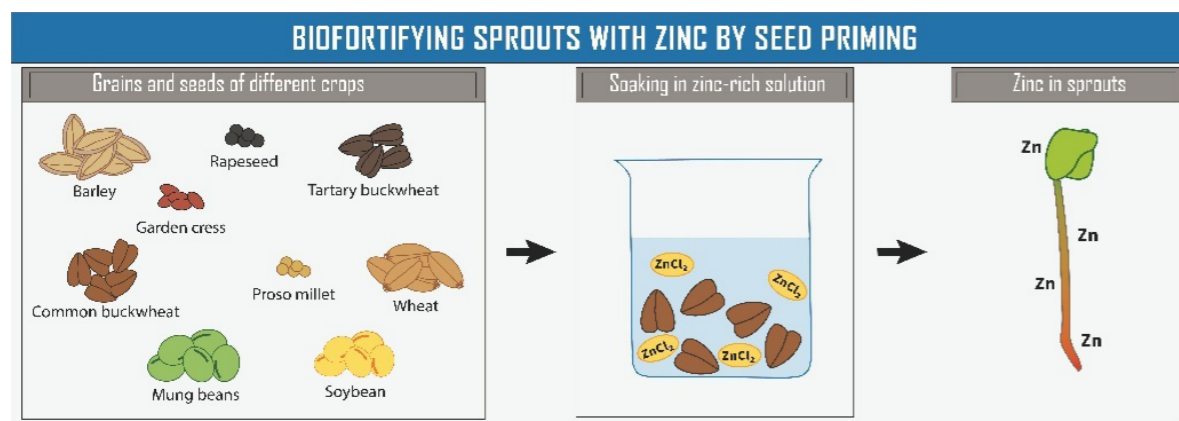
Many humans lack sufficient zinc (Zn) in their diet for their wellbeing and increasing Zn concentrations in edible produce (biofortification) can mitigate this [1]. Increasing the concentration of Zn in grain, seed and tubers is limited by Zn mobility within plants [2], thus, physiologically, leafy vegetables are better candidates for Zn biofortification [3,4]. Soaking grain and/ or seeds in Zn-rich solutions has been proposed as a promising approach to increase Zn concentration in edible sprouts (or microgreens) [5]. Therefore, the aim of the study was to assess the level of increase in Zn concentration in sprouts of different crops after their grains and/ or seeds were primed by soaking in different Zn-rich solutions.

Grain of common (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and proso millet (*Panicum miliaceum* L.), and seeds of garden cress (*Lepidium sativum* L.), soybean (*Glycine max* (L.) Merr), mung bean (*Vigna radiata* (L.) R. Wilczek) and rapeseed (*Brassica napus* L.) were soaked in different concentrations of $ZnSO_4$ or $ZnCl_2$ for 16-24 h at room temperature. Grains and seeds were rinsed in tap water and transferred to sprouters (EasyGreen® MicroFarm System, Easy-Green Factory Inc., Nevada, USA) where sprouts were grown for 7 days. Sprouts were watered with tap water by automatic misting for 30 min every eight hours. At harvest, shoots were weighed (fresh weight) and dried in an oven at 60°C for three days, after which the shoots were weighed again (dry weight). The dried shoots were finely ground with pestle in a mortar and pressed into pellets with a hydraulic press. Concentrations of Zn were measured using X-ray fluorescence spectrometer (Peduzo T02, Jožef Stefan Institute, Ljubljana, Slovenia) as described in detail previously [6].

Results indicate that there is a large variation in the level of increase in Zn concentration in different sprouts with the largest increase (more than 11-fold) in garden cress and rapeseed and the smallest (1.5-fold) in proso millet. Occasionally, there was growth penalty observed, but this was not consistent for crops tested, indicating that Zn concentration (and time of soaking) used for grain and seed priming should be optimised for each species individually. Results for each plant species will be presented and discussed further.

Acknowledgements

References

[1] P. J. White, M. R. Broadley (2009) *New Phytologist*, 182 (1), 49-84.

[2] P. J. White, M. R. Broadley (2011) *Frontiers in Plant Science*, 2, 80.

[3] C. V. Buturi, R. P. Mauro, V. Fogliano, C. Leonardi, F. Giuffrida (2021) *Foods*, 10, 223.

[4] P. J. White, P. Pongrac, C. C. Sneddon, J. A. Thompson, G. Wright (2018) *Agriculture*, 8, 32.

[5] R. Bączek-Kwinta, A. Baran, M. Simlat, J. Lang, M. Bieniek, B. Florek (2020) *Agriculture*, 10 (7), 937.

[6] M. Nečemer, P. Kump, J. Ščančar, R. Jaćimović, J. Simčič, P. Pelicon, M. Budnar, Z. Jeran, P. Pongrac, M. Regvar, K. Vogel-Mikuš (2008) *Spectrochimica Acta Part B: Atomic Spectroscopy*, 63 (11), 1240-1247.

Survey of PFAS in Farmed Marine Shellfish in North Greece. Do PFAS Pose a Threat to Marine Biota and Human Health?

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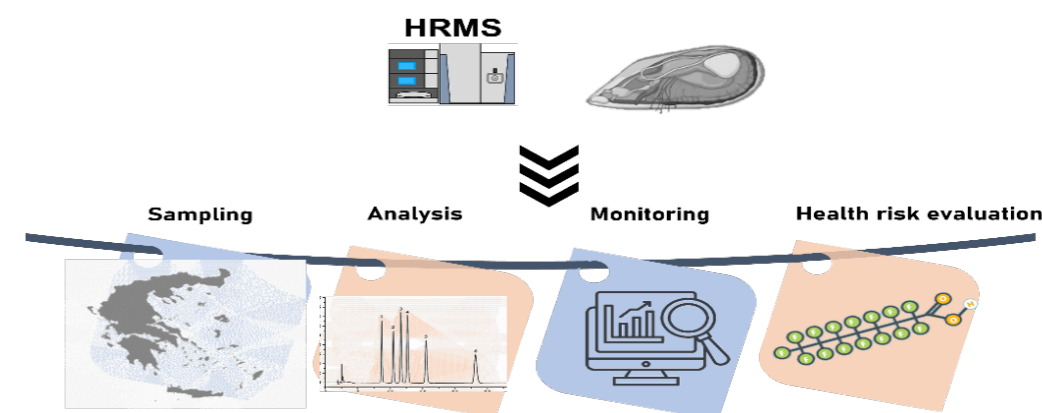
Perfluorinated alkyl substances (PFAS) are man-made chemicals and due to their special physical characteristics PFAS have been utilised in many commercial and industrial products. However, their wide application comes along with their release in environment. They present excellent physical and chemical stability which leads to their accumulation in the environment. Although, PFAS are widely reported in many environmental matrices [1], a shift of focus on the marine environment has been noticed lately. PFAS are now detected in higher trophic levels which clearly underlines PFAS trophic transfer through marine food chain. Their ubiquitously occurrence and trend of bioaccumulation is of high concern especially when considering the risks for humans as top consumers of the trophic chain. As a result, biomonitoring of marine biota is of high importance in order to assess the possible toxicological effects for organisms.

To this end, the present study provides a preliminary investigation of legacy and emerging PFAS in 13 marine farms of shellfish in Greece. For the pretreatment step, two different QuEChERS based protocols were evaluated in order to select the most appropriate according to LODs, LOQs and recoveries [2,3]. Target and suspect High Resolution Mass Spectrometry (HRMS) screening methods (Orbitrap LC-MS/MS system, Negative ionization mode) were applied in order to identify the potential PFAS.

In order to cover the broad spectrum of PFAS released in the marine environment Norman databases were combined and utilized for the identification of the "suspects". PFAS revealed by suspect screening were quantified when standard was available.

The first results obtained by these two protocols, indicate the presence of PFOA as well as PFDoA in concentrations not higher than 7 ng/g and 1 ng/g respectively. According to these findings no threat is posed to human health, however since this study is in preparatory stage further investigation is required. The data obtained will serve as a reference for future studies, either on a Greek or European scale since data on marine shellfish are still scarce.

Acknowledgements

References

[1] L. -A. Koronaiou, C. Nannou, N. Xanthopoulou, G. Seretoudi, D. Bikiaris, D. A. Lambropoulou (2022) *Microchemical Journal, 179*, 107457.

[2] B. Cara, T. Lies, G. Thimo, L. Robin, B. Lieven (2022) *Environmental Pollution, 311*, 119907.

[3] A. Podder, A. H. M. A. Sadmani, D. Reinhart, N. Chang, R. Goel, *J. Hazard. Mater.* 2021, *419*, 126361.
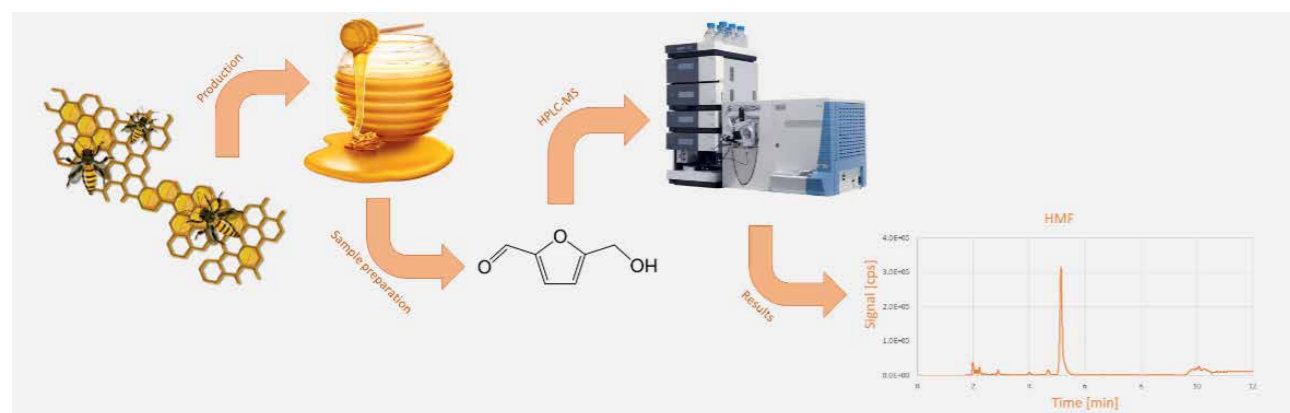
Determination of 5-Hydroxymethylfurfural in Honey by High-Performance Liquid Chromatography

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5-hydroxymethylfurfural (5-HMF) is mostly formed in honey through the sugars present in the honey [1]. The formation of HMF is natural process, which, however, is accelerated by heating the honey, during processing, production and with improper storing [2-3].

In this work, several honey samples were analysed and the content of 5-HMF in different samples was determined. Analysis was performed using LC/MS HPLC equipment. Various parameters of the analytical method were determined, such as repeatability, linearity and accuracy of the method but also the stability of the standards and the sample.

The analysis was performed with both MS and UV detection. It was found that due to the poor ionisation of 5-HMF, the sensitivity is much better using UV detection at wavelength 285 nm. The optimised method was linear from 0.1 to 10 mg/L of 5-HMF. Recoveries were tested on real samples and were found to be between 95 % and 113 %. 5-HMF content between honey samples from different botanical species was compared. The determined concentrations were in accordance with other literature data, the concentration in honey ranging from 1.2 mg/kg to 96 mg/kg. In addition to that also some samples of creamed honey with additives were analysed showing similar content of 5-HMF.

Experiment with the sample kept at elevated temperature (65 °C) showed that there is significant increase of 5-HMF content already in few days of heating the sample at 60 °C. The content of 5-HMF increased from 1-10 mg/kg to 150-250 mg/kg. This is an indication that 5-HMF is an important marker in honey showing improper storage temperature of the samples. The content of 5-HMF in samples of honey produced in 2018 and in samples of honey produced in 2022 was also compared. The content of 5-HMF in samples produced in 2022 was relatively low (around 1 mg/kg), while the content of 5-HMF in samples of honey produced in 2018 was significantly higher (between 6 mg/kg and 62 mg/kg). Although improper storage cannot be excluded, there is still an indication that the content of 5-HMF increases during storage of honey.

In addition to that, HMF content is of importance in helping consumer to indicate not only improper storage temperature but also age of the honey and honey processing at elevated temperatures.

References

[1] U. M. Shapla, Md. Solayman, N. Alam, Md. I. Khalil, S. H. Gan (2018) *Chemistry Central Journal*, 12, 35.

[2] H. Kurtagić, E. Sarić, N. Abdelfattah, N. Murtabegović, (2021) *International Journal of Environmental Sciences & Natural Resources*, 26, 154-159.

[3] A. Kesić, A. Crnkić, Z. Hodžić, N. Ibrišimović, A. Šestan (2014) *Journal of Scientific Research & Reports*, 3 (8), 1057-1066.

Chemicals Migrating from Beer Cans: Non-Targeted and Suspect Analysis

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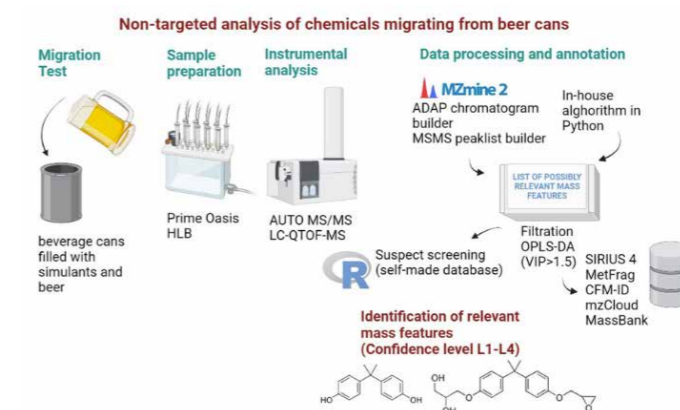
This study aimed to address the issue of food safety by investigating the migration of chemicals from food contact materials (FCM), particularly epoxy resin-coated beer cans [1]. Identifying potentially migrating chemicals, including non-intentionally added substances, is critical for the assessment of FCM safety and for reducing human exposure to potentially harmful chemical substances. For this reason, non-targeted analytical investigations were performed using high-performance liquid chromatography coupled with hybrid quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) on samples prepared from beer cans coated with epoxy resins (n = 12) using a suitable simulant (20% ethanol), and beer not yet in contact with packing material. The instrumental analysis and post-acquisition data processing were optimized based on 30 target chemicals to enrich a broad range of compounds from tested matrices.

To confirm the consistency of data processing using different software tools, the same raw data files were analyzed using MZmine2, ADAP chromatogram builder and MSMS peak list builder algorithm and an in-house developed algorithm in Python. The results showed a high coherence, with over 80% of mass features overlapping between different processing tools. The number of potentially relevant mass features from all tested workflows was reduced to approximately 370 in negative ionization mode using OPLS-DA (VIP number > 1.5). Initially, the annotation of filtered mass features was based on suspect screening (expanded csv database of possible leaching chemicals from FCM) performed in R using the modified Shiny app. Next, MS2 spectra of relevant mass features were submitted to the computational tool SIRIUS 4 to annotate the potential elemental composition and, where possible, the chemical composition of relevant mass features. The results from SIRIUS were compared with an in-silico prediction using the open source software (MetFrag and CFM-ID) and MS2 libraries (MassBank and mzCloud). The annotation was successful with more than 40 mass features identified at confidence levels 1 to 4, e.g., bisphenols, BADGE derivatives, 4-[11-(4-hydroxyphenyl)-1-methylbenzo[b]fluoren-11-yl] phenolate and phthalates derivatives. The presence of annotated mass features will be searched for in the beer sample extracts.

In conclusion, the non-target and suspect screening approaches developed in this study, which utilizes advanced mass spectrometry-based methods and includes strategies for data reduction and annotation of unknown compounds, has the potential to significantly reduce the uncertainty associated with identifying non-targeted compounds. This strategy can contribute to developing more effective food safety regulations by comprehensively understanding the chemicals present in FCM and their potential migration into food products.

References

[1] A. Kovačič et al. (2020) *Food Chemistry*, 331, 127326.

Heavy Metal Content in Crops Grown Near Former Metal Mining Sites and Potential Risk to the Local Population

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Heavy metals are potential health risk, especially in mining sites where they deteriorate from sulfide-rich ore bodies. Soil contamination with heavy metals due to mining activities poses risks to ecological safety and human well-being. When soil is contaminated with toxic heavy metals, its quality and productivity decrease with a reduction in food safety [1,2]. Studies have shown that fruit and vegetable consumption is the primary pathway of human exposure to heavy metals [3,4]. These metals can be admitted into the human body by several input and have several toxicity mechanisms and effects [5].

The present study focused on contamination of plants cultivated in soils located nearby an abandoned Pb-Zn mining area, which is of high concern with respect to the contamination of food chain. The investigation has been operated on the edible parts of both leafy (Chard, Spinach, Coriander and Salad) and bulbous (Carrot, Fennel and Radish) plants.

Results show that these plants bear high concentrations of Zn, but lesser amounts of Pb and Cd.

The values of the Transfer Factor (TsF<< 1 for Cd, Cu and Zn and even less for Pb) for contaminated plants, are too low compared with those of the control plants, which indicates that the plants which grow in contaminated areas react to metal stress absorbing small amounts of metals.

In addition, all plants with edible leaves, are characterized by TF>1 for Cd, Cu and Zn, indicating an accumulation of these elements in leaves, and TF<1 for Pb, indicating an accumulation of this element in roots.

However, in most plants with edible bulbs the values of the Translocation Factor (TF>1) for Cd, Zn, Pb and Cu show that these plants accumulate preferentially these elements in leaves.

The leaves of leafy and bulbous plants are the essential storage organs for most metals with the exception of Pb for leafy plants which is preferably stored in the roots.

As a matter of fact, the consumption of leafy plants seems to more hazardous to local population than bulb plants as long as they bear higher amounts of Cd and Zn.

Acknowledgements

References

[1] B. J Alloway, in Heavy Metals in Soils, B. J. Alloway (Ed.), Dordrecht (The Netherlands), *Springer*, 2013, 11-50.

[2] R. A. Wuana, F. E. Okieimen, (2011) *International Scholarly Research Network*, 2011, 20.

[3] C. Y. Chang, H. Y. Yu, J. J. Chen, F. B. Li, H. H. Zhang, C. P. Liu (2014) *Environmental Monitoring and Assessment*, 186, 1547-1560.

[4] B. Girmaye (2014) *African Journal of Environmental Science and Technology*, 8 (7), 428-434.

[5] M. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew, K. N. Beeregowda (2014) *Interdisciplinary Toxicology*, 7 (2), 60-72.

Functionalization of PLA Foils Using Chitosan and Plant Extracts as a Bilayer Coating

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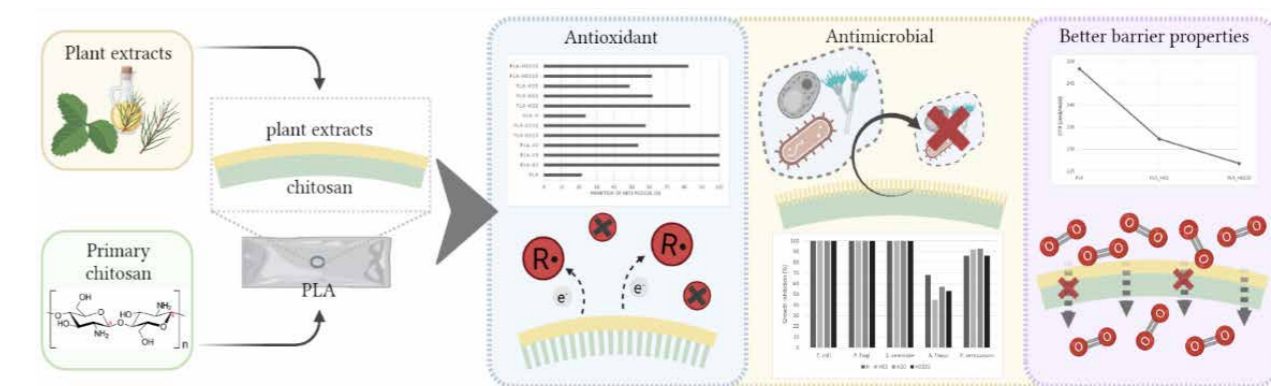
Due to the uncontrolled use of petroleum-based plastics in food packaging and the requirements for better food safety, there is a great need for the development of active packaging made of biodegradable materials. In this study, the antimicrobial polysaccharide chitosan was used in combination with plant extracts of blackberry leaves, *Rubus fruticosus*, needles of prickly juniper, *Juniperus oxycedrus*, and by-product of essential oil distillation of common juniper, *J. communis*, as a surface coating for PLA packaging material in a bilayer coating approach [1]. A solution of macromolecular primary chitosan was applied as the first layer, while the plant extract was applied as the second layer on top of the chitosan. Zeta potential measurements were performed after the coating was applied. In addition, parameters important for practical application such as contact angle and oxygen barrier properties were also investigated. The functionalized films were analysed from a bioactive point of view, i.e., with respect to their antimicrobial and antioxidant activity. The antimicrobial activity was tested against a range of microorganisms, i.e., Gram-negative bacteria (*Escherichia coli, Pseudomonas fragi*), fungi (*Aspergillus flavus, Penicillium verrucosum*) and yeasts (*Saccharomyces cerevisiae*) according to the ISO 22196. Using the ABTS assay, antioxidant activity was determined spectrophotometrically.

The zeta potential measurements proved the adhesion of the coating to the surface due to the shift of the isoelectric point to the theoretical point of chitosan (i.e., isoelectric point between 5.8 and 6.6). Contact angle measurements showed that the coating with chitosan converted the hydrophilic character of PLA into a hydrophobic one, while the extract as the second coated layer decreased the contact angle by 20-50%, improving wettability and thus antifogging activity. The barrier properties were investigated only for the optimal films, where the oxygen permeability decreased by about 8%, slightly improving the oxygen barrier properties. It was found that the good antioxidant activity of the films, which ranged from 50-83%, was due to the additive effect of the antioxidant plant extracts to the chitosan and vice versa on the antimicrobial activity.

All functionalized films showed the dominant effect of chitosan by inhibiting microbial growth, as the films with only plant extracts showed less inhibition than those with chitosan and extracts. Films with chitosan and extracts in a bilayer showed high inhibition of bacteria and yeast of 100%, while inhibition of fungi was slightly lower at 89% for *P. verrucosum* and 56% for *A. flavus*.

Acknowledgements

References

[1] L. Fras Zemljič, T. Kraševac Glaser, O. Plohl, I. Anžel, V. Šimat, M. Čagalj, E. Mežnar, V. Malin, M. Sterniša, S. Smole Možina (2022) *Journal of Functional Biomaterials*, 13, 228.

A Newly Synthetized Chitosan Derivative as a Promising Sorbent for Fungicides Enrichment from Wine Samples

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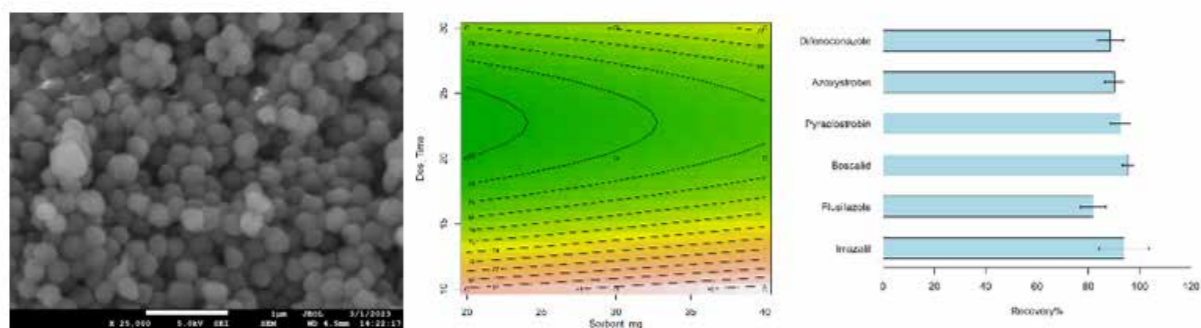
Investigation on new sorbent materials for sample pretreatment to apply in food analysis is an active research field. Chitosan (CS) (poly-b-(1 4)-2-amino-2-deoxy-d-glucose) is an amino-polysaccharide generated by the N-deacetylation of chitin, it is abundant, non-toxic, and exhibits a good adsorbent aptitude [1]. Due to easy formation of electrostatic interactions and coordination bonds between its amino and hydroxyl groups, CS has been largely studied for its possible use as a powerful sorbent material [2]. However, the applications of CS are mostly focused on compounds removal rather than recovery, due to its outstanding performance in adsorption, while desorption remains a more challenging process.

In wine science the application of CS and derivatives has been reported for removal of metals i.e. lead and cadmium, or organic contaminants i.e. ochratoxin A [3].

In the present study a new, inexpensive co-polymer of CS grafted with N-isopropylacrylamide, acrylic acid and triethylene glycol dimethacrylate was synthetized through free-radical polymerization, avoiding the use of harmful solvents. After characterization through Fourier transform infrared spectroscopy and scanning electron microscopy, it was tested as an innovative sorbent for dispersive solid phase extraction (d-SPE) followed by liquid chromatographic analysis (LC-MS) of wine samples spiked at trace level with fungicides frequently used on grapes. Several fungicides belonging to different chemical classes were considered (imazalil, flusilazole, boscalid, pyraclostrobin, azoxystrobin and difenoconazole). Some important extraction parameters (pH, sorbent amount, desorption solvent, adsorption/desorption time) were screened by mixed-levels Taguchi orthogonal array fractional factorial design of experiments and then further optimized by Box-Behnken design. Through design of experiments, an unexpected inverse relationship between the desorption time and the recovery was found. Despite the vast majority of materials employed in d-SPE, the CS derivative synthetized in the present study, exhibited higher recoveries at lower desorption times. The authors suggest this behaviour may be related to modification occurring in the sorbent when dispersed in an organic solvent for a prolonged time, which may lead to a reuptake of the analytes followed by a hindered release of the same. Under optimal conditions, the developed d-SPE-LC-MS method showed good methodological indices (extraction recoveries in the range 77.42 - 104.01 % for all the fungicides and RSD% between 2.34 and 10.19). The established method optimized for fungicides in wine samples, suggests the developed CS derivative is a promising material for pesticides enrichment from complex matrices.

Acknowledgements

References

[1] M. Rinaudo (2006) *Progress in Polymer Science*, 31, 603-632.
[2] X. Hai, F. Shi, Y. Zhu, L. Ma, L. Wang, J. Yin, X. Li, Z. Yang, M. Yuan, H. Xiong, Y. Gao (2023) *Food Chemistry*, 410, 135338.
[3] J. Zhang, R. Cai, T. Yue, Y. Yuan, Z. Gao, Z. Wang (2022) F*ood Chemistry*, 373, 131592.

Selenium Biofortification of Wheat Plants by Foliar Application of Liposomes: Selenium Speciation by Synchrotron Radiation Techniques

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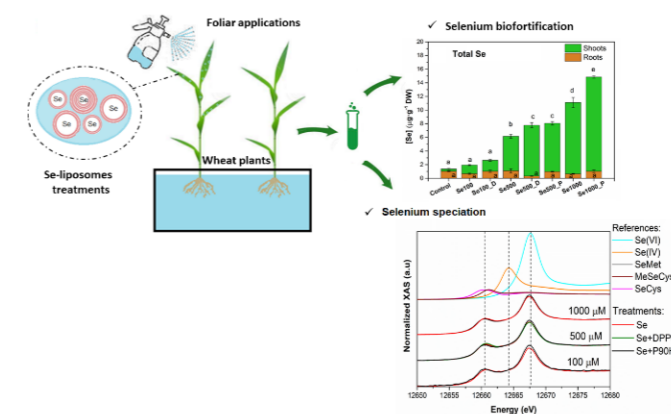
Selenium (Se) is an essential element for humans and animals [1]. Worldwide, the Se deficiency is considerable, and it has been estimated that 1 billion people have a scarce Se dietary intake due to low concentrations in soils [2]. To overcome this issue, Se agronomic biofortification practices are extensively investigated. Foliar Se fertilization is up to 8 times more effective than direct soil Se supplementation [3]. To enhance the yield of foliar feeding and to minimize the stress or toxicity to crops, nanocarrier delivery technologies have been proposed [4].

In the present work, liposomes have been used as nanocarriers in the biofortification of wheat plants with selenium using foliar application. Liposomal formulations based on 1,2-dipalmitoyl-sn-glycero-3-phosphocholine 16:0 PC (DPPC) or hydrogenated Phospholipon®90H (P90H) were prepared (mean size ~100 nm), loaded with different selenium concentrations, and applied at the plants' vegetative stage of growth (two applications). After the harvest, total Se was determined in wheat showing that this element was successfully incorporated into the plants without affecting their biomass production and photosynthetic pigments. Also, Se chemical speciation was studied by X-Ray Absorption Spectroscopy (XAS) using synchrotron radiation.

Highest Se enrichment was obtained by using the DPPC phospholipid and 1000 μmol·L-1 of Se concentration after two foliar applications. The speciation analysis of the XANES spectra revealed selenium in wheat to be mainly present as organic selenium and the application using liposomes influences the proportion of these organic Se species. This information will be useful to minimize both plant toxicity and economic costs towards a more effective Se supplementation.

### Acknowledgements

References

[1] M. Gupta, S. Gupta (2017) *Frontiers in Plant Science*, 7, 2074.
[2] M. Schiavon, S. Nardi, F. Dalla Vecchi, A. Ertani (2020) *Plant and Soil*, 453, 245-270.
[3] G. H. Ros, A. M. D. Van Rotterdam, D. W. Bussink, P. S. Bindraban (2016) *Plant and Soil*, 404, 99-112.
[4] P. L. Chariou, O. A. Ortega-Rivera, N. F. Steinmetz (2020) *ACS nano*, 14, 2678-2701.

The Importance of Experimental Design for the Evaluation of Polymer Nanocomposites as Antimicrobial Coatings on Food Contact Surfaces

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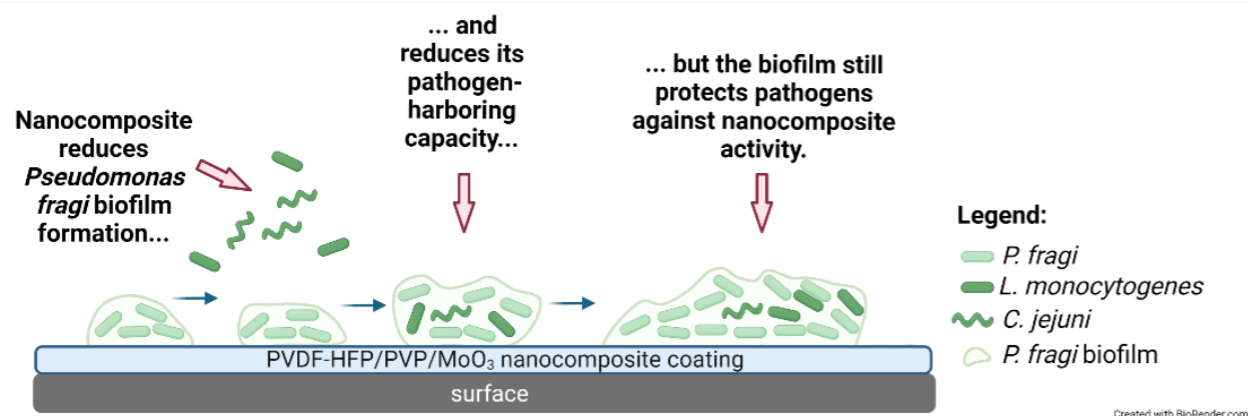
The development of **innovative nanocomposites** for use in the food industry has increased significantly in recent years. One of their potential applications is also on steel surfaces that come into contact with food. **Bacterial biofilms** are a major problem here. They represent a protective survival strategy of bacteria in a harsh environment, whose characteristic is also an increased resistance to the antimicrobial substances used to combat them [1].

Currently, there is **no existing standard method** for evaluating the activity of polymer nanocomposites on microorganisms. Thus, a number of in-house developed or adapted methods are used for such purposes in which different target microorganisms, media (liquid / solid, nutritional / nutrient restricted / nutrient depleted), incubation conditions and detection methods are used. The complexity of experiment increases with the additional evaluation of the performance in food-related environments – mainly due to the presence of nutrients from foods that can interact with antimicrobial agents and due to the presence of mixed microbial communities.

In our study, we focused on **evaluating the activity of the polymer nanocomposite on the stainless-steel surface in an environment mimicking food industry condition.** The coating we chose was a **PVDF-HFP/PVP/MoO3 nanocomposite** with well-characterised physicochemical properties and known antimicrobial activity [2]. The emphasis was on the test temperatures (5 °C / 15 °C) and the media used (phosphate buffered saline / fish meat homogenate / chicken meat homogenate). The selection of bacteria and their combinations was another factor. Here we focused on the combination of a bacterium that causes food spoilage and is widely distributed in the production environment of food of animal origin – *Pseudomonas fragi* and two pathogens that may be present in this environment – *Listeria monocytogenes* and *Campylobacter jejuni*. Quantification was performed by determining the viable cells – we determined the adherent / biofilm cells on the tested surface and the planktonic cells in the inoculum.

We have shown that the c**hoice of medium is critical for evaluating the performance of the polymer nanocomposite,** as the activity was lower in food homogenates, but a reduction in bacterial load of more than 95% was still achieved. In addition, we have shown that testing in mixed microbial communities is also important, as the **biofilm of** *P. fragi* **protected pathogenic bacteria from the activity of the nanocomposite** [3].

References

[1] H.-C. Flemming, J. Wingender (2010) *Nature Reviews Microbiology*, 8, 623-633.

[2] U. Gradišar Centa, M. Sterniša, B. Višić, Ž. Federl, S. Smole Možina, M. Remškar (2020) *Surface Innovations*, 9 (5), 256-266.

[3] M. Sterniša, U. Gradišar Centa, A. Drnovšek, M. Remškar, S. Smole Možina (2023) *International Journal of Food Microbiology,* in review.

Simultaneous Detection of Antimicrobial and Anti-Biofilm activity of Novel Compounds Using Salmonella Infantis – Improving Data Analytics Through Machine Learning

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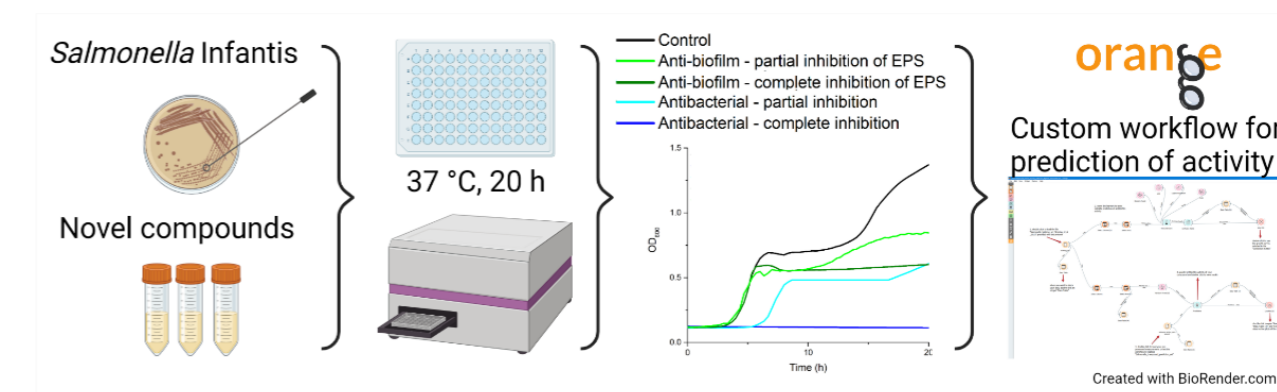
Salmonellosis is the second most common food-borne zoonosis and the most frequently identified cause of food-borne outbreaks in the EU [1]. A major problem in the food industry are **biofilms** – highly organized matrix-embedded structures protecting bacteria against adverse environmental conditions that provide a continuous source of contamination.

Much attention is given to finding new **anti-*Salmonella* – antimicrobial and anti-biofilm – agents.** As antimicrobial agents, these compounds inhibit bacterial growth or kill bacteria; as specific anti-biofilm agents, they may interact with biofilm-related metabolic processes or quorum sensing without affecting bacterial growth. These two concepts must be addressed separately, as agents that simultaneously affect growth and biofilms may also contribute to the further development of bacterial resistance. Therefore, there is a need to target biofilms and their formation without affecting bacterial growth [2].

We studied the bioactivity of cold- water fungal extracts. In doing so, we developed **a method for simultaneous detection of antimicrobial and anti-biofilm activity – SIMBA** (patent pending) [3]. The method combines the testing of both types of activity against bacteria with the evaluation of the 20-h growth curve of *Salmonella* Infantis ŽM9' strain determined by absorbance measurements at 600 nm in a 96-well plate. SIMBA method thus shortens the time required to determine the bioactivity of novel compounds, reduces material consumption, and eliminates the need for additional reagents compared to existing methods.

Because of the specific growth curve shapes that emerged, existing programs for processing these data do not provide fast or adequate interpretation of them. Therefore, the initial data analysis was by **visualization of** the growth curves complemented by **quantitative evaluation** of antimicrobial and anti-biofilm activity by calculating growth rate parameters and end-point OD600, respectively. **Machine learning** was leveraged to predict the possible activity by using the open-access tool Orange [4]. Experimentally verified data were used to train the model, which was further used to **predict the activity of the unknown compound** just by providing OD_{600} to the workflow.

References

[1] EFSA, ECDC (2019) *EFSA Journal*, 17, 5926.

[2] L. Dieltjens et. al. (2020) *Nature Communication*, 11, 107.

[3] M. Sterniša, J. Sabotič, A. Klančnik (2022) *International Journal of Food Microbiology*, 364, 109520.

[4] J. Demšar et al. (2013) *Journal of Machine Learning Research*, 14, 2349-2353.

Effects of *Lavandula* Essential Oil and Pure Phenolic Compounds on Invasion Mechanisms and Protein Profile of *Campylobacter jejuni*

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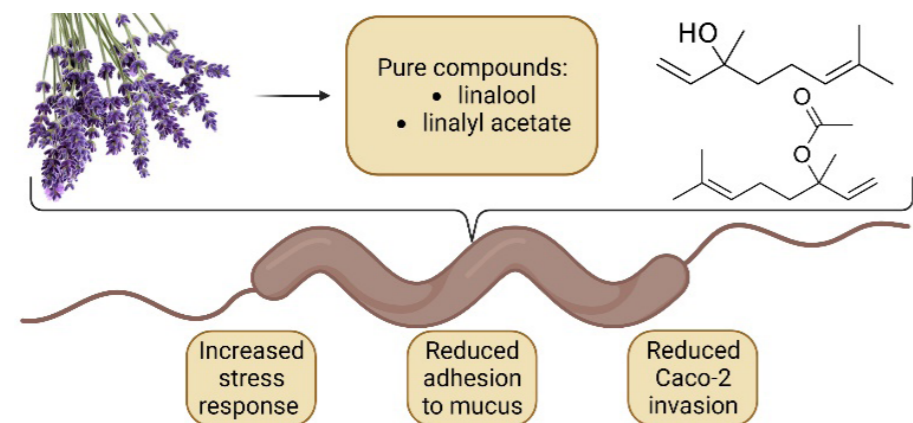
Campylobacter jejuni is a foodborne pathogen that is one of the most common causes of bacterial gastroenteritis worldwide. It is commonly found in the intestinal tract of poultry and can contaminate meat products during processing. In addition, *C. jejuni* can contaminate other foods such as milk, water and fresh produce, making contaminated food the main route of transmission of *C. jejuni* to humans.

Preventing the spread of *C. jejuni* in food is critical to reducing foodborne illness. The importance of alternative antimicrobial agents of natural origin *C. jejuni* is increasing because widespread antibiotic resistance to a variety of antibiotics and various biocides used in the food industry is a major problem and challenge for the food industry and researchers alike [1].

The genus *Lavandula* is a rich source of phytochemicals for the production of essential oil and pure phenolic compounds, which have great potential to use in the food industry. Our group has already demonstrated that they are effective in reducing adhesion and biofilm formation on abiotic surfaces commonly found in the food industry [2,3].

Thus, we continue to investigate the changes in protein profile and biological functions targeted by *Lavandula* essential oil and its pure phenolic compounds. We are also investigating their effects at subinhibitory concentrations on *C. jejuni* adhesion to various biotic surfaces and the invasion assay of the Caco-2 cell line, which serves as a model for the enterocytic brush border layer in the small intestine.

The results show that the compounds can reduce adhesion to the mucus surface, highlighting the specificity of surface attachment of *C. jejuni*. While the pure compounds were not able to reduce *C. jejuni* adhesion to Caco-2 cells, they were able to reduce cell invasion by more than 90%. Proteomic analysis showed us that *C. jejuni* treated with essential oil and the pure compounds linalool and linalyl acetate had lower biosynthesis of new amino acids and translation factors (NusG), but produced more of the following proteins: stress response (GroEL) to refold misfolded proteins, oxidative stress response (Dps, Cft) to prevent free radical damage and increase ATP synthesis (AtpD). Interestingly, they also produced more of the adhesin PorA, which may be involved in adhesion to intestinal cells, but overall it did not increase the adhesion of treated cells to Caco-2 cells.

We can conclude that *Lavandula* compounds have the potential to be used as natural alternatives to prevent and control *C. jejuni* by limiting its ability to establish itself in the intestine, thus preventing the possibility of invasion and disease.

References

[1] A. Mavri, S. Smole Možina (2013) *International Journal of Food Microbiology,* 160, 304-312.

[2] D. Ramić, F. Bucar, U. Kunej, I. Dogša, A. Klančnik, S. Smole Možina (2021) *Applied and Environmental Microbiology*, 87 (19), e01099-21.

[3] D. Ramić, J. Ogrizek, F. Bucar, B. Jeršek, M. Jeršek, S. Smole Možina (2022) *Antibiotics*, 11 (7), 854.

Cr Speciation in Wine and Beer using HPLC-ICP-MS and Enriched Stable Isotopic Tracers of ^{50}Cr(VI) and ^{53}Cr(III)

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Hexavalent chromium (Cr(VI)) compounds are highly toxic, carcinogenic, mutagenic and can cause dermatitis. In contrast, trivalent chromium (Cr(III)) compounds are far less toxic than Cr(VI) [1].

Exposure to Cr(VI) through the consumption of foodstuffs of animal or plant origin, as well as beverages, is unlikely to occur since the presence of reducing substances and organic matter in food prevents the existence of Cr(VI) [2]. The only possible source of exposure to Cr(VI) in beverages is through the consumption of contaminated water.

Among trace elements, Cr is also present in wine and beer. The specific behaviour of Cr in complex matrices was not taken into account, leading to an overestimation of the Cr(VI) content reported in scientific literature [2]. False statements have also been reported regarding the presence of Cr(VI) in wine and beer [3,4]. The main reason for reporting erroneous results came from the characteristics of Cr(III), as it can form negatively charged complexes with organic ligands that behave similarly to negatively charged chromate. If Cr(VI) were present in wine and beer, their consumption would pose a chronic long-term exposure and health hazard to humans. Since wine and beer contain polyphenols and other antioxidants [5], it can be assumed that Cr(VI) cannot exist in these beverages.

To test this hypothesis, the Cr(VI) content in wine and beer samples was determined with speciation analysis based on high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The behaviour of Cr(III) and Cr(VI) was followed using enriched ^{50}Cr(VI) and ^{53}Cr(III) stable isotopic tracers added to wine and beer samples.

The results showed that wine and beer do not contain toxic Cr(VI). Its concentrations were below 0.06 ng/mL. By using stable isotopic tracers of Cr, it was further demonstrated that the added ^{50}Cr(VI) was completely reduced by the polyphenols and organic matter, while the formed ^{50}Cr(III) was complexed by the organic ligands. The results are consistent with the known behaviour of Cr in plant material. The use of enriched stable isotopic tracers of Cr in combination with HPLC-ICP-MS significantly improves the reliability and validity of the analytical data. These findings highlight the importance of selecting an appropriate method for Cr speciation to accurately determine the forms of Cr present in food and to properly interpret the resulting analytical data. The availability of reliable analytical data is crucial for the evaluation of food safety.

References

[1] Y. Wang, H. Su, Y. Gu, X. Song, J. Zhao (2017) *OncoTargets and Therapy*, 10, 4065-4079.

[2] R. Milačič, J. Ščančar (2018) *Food Chemistry,* 254, 78-79.

[3] I. López-García, Y. Vicente-Martínez, M. Hernández-Córdoba (2015) *Talanta* 132, 23-28.

[4] E. Vieira, M. E. Soares, M. Kozior, Z. Krejpcio, I. M. Ferreira, M. L. Bastos (2014) *Journal of Agricultural and Food Chemistry,* 6, 9195-9200.

[5] M. Ivanović, M. Islamčević Razboršek, M. Kolar (2016) *Acta Chimica Slovenica 63*, 661-669.

The Risk of Plants Contamination by Potentially Toxic Elements Near Mining Sites

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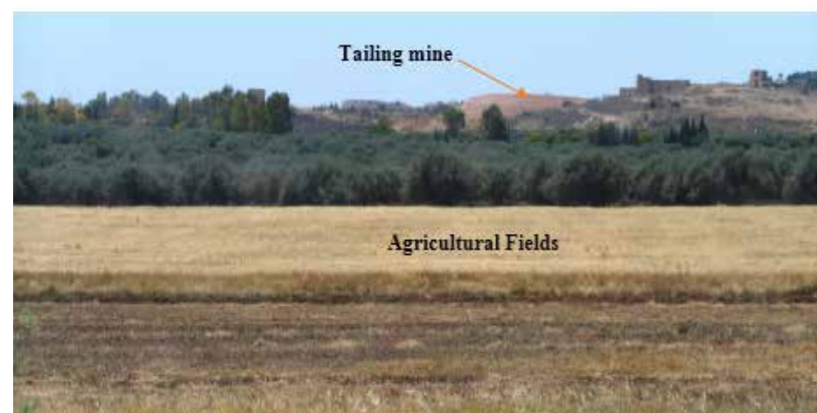
Several works have shown that the presence of high levels of metals in soils contaminated by mining waste can cause disturbances in the development and functioning of plants grown on site [1,2,3,4]. Understanding the behavior of these contaminants in the soil/plant system is necessary to assess the risk to ecosystems.

In this study we are interested in metals (Pb, Zn, Cd) and metalloids (As and Sb) that can be absorbed by plants, grown around former mining sites (Jalta, Jebel Hallouf-Sidi Bouaouane and Jebel Ghozlane) in north-west of Tunisia. Three edible plants were considered; e.g., Wheat (Triticum durum salevum), Barley (Hordeum vulgare) and caper (Capparis spinosa).

Results showed that these soils are characterized by a fine texture and highly contaminated by PTE (Pb, Zn, Cd, As, Sb). The levels of these elements in the organs of the plants (roots, stems, leaves and seeds) grown on contaminated soils are higher compared to the levels in the control plants. It was also noted that the contents of Pb, Zn and Cd are more concentrated in the roots than in the aerial organs, whereas As and Sb are concentrated in both root and aerial organs.

Therefore, the presence of PTE in the soil is harmful with respect to the food chain and human health.

Keywords: contamination; edible plants, mining sites, soil, potentially toxic elements (PTE), northern Tunisia.

References

[1] H. K. Chakroun, F. Souissi, J. L. Bouchardon, R. Souissi, J. Moutte, O. Faure,…, S. Abdeljaoued (2010) *African Journal of Environmental Science and Technology*, 4 (10), 651-659.

[2] A. Kabata-Pendias, H. Pendias in *Trace Elements in Soils and Plants*, A. Kabata-Pendias (Ed.), 3rd Edition, Boca Raton (US), CRC Press, 2001, 331.

[3] J. Wang, J. Su, Z. Li, B. Liu, G. Cheng, Y. Jiang, …, W. Yuan (2019) *Environmental Pollution*, 254, 113089.

[4] L. Chen, M. Zhou, J. Wang, Z. Zhiqin, D. Chengjiao, W. Xiangxiang, …, L. Fang (2022) *Science of the Total Environment*, 155441.

Thermal and Rheological Properties of Nut Butters as a Tool for Assessing Butter Quality

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Meals following a healthy lifestyle usually consist of nuts or their products, since nuts are high in proteins, fibres, essential fatty acids, and unsaturated fats.

The rheological and thermal properties have great importance on various factors, including determination of the product's sensory characteristics, texture, consistency, industrial process parameters, storage conditions. Foods usually exhibit thixotropic rheological behaviour, which means that their rheological properties depend on the flow history of the product [1]. For example, the viscosity of nut butter is affected by the size of solid particles in the product. Moreover, the rheological properties of nut butters depend also on the chemical composition or content of macronutrients such as proteins, fibres, lipids, and the type of fatty acids.

In the present work, peanut, almond and cashew butters with and without added oil were used for the analysis. The thermograms of nut butters showed that the thermal properties depended on the chemical composition of the nuts. The saturated fat content altered the glass transition temperature (Tg) [2], while the protein content changed the enthalpy [3]. Cashew butter exhibited the highest content of saturated fats and therefore the highest Tg (– 5.5 °C), while the content of fats in almond butter was low and therefore exhibited the lowest Tg (– 12.6 °C). Moreover, the lowest protein content was observed in cashew butter without added oil, which also exhibited the lowest enthalpy (29.36 J/g). The results showed that the rheological properties of the butters depend also on chemical composition; viscosity decreased with increasing fat content [4], yield strength increased with narrowing the particle size distribution [5], storage and loss modulus decreased intensively in case of increased amount of dietary fibre [6].

The effect of oil addition to cashew butter was also studied. The addition of oil decreased the values of storage and loss modulus at frequency and amplitude tests. Particle size analysis showed that the largest particle size was observed in almond butter, while the smallest particle size was detected in peanut butter. The particle size distribution was the widest for the cashew butter with no added oil, and the narrowest for the almond butter.

References

[1] G. P. Citerne, P. J. Carreau, M. Moan (2001) *Rheological Acta*, 40, 86-96.

[2] K. M. Barbosa, L. P. Cardoso, A. P. B. Ribeiro et.al. (2018) *Journal of Food Science and Technology*, 55 (3), 1104-1115.

[3] J. Jiang, Z. Song, Q. Wang et. al. (2019) *Ultrasonics Sonochemistry*, 58, 104641.

[4] O. Sakiyan, G. Summu, S. Sahin, G. Bayram (2004) *European Food Research and Technology*, 219, 635-638.

[5] N. H. Mohd Rozalli, N. L. Chin, Y. A. Yusof (2015) *International Journal of Food Properties*, 18 (9), 1888-1894.

[6] A. Aydogdu, G. Sumnu, S. Sahin (2018) *Journal of Food Science and Technology*, 55 (2), 667-677.

Determination of Regulated and Emerging Mycotoxins in Organic and Conventional Gluten-Free Flours by LC-MS/MS

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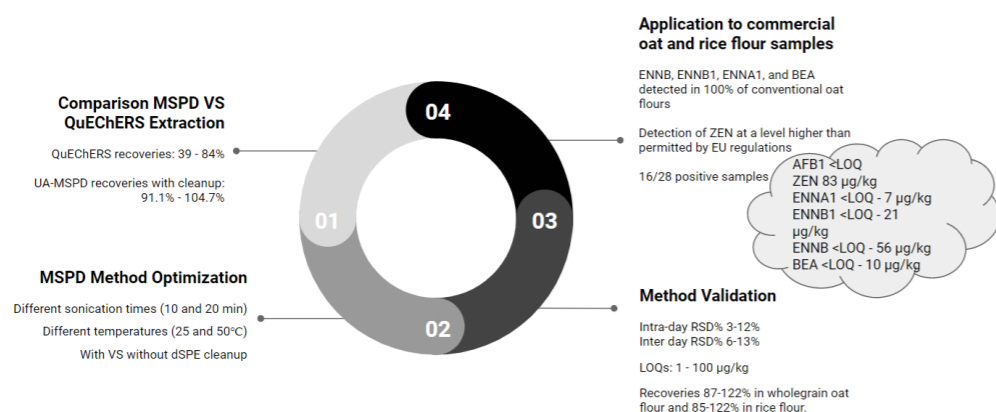
The number of known mycotoxins exceeds 300 and, given their toxicological effects, several countries have legally set limits as to their acceptable concentrations in food products. However, the information on the levels of emerging mycotoxins remains scarce. Considering the continuous rise in the popularity of gluten-free and organic products, our aim was to develop an efficient, sensitive, and selective method for the detection of four emerging (beauvericin and enniatins A1, B, and B1) and three regulated (aflatoxin B1, zearalenone, and deoxynivalenol) mycotoxins in gluten-free organic and conventional flours. Ultrasound-assisted matrix solid-phase dispersion (UA-MSPD) and QuEChERS methods were applied for extraction, with the first resulting the most promising. The validated UA-MSPD method was utilized for the LC-MS/MS analysis of conventional and organic wholegrain oat and rice flours. All target mycotoxins, except for deoxynivalenol were detected in these samples. Multi-mycotoxin contamination was found in all flour types, with the highest frequency of occurrence in conventional wholegrain oat flour.

Despite their limited presence in rice flour, one sample was found to contain zearalenone at a concentration of 83.2 µg/kg, which was higher than the level set by the European Commission for cereal flours. The emerging mycotoxins had the highest detection frequencies; enniatin B was present in 53% of the samples at a maximum concentration of 56 µg/kg, followed by enniatin B1 and beauvericin which were detected in 46% of the samples, and at levels reaching 21 µg/kg and 10 µg/kg, respectively. These results highlight the need to improve current knowledge and regulations on the presence of mycotoxins, particularly emerging ones, in gluten-free flours and cereal-based products.

Acknowledgements

Microbial Communities on Microplastics from Nearshore Seawater and Mediterranean Mussels *Mytilus galloprovincialis* in Slovenia

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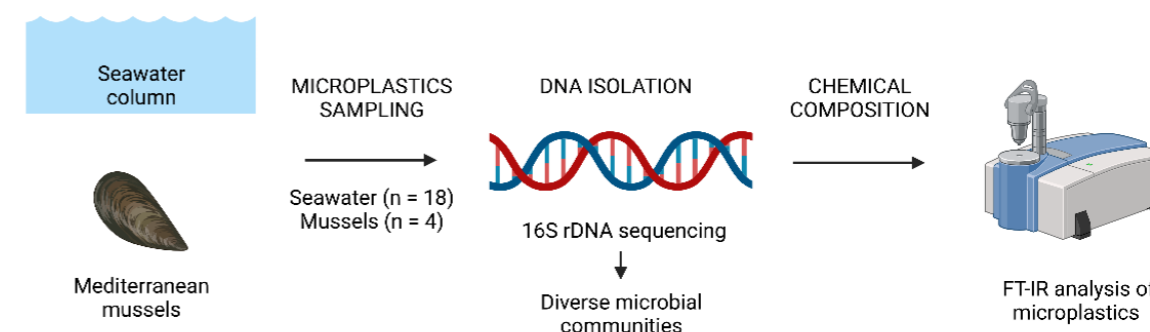
Microplastics (MPs) are an increasing anthropogenic pollutant of terrestrial and aquatic ecosystems. The long half-life and hydrophobic surface of plastics allow microbes to colonise MPs and form biofilms distinct from the free-flowing marine microbiome [1]. Recent research has highlighted the accumulation of microplastics through trophic transfer and its role as a vector for pathogen transport in the marine environment [2]. In the present study, we investigated the presence of MP particles in coastal waters and Mediterranean mussels (*Mytilus galloprovincialis*), determined the microbial communities on the particles, and characterised the microplastic particles by imaging and chemical analysis.

Water samples (n=3) were collected from the seawater column (0-2 m depth) using a water pump, filtered (100 µm) through a filtration system on the boat in Debeli Rtič Bay, Slovenia, and frozen. Mussels (n=126) were collected from a mollusc farm at Debeli Rtič, and frozen. MPs were isolated from mussels and water samples by visual inspection under a stereomicroscope using tweezers (water) and dissection equipment (mussels). DNA was extracted from each MP particle found (mussel and water samples), and sequenced using Illumina 16S rDNA amplification. Chemical characterization of MPs was performed using FT-IR analysis.

A total of 18 MP particles were isolated from seawater and four particles were isolated from mussels. Sufficient DNA was obtained from five seawater and one mussel particle for microbiome analysis. Bacteria from more than 11 different phyla were detected on the examined MPs. The predominant phyla in all samples were Proteobacteria,

Firmicutes, Cyanobacteria, Bacteroidetes, and Actinobacteria. At the genus level, the predominant bacteria in all samples were *Pseudomonas*. Bacteria of the genera *Campylobacter, Klebsiella,* and *Streptococcus* were detected at a low proportion in 5 individual MP samples. Analysis of the chemical composition of MPs revealed that the particles consist of polyvinyl chloride, high-density polyethylene, and polypropylene, among others, indicating different sources of pollution.

Microplastics are present in nearshore seawater and are ingested by filter feeders such as mussels to transfer MPs through the food chain. The microbial communities inhabiting the studied plastisphere were diverse within each sample and varied between samples. The transmission of microplastics in the food chain not only contaminates foods with plastic particles, but also facilitates the transmission of potential pathogens.

Acknowledgements

References

[1] E. R. Zettler, T. J. Mincer, L. A. Amaral-Zettler (2013) *Environmental Science and Technology*, 47, 7137-7146.
[2] M. Kovač Viršek, M. N. Lovšin, Š. Koren, A. Kržan, M. Peterlin (2017) *Marine Pollution Bulletin*, 125, 301-309.

Evaluation of Naturally Adhered Bacteria on Lettuce *(Lactuca sativa)* Leaves

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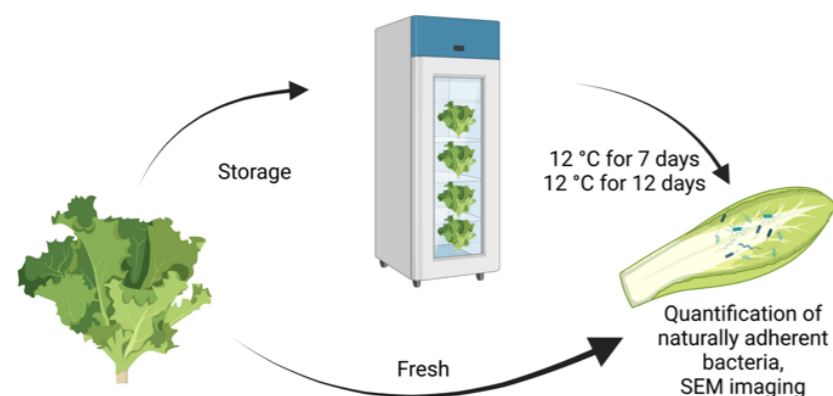
Foodborne disease outbreaks are a pervasive threat in the food chain. Outbreaks associated with fresh produce can occur across countries, as the path from farm-to-fork has lengthened, providing more opportunities for contamination with bacterial pathogens, such as *Escherichia coli, Salmonella enterica, Listeria monocytogenes*, and others [1]. To prevent contamination of vegetables with pathogenic bacteria, it is necessary to ensure not only good agricultural and production practices but also appropriate conditions during distribution, storage and processing.

The aim of the present study was to evaluate the change in the number of naturally adhered bacteria during storage of lettuce (*Lactuca sativa*). The adherent aerobic mesophilic bacteria (AAMB) naturally present on lettuce leaves were determined [2], and the bacterial cells on the lettuce leaf were imaged by scanning electron microscopy (SEM).

Lettuce was purchased from various stores and analysed fresh or after incubation at 12 °C for 7 and 12 days. After storage, the number of naturally adhered bacteria was determined on a central part of the lettuce leaf, which was aseptically excised and placed in a sterile pot, and physiological solution (0.9 % NaCl, 100 ml) was added. The leaf was washed by mixing (100 RPM for 2 min), allowing the non-adherent bacteria to detach from the surface. After washing, the leaf was used to quantify the adherent bacteria applying plate count method (PCA; plate count agar). AAMB were quantified after incubation at 30 °C for 24 h.

Washing the lettuce with sterile physiological solution in the pot affects the reduction of AAMB. After 1-day storage, the AAMB after rinsing amounted to 3.4 ± 0.4 log cfu/g, while without rinsing it amounted to 4.4 ± 0.1 log cfu/g. After 7-day storage of samples the results were similar, the AAMB was determined to be 5.3 ± 0.1 log cfu/g whereas without rinsing it was 5.9 ± 0.2 log cfu/g. The average number of AAMB increased by almost 2 log units after 7 days of storage at 12 °C. Similar results were obtained with the second set of lettuce samples, but the results showed that the adhesion of naturally occurring bacteria increased more during the first 7 days, while their numbers increased more slowly after 9 and 12 days. The results were also visible in SEM images of leaves.

Fresh produce can get contaminated with pathogens at many stages from pre-harvest, harvest, post-harvest and during processing [3]. As we have shown, the number of adhered bacterial cells increases with increasing storage time in mild temperatures. Obviously, required nutrients are present on the surface that stimulate the growth of the naturally occurring microbiota under favourable conditions.

References

[1] R. M. Callejón, M. I. Rodríguez-Naranjo, C. Ubeda, et al. (2015) *Foodborne Pathogens and Disease*, 12, 32-38.

[2] *Adhezija bakterij vrst L. monocytogenes in E. coli na zeleno solato (Lactuca Sativa),* G. Svetelj, Ljubljana (Slovenia), Biotehniška fakulteta, 2017.

[3] C. K. Carstens, J. K. Salazar, C. Darkoh (2019) *Frontiers in Microbiology*, 10, 2667.

Selected Non-Protein Nitrogen Compounds in Insects for Food and Feed Purposes

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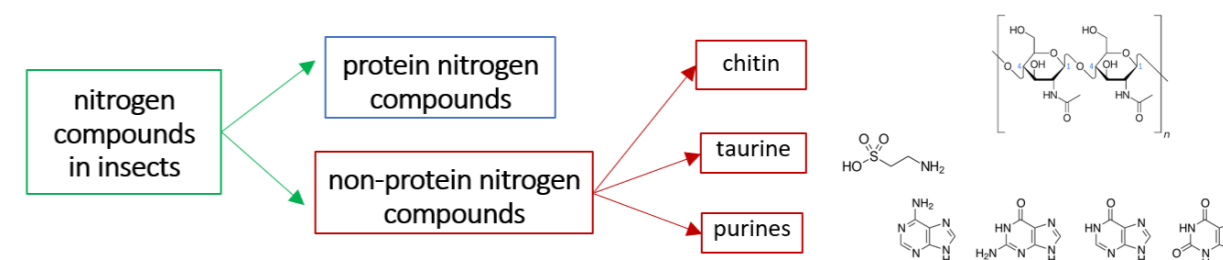
Insects are considered a nutritionally valuable source of alternative proteins [1]. The protein content in the literature is mainly based on nitrogen content using the nitrogen-to-protein conversion factor of 6.25 generally used for proteins. Besides true protein, there is a significant proportion (11–26%) of non-protein nitrogen (NPN) compounds in insects which should be considered [2]. These NPN compounds are for example, chitin, nucleic acids, phospholipids, and excretion products (e.g., ammonia) in the intestinal tract.

Our studies in the field of NPN substances were focussed on chitin, purines (adenine, guanine, xanthine, and hypoxanthine) and their metabolite (uric acid), and taurine in various insect species intended for food and feed purposes.

Purines and uric acid content were determined by RP-HPLC with UV detection [3], the chitin present in the insect samples was converted to glucosamine and determined by spectrophotometric method [4], taurine was determined by an electrophoretic method [5].

The chitin content in the samples ranged from 1.64 to 3.03 g/100 g fresh weight; larvae had the lowest values as compared to other developmental stages [4].

Compared to meat, edible insects contained equivalent or higher amounts of total purine (3.23 13.22 g/kg edible insect dry matter vs. 3.61 5.53 g/kg meat dry matter). The uric acid content was significantly affected by the developmental stage and species [6].

Amongst the tested samples, *Gryllus assimilis* contained the highest levels of taurine (121.0 ± 10.2 mg/100 g fresh weight). On the contrary, the lowest taurine level was detected in *Schistocerca gregaria* (5.4 ± 0.6 mg/100 g fresh weight) [4].

As non-protein nitrogen compounds account for a relatively high percentage of the total nitrogen in insects, the amount of nitrogen present from NPC compounds should be considered when calculating the true protein content from the total amount of nitrogen substances.

References

[1] *Edible Insects. Future Prospects for Food and Feed Security.* A. van Huis, J. van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, P. Vantomme, Rome (Italy), FAO, 2013.

[2] R. H. Janssen, J. -P. Vincken, L. A. M. van den Broek, V. Fogliano, C. M. M. Lakemond (2017) *Journal of Agricultural and Food Chemistry*, 65, 2275-2278.

[3] M. Sabololová, M. Kulma, L. Kouřimská (2021) *Journal of Food Composition and Analysis*, 96, e103746.

[4] L. Kourimska, F. Kvasnička, M. Kurečka, A. Rajchl, P. Škvorová, M. Kulma (2023) *Journal of Insects as Food and Feed,* accepted for publication.

[5] F. Kvasnička, A. Rajchl (2021) *Journal of Chromatography A*, 1645, e462075.

[6] M. Sabolová, M. Kulma, P. Škvorová, K. Veselá, M. Kurečka, L. Kouřimská (2023) *Journal of Insects as Food and Feed*, 9, 77-85.

Stable Isotope Ratio Analysis of H, C, N and S in Edible Insect Samples from Europe, Asia and Africa

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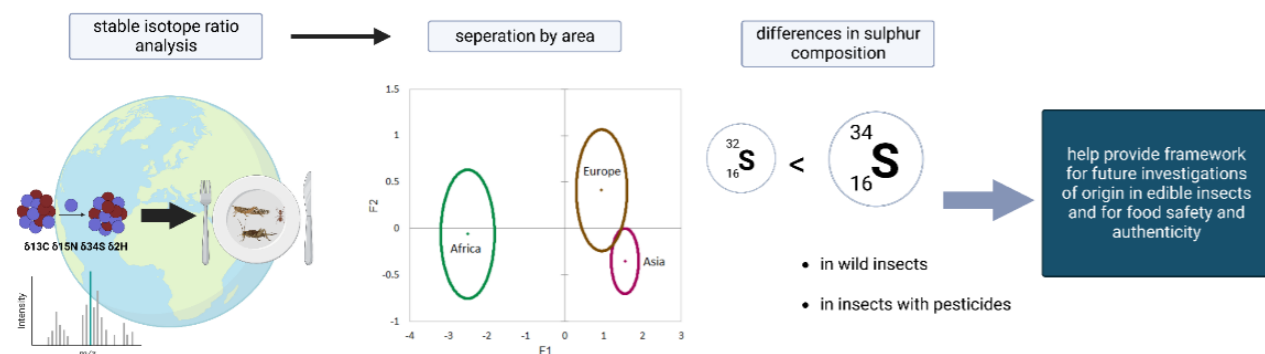
With a continuously growing world population, alternative food sources with high nutritional value are becoming increasingly important and will be a crucial factor in the global food system. [1] Insects have already proven to be a valuable protein alternative, due to their high nutritional value and their limited use of land, water, energy, and reduced greenhouse gas emission compared to common livestock. [2] In recent years, the European Union has added edible insects to the list of novel foods, allowing an increasing number of insect-based food products into the European market. While a first background reference stable isotopes ratio analysis has previously been provided by *Pianezze et al.* [3], extensive datasets for stable isotopes in edible insects have not yet been established. In a follow-up study, we conducted stable isotope ratio analyses of different edible insect samples (n=49) purchased from Asia, Africa and Europe. δ^{13}C, δ^{15}N, δ^{34}S and δ^2H isotopic compositions of the defatted samples were analysed using a Sercon 20-22 Isotope Ratio Mass Spectrometer coupled to an elemental analyser (ISOEarth+) for simultaneous analysis of ^{13}C/^{12}C, ^{15}N/^{14}N and ^{34}S/^{32}S ratios and a pyrolizer (Vecstar HT furnace) for measurement of ^{2}H/^{1}H ratios. Differences in isotope ratios between countries, wild and farmed insects, and samples containing pesticides were investigated using descriptive statistics, Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Linear Discriminative Analysis (LDA).

There were significant differences between countries in δ^{13}C- (p < 0.01) and δ^{15}N- (p < 0.01) values, while δ^{34}S and δ^2H were not significant. However, δ^2H had a p-value of 0.055, and might thus show differences between countries when considering a larger dataset. While it was not possible to distinguish between countries using PCA and LDA, LDA could be used

to distinguish between larger areas (i.e. Africa, Europe and Asia). In general, African samples had a more distinct isotopic profile compared to Europe and Asia. As for wild vs farmed insect samples, it was observed that wild samples contained heavier sulphur than farmed samples. When comparing the isotopic compositions of samples containing pesticides with samples with no detected pesticides, differences in sulphur compositions could be observed. Additionally, LDA was able to correctly classify the presence of pesticides in a sample with 76% correct classification based on the sulphur composition. These findings could help to provide a framework for future investigations on origin of edible insects, as well as food safety and authenticity and to indicate eventual pesticide occurrence.

Acknowledgements

References

[1] FAO (2018) *Future of Food and Agriculture: Alternative Pathways to 2050*, Rome, Italy.

[2] FAO (2013) *Edible insects: Future prospects for food and feed security.* FAO forestry paper, 171, Rome, Italy.

[3] S. Pianezze, M. Perini, L. Bontempo, E. Piasentier, G. Poma, A. Covaci, F. Camin (2021) *Journal of Food and Feed*, 7, 955-964.

Multiresidue Determination of Current-used Pesticides in Edible Insects by Orbitrap HRMS Target, Suspect and Non-target Approaches

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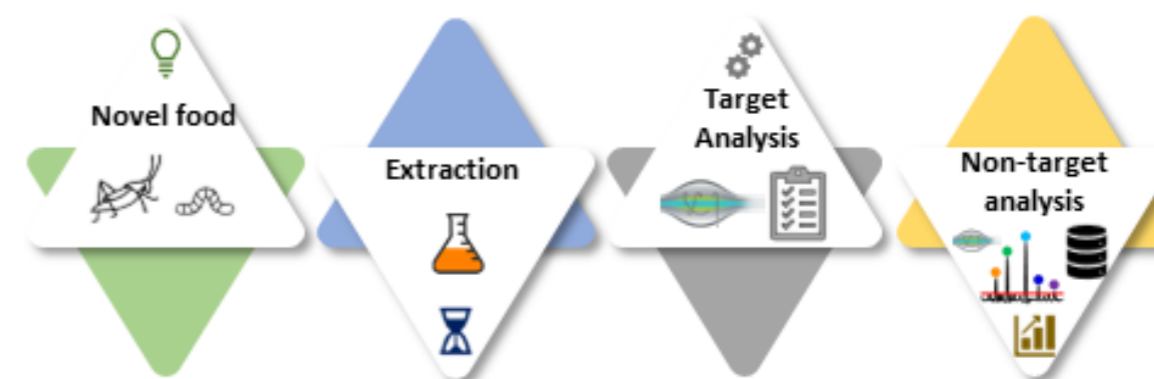
Edible insects are emerging as a sustainable alternative that can satisfy protein needs while promoting environmental sustainability [1,2]. However, there is a crucial need to study the safety of farmed insects in the EU, considering the growing interest in using them as a sustainable protein source for human and animal consumption.

Furthermore, additional safety information on edible insects will help redirect current perceptions and concerns regarding entomophagy [3,4].

In this study, ultra high-pressure liquid chromatography (UHPLC) tandem high-resolution mass spectrometry (HRMS) was employed to provide high mass accuracy data on extracts from edible insects. Three species (yellow mealworm, house cricket, and locust, and their respective feed) were studied. A modified QuEChERS methodology was followed, and samples were injected into a Q Exactive Focus Orbitrap MS system equipped with a heated ESI source, acquiring spectra in both positive and negative ionization mode, operating in data-depended Discover mode. Three approaches were exploited target, suspect screening, and non-target analysis.

Target analysis was used to study the qualitative and quantitative identification of a mixture of more than 300 compounds, including currently used pesticides. The applied method was investigated regarding linearity, precision, and method detection and quantification limits. The method yielded acceptable recoveries (70%) for most studied compounds.

Regarding suspect and non-target analysis, comprehensive workflows were designed in Compound Discoverer. A plethora of nodes for peak picking, feature alignment, and compound annotation connected with online spectral databases enabled the

tentative annotation of pesticides with high confidence based on fragmentation pattern and isotopic profile.

Finally, a matrix-effect study was performed due to the complexity of the matrix. The study was also expanded into the non-target approach by setting spiked samples and a spiked solvent as unknowns. Results demonstrated that the use of matrix-matched calibration aids in accurate quantification.

Acknowledgements

References

[1] G. Poma, S. Yin, B. Tang, Y. Fujii, M. Cuykx, A. Covaci (2019) *Research*, 127, 1.

[2] G. Poma, M. Cuykx, E. Amato, C. Calaprice, J. F. Focant, A. Covaci (2017) *Food and Chemical Toxicology*, 100, 70.

[3] G. Poma, Y. Fujii, S. Lievens, J. Bombeke, B. Gao, Y. Jeong, T. J. McGrath, A. Covaci (2021) *Food and Chemical Toxicology*, 154, 112311.

[4] G. Poma, S. Yin, B. T. Folarin, A. M. Schönleben, J. Bombeke, J. C. Altamirano, G. Ssepuuya, D. Nakimbugwe, T. Oluseyi, A. Covaci (2022) *Journal of Environmental Exposure Assessment*, 1, 22.

Descriptive Sensory Analysis of Edible Insects in Czechia: Tasty or Nasty? And Does Size Really Matter?

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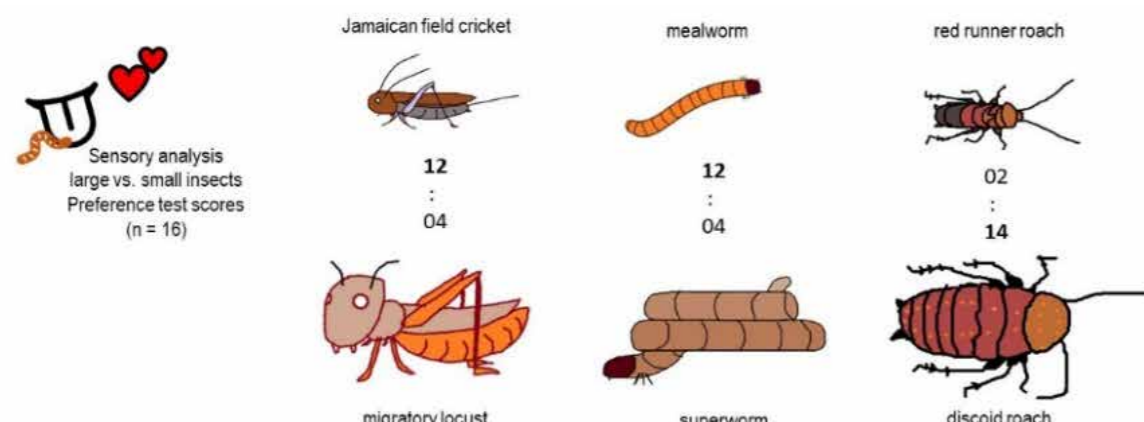
During the last few decades, intensive research has shown that insects are one of the most promising alternative foods. Generally, insects are rich sources of high-quality proteins and lipids, as well as micronutrients [1]. Because of the low environmental impact, high biomass gain, and valorization of by-products or waste from the food industry, the production of insects might be both feasible and sustainable [2]. To promote insects as food, the introduction and establishment of insect foods in the European market represents a serious challenge in the near future. The willingness to buy insect-based products can be influenced by many factors such as food type, labeling, price, and taste [3]. In general, insects are available either whole or ground, when the perception of insects by humans varies. Therefore, species selection is one of the first and most crucial steps when designing insect-based food.

Our recently conducted questionnaire survey [4] focused on the acceptance of insects by Czech respondents revealed different preferences between the consumption of whole and ground insects. Regarding preferences, ground insects or insect meal were the favorite forms of consumption, regardless of the species. In case of eating of whole insects, processed crickets, katydids and locusts were the top-rated insects, and cockroaches were the least popular choice.

To identify whether the species and size may affect the acceptance of insects, small and large species of Blattodea, Coleoptera, and Orthoptera were assessed in their whole form and as ground meals in white bread and chocolate cookies by a panel of 16 panelists. The hedonic ranking test of whole fried and salted insects showed that *Tenebrio molitor* was the best

choice. According to the paired preference test, smaller insects were preferred in the case of Orthoptera and Coleoptera, but larger cockroaches were rated better than smaller ones. The overall acceptability of insect meal in food products was higher than that of whole insects. Only bread containing *Tenebrio molitor* and *Gryllus assimilis* meals were comparable to those of the control, whereas no significant differences were detected among the cookie samples.

Conclusively, it was proven that the acceptance of whole insects is influenced more by species than size, and all tested meals can be used to fortify confectionary products with no adverse effects on their liking.

Acknowledgements

References

[1] D. G. A. B. Oonincx, M. D. Finke (2021) *Journal of Insects as Food Feed*, 7 (5), 639-659.
[2] A. van Huis (2022) *Entomological Research*, 52 (4), 161-177.
[3] S. Mancini, R. Moruzzo, F. Riccioli, G. Paci. (2019) *Food Research International*, 122, 661-678.
[4] M. Kulma, V. Tůmová, A. Fialová, L. Kouřimská (2020) *Journal of Insects as Food and Feed*, 6 (5), 525-535.

Motivation and Barriers to the Adoption of New Alternative Foods among European Consumers

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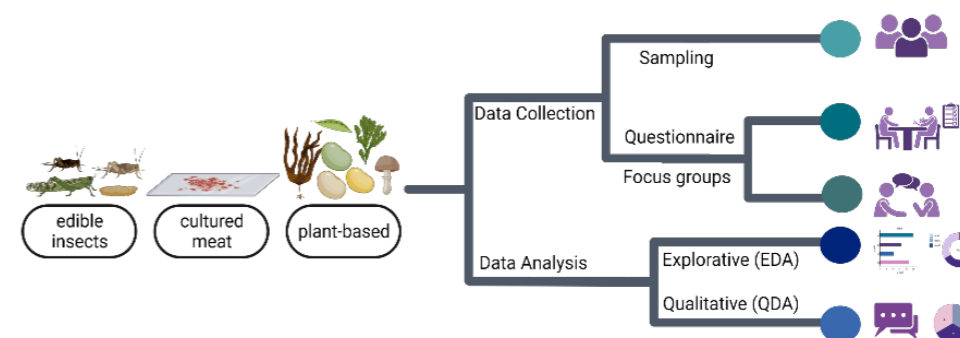
"Alternative protein foods" are derived from plants, microorganisms, insects, and cultured meat [1,2]. They are also referred to as "novel" proteins [3] or "non-traditional" as they are not typically consumed as the primary source of protein in Western diets [1,4].

The literature identifies that alternative protein sources offer improved health and nutrition and have a greater positive impact on sustainability and the environment as compared to traditional animal protein sources [1,2,3]. However, identifying the positive effects of alternative proteins is insufficient for successful market integration [1,2,3,4]. This requires a thorough understanding of factors that influence consumer acceptance and adoption. These factors include perceptions of taste, texture, price, health benefits, environmental sustainability, social, and cultural influences [1,2,3,4]. Furthermore, there is limited understanding of why some alternative protein sources are preferred over others.

This study sought to investigate consumer motivations, identify the root causes of barriers, particularly negative attitudes, and suggest potential solutions to increase the acceptance and adoption of alternative foods in Europe.

The study utilised a mixed-methods research approach, starting with a questionnaire, pilot-tested and distributed via online/offline channels in Europe. The collected data was analysed using explorative data analysis (EDA) to identify patterns and trends. The study further employed a focus group study to provide additional insights, which will be analysed using qualitative data analysis (QDA). The preliminary results show consumers' varying opinions on introducing alternative foods. Majority cited demands for healthier options (57%) and sustainability (46%), while a significant proportion believed it was to promote healthy eating (41%) and sustainable consumption (49%). There was also

a difference among demographics such as 26% of UK consumers marked "neutral/not sure" and 20% of Slovenians marked "agree" regarding the safety of consuming edible insects.

Plant-based alternatives were the most popular among consumers, with 97% having heard of it and 96% having tried it. However, both cultured meat and insect-based foods had lower levels of awareness and acceptance. Overall, cultured meat was found to be more acceptable than insects as cited in literature [1,2].

The study further revealed consumers' willingness to try new foods and emphasised the impact of cultural factors and safety concerns on attitudes and barriers. These findings can aid in developing effective strategies to address the barriers to adopting alternative protein sources.

Acknowledgements

References

[1] M. C. Onwezen, E. P. Bouwman, M. J. Reinders, H. Dagevos (2021) *Appetite*, 159, 105058.
[2] S. Anusha Siddiqui, N. A. Bahmid, C. M. Mahmud, F. Boukid, M. Lamri, M. Gagaoua (2022) *Critical reviews in food science and nutrition*, 1-22.
[3] I. Ismail, Y. H. Hwang, S. T. Joo (2020) *Journal of animal science and technology*, 62 (2), 111.
[4] M. Henchion, M. Hayes, A. M. Mullen, M. Fenelon, B. Tiwari (2017) *Foods*, 6 (7), 53.

Optical Sensors for Food Freshness and Pesticides detection

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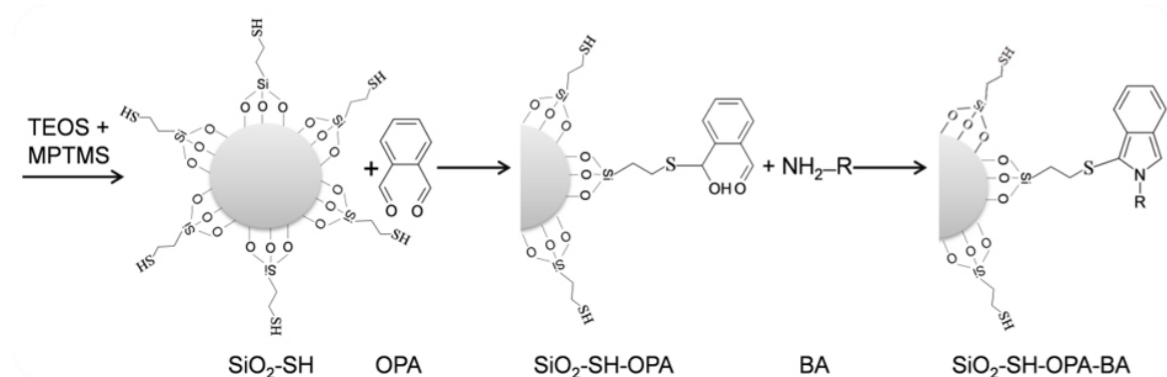
Sensors are devices that obtain a physical quantity and convert it into an analytical signal (i.e. mechanical, optical, or electrical). The field of sensor technology is extremely broad, and its current development is primarily based on simplicity, rapid response, and cost-effectiveness. Food safety is a scientific area that requires advanced handling, storage, and preparation. The primary techniques for food analysis are time-consuming and laborious, whereas sensors and probes offer a faster, easier, functional, and more economical solution.

Biogenic amines are nitrogenous organic compounds that may occur naturally in foods and beverages, particularly in products that involve a ripening or fermentation period such as meat products, fish, cheese, beer, and wine [1]. They are generated either because of endogenous amino acid decarboxylase activity in raw food material or by the growth of decarboxylase-positive micro-organisms under conditions favourable to enzyme activity. Although they are involved in important metabolic and physiological functions in every living organism, consumption at high concentrations may cause undesirable physiological effect [2]. The control of biogenic amines in foods is gaining importance in order to monitor production processes, to ascertain their quality and freshness, and to monitor food safety [3]. During this conference several sol-gel as well SiO2 nanoparticle based optical sensors will be presented for detection of various biogenic amines [4-6].

Another important analyte to monitor food safety are organophosphorus (OP) pesticides. They have been used widely as agricultural and household pest control agents for almost five decades and persist in water resources, fruits, vegetables and processed food as health and environmental hazardous compounds. Thus, detection of these harmful OP pesticides at an ease with high sensitivity and selectivity [7] is the need of hour.

Here we will present several approaches to design applicable optical based pesticides sensors which can be sensitive as well selective enough to be used in the food safety control [8-9].

References

[1] S. Yeğin and A. Üren, (2018) *Food Chem.*, 4, 983–987.

[2] M. Papageorgiou, D. Lambropoulou, C. Morrison, J. Namieśnik, and J. Płotka-Wasylka, (2018), *Talanta*, 183, 276–282.

[3] J. L. Ordóñez, A. M. Troncoso, M. D. C. García-Parrilla, and R. M. Callejón, (2016), *Anal. Chim. Acta*, 939, 10–25.

[4] P. Nedeljko, M. TUREL, A. LOBNIK, (2017), *Sensors and actuators. B, Chemical.* 3, 1066-1073.

[5] T.Mastnak, A. Lobnik, G. Mohr, M.Turel, (2018) *Sensors.* 18, 1-13.

[6] P.Nedeljko, M.Turel, A.Lobnik, (2018) *Journal of sensors,* 3, 1-9.

[7] C.S. Pundir, A. Malik, Preety, (2019) *Biosensors & Bioelectronics*, 140, 111-348.

[8] N.Frančič, M.Bellino, G.Soler Illia, A.Lobnik, (2014) *Analyst.* 139, 3127-3136.

[9] N. Frančič, A. Košak, I. Lyagini, E. Efremenko, A.Lobnik, (2011), *Analytical and bioanalytical chemistry.* 401, 2631-2638.

The Potential of Single Particle Inductively Coupled Plasma Mass Spectrometry for Studying Micro- and Nanoscale Particles in Water and Food

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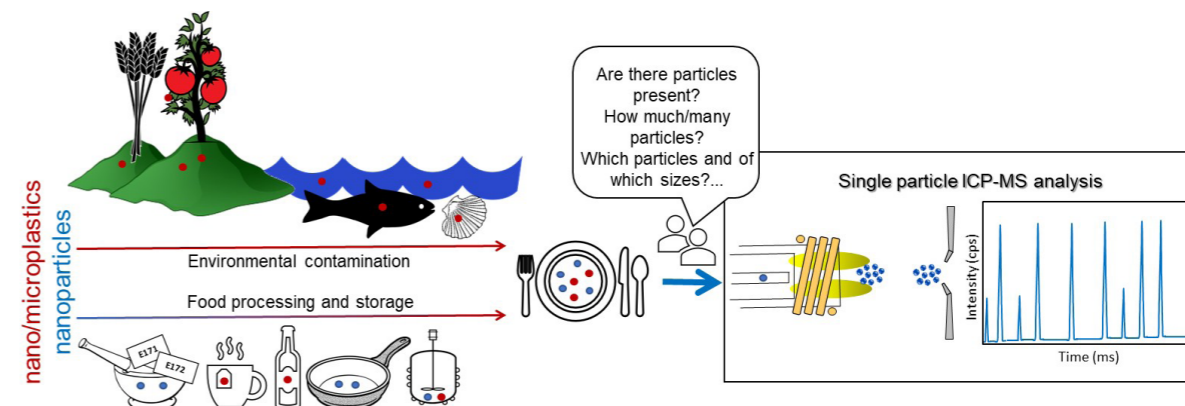
Expanded production and use of metal-based nanoparticles in various applications have resulted in their increased presence in consumer products, including food, and their release into different environmental compartments. Similarly, the widespread environmental pollution with microplastics (polymer particles <5 mm) and nanoplastics (polymer particles <1 μm) has led to the nano/microplastics entering the food chain. Other sources of nanoparticles and nano/microplastics in food include their migration from food contact materials and formation during food production, processing, storage, preparation, and serving. As a consequence, humans can be exposed to metal-based nanoparticles and nano/microplastics either through contaminated environment or by consuming contaminated food, which raises concerns about their potential health hazards. However, very limited exposure data for risk assessment exists so far due to the lack of suitable analytical methods. Nanoparticles and nano/microplastic detection in water and food poses a great analytical challenge due to the complexity of the analytes and sample matrices as well as the small size and expectedly low concentration of the particles.

Inductively coupled plasma mass spectrometry (ICP-MS) operated in single particle mode (spICP-MS) has become a well-established technique for the detection of metal-based nanoparticles in various complex matrices due to its high sensitivity, selectivity and fast analysis [1]. The spICP-MS provides information on particle elemental composition, size (spherical equivalent diameter) and size distribution, particle number density and mass concentration. Very recently, a potential of spICP-MS technique for the detection of microplastics in the size range of 1-5 μm has been demonstrated, when monitoring the signal of ^{13}C or ^{12}C isotopes [2]. As an alternative, labelling of nano/microplastics with metal probes, followed by spICP-MS detection of the corresponding metals, offers more sensitive and selective approach [3].

The talk will focus on the feasibility of spICP-MS for the analysis of metal-based nanoparticles and nano/microplastic in a variety of samples, from waters to foods. In addition to its principles, main features and advantages, some limitations and analytical challenges commonly encountered in spICP-MS analysis of particles in complex samples will also be discussed. Finally, the applicability of the spICP-MS technique will be demonstrated on selected examples of nanoparticles and nano/microplastics analysis in water and food samples.

References

[1] J. Vidmar, in *Analysis and characterisation of metal-based nanomaterials*, R. Milačič, J. Ščančar, H. Goenaga-Infante, J. Vidmar (Eds.), Amsterdam (The Netherlands), Elsevier, 2021, 10.

[2] E. Bolea-Fernandez, A. Rua-Ibarz, M. Velimirovic, K. Tirez, F. Vanhaecke (2020) *Journal of Analytical Atomic Spectrometry*, 35, 455-460.

[3] Y. Lai, L. Dong, Q. Li, P. Li, Z. Hao, S. Yu. J. Liu (2021) *Environmental Science and Technology*, 55, 4783-4791.

The Possibility of Using Olive Leaves as a Source of Polyphenols in the Light of Circular Economy

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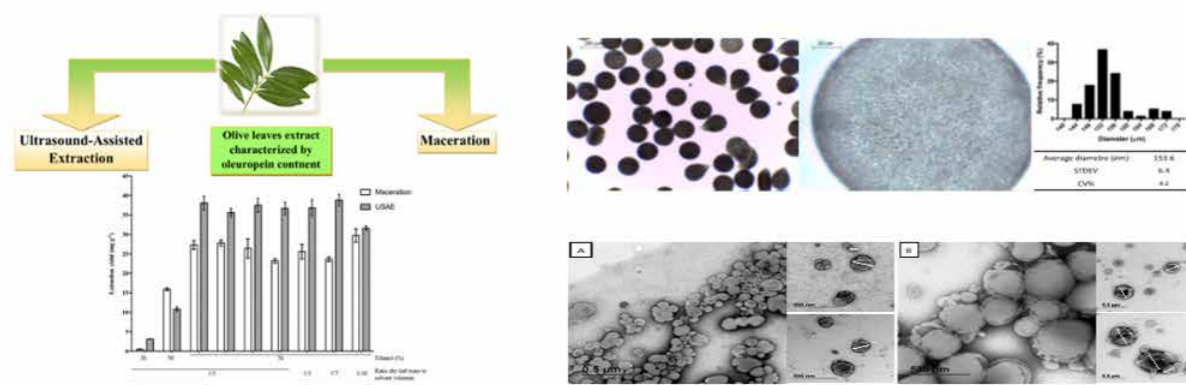
In recent years, the agronomic, cosmetic and pharmaceutical industries have increased their interest in natural extracts from plants and by-products rich in bioactive compounds. This is due to the need to meet the growing demand for natural preservatives and to create novel functional foods with significant health benefits. In this regard, olive leaves are a promising vegetable matrix from which to obtain natural extracts rich in a variety of bioactive compounds as they are rich in phenolic compounds. The main components of olive leaves are secoiridoids (e.g. oleuropein, ligstroside, dimethyloleuropein) and flavonoids (e.g. apigenin, kaempferol, luteolin) along with other phenolic compounds (e.g. hydroxytyrosol, tyrosol, caffeic acid). Oleuropein is the most representative polyphenolic component of olive leaves as the compound is responsible for the bitterness of both table olives and extra virgin olive oil. Several studies have demonstrated a variety of in vitro and in vivo properties for oleuropein, including antioxidant, antiviral, antibacterial, and anti-inflammatory activities. In order to maximize the extraction yield of oleuropein from olive leaves and reduce operating costs with the possibility of lower solvent volumes and lower extraction times and temperatures, we investigate the application of USAE and the various extraction parameters such as type of solvent, olive leaf mass to solvent volume ratio and extraction time and -temperature. For comparison, parallel extractions were carried out using maceration.

The use of oleuropein or olive leaf extracts from natural raw materials in food, pharmaceuticals and cosmetics is often challenging due to their low solubility, sensitivity to environmental conditions (e.g. temperature, pH, light) and often poor sensory properties. For example, oleuropein is a bitter compound that is broken down into the mild-tasting hydroxytyrosol and elenolic acid, generally at acidic and alkaline pH values during the manufacture of table olives. To overcome these problems of phenols, various microencapsulation and nanoencapsulation techniques have been developed over the past decades to improve their utility and bioavailability. Here we present the results of liposomal encapsulation of oleuropein and olive leaf extracts. The morphological and physicochemical properties of these liposomes with actively encapsulated oleuropein are discussed together with the oleuropein stability under different pH conditions in a food model and a real food system.

Design of Novel Molecularly Imprinted Polymers as Effective Extraction Media for Selective Determination of Triazole Fungicides in Juice Samples

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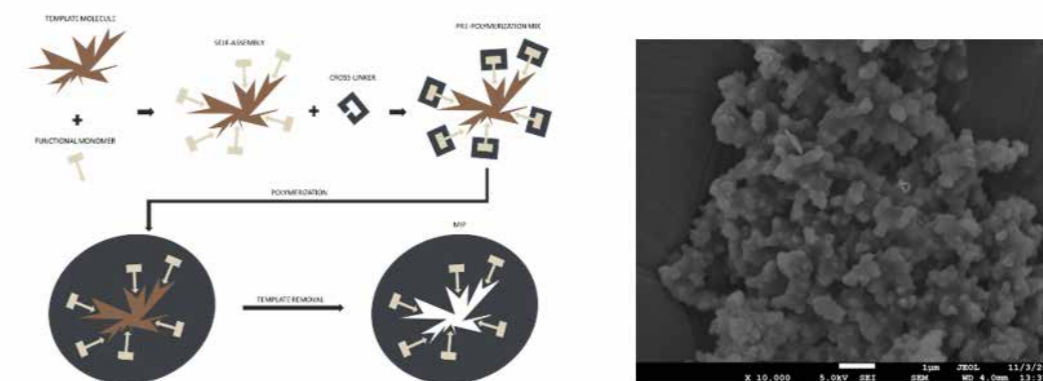
The one of triazoles is a class of fungicides containing the 1, 2, 4-triazole ring in their structure. For their excellent activity and low resistance, triazole fungicides have been largely used as preventive and therapeutic fungicides on a wide range of crops. Penconazole belongs to the group of triazole pesticides, it is related to high persistence in soils and alert on human health due to its action as endocrine disruptor [1].

Molecularly imprinted polymers (MIPs) are specifically designed sorbents that display enhanced discernment towards a certain structure, allowing selectivity toward a target molecule or a family of compounds [2]. MIPs synthesis consists generally in a co-polymerization between a functional monomer and a cross linker in the presence of a template molecule which can interact with the functional monomer in the pre-polymerization mixture, generally through non-covalent interactions, and that is secondly washed from the material after the polymerization takes place. Due to their inherent properties (selectivity, resistance, thermal stability, reduced cost) MIPs have been widely used for analytical applications. However, the synthesis of a polymer molecularly imprinted with penconazole to the best of authors' knowledge, has never been reported.

In the present study, novel molecularly imprinted polymers were prepared using penconazole as template and were evaluated for multicomponent analysis of triazole compounds in different matrices such as fruit juices (orange, strawberry, apple). Different combinations of functional monomers (acrylic acid (AA) and N-Isopropylacrylamide (NIPAM)) and cross-linkers (ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM)) were tested by Plackett-Burman design of experiments. The synthesized materials were fully characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and Brunauer-Emmett-Teller analysis. Adsorption behaviours in solution were also studied both at the equilibrium (Langmuir, Freundlich and Temkin models), and at the non-equilibrium (pseudo-first, pseudo-second order kinetics).

Among the synthetized ones, the MIP deriving from the combination NIPAM-EGDMA showed the best recognition mechanism towards compounds containing the triazole ring, and it was employed for the development of an analytical procedure for selective enrichment of azole compounds in commercially available juice samples, through dispersive solid phase extraction.

Good methodological parameters were obtained: high recovery (93.35% - 112.36%) high repeatability (RSD < 5.2 %), reproducibility (RSD < 7.2), and selectivity toward triazole fungicides in comparison to other compounds not containing the triazole ring in their structure.

Acknowledgements

References

[1] K. A. Lewis, J. Tzilivakis, D. J. Warner, A. Green (2016) *Human and Ecological Risk Assessment*, 22, 1050-1064.
[2] G. Z. Kyzas, D. N. Bikiaris, N. K. Lazaridis (2009) *Chemical Engineering Journal*, 149, 263-272.

Optimization of Experimental Parameters for Electrochemical Detection of Neonicotinoids in Honey with Modified Screen-Printed Carbon Electrodes

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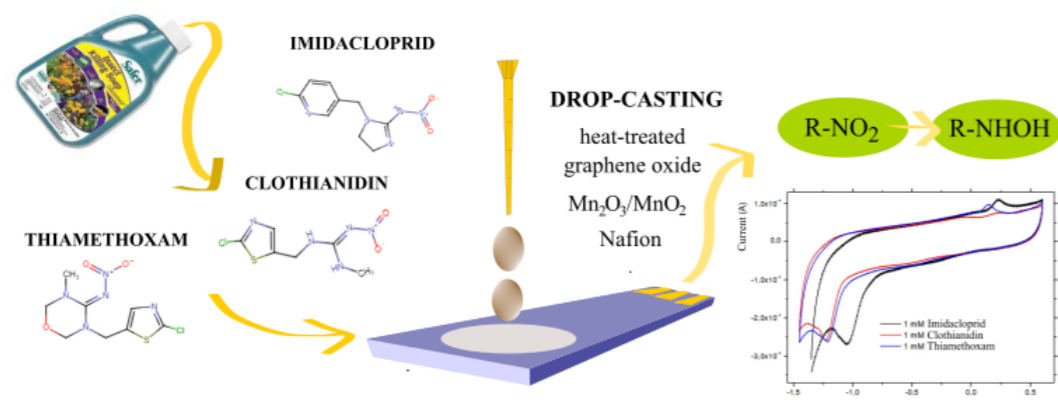
Neonicotinoids (NNI) are systemic pesticides with selective activity on acetylcholine receptors of insects. In the last decade they have been a target of many critics for their toxicity against pollinators, such as bees [1]. Established chromatographic methods for the determination of these insecticides have long procedure times, high price, and require large amounts of solvents. Many recent research papers have been focused on fast and selective electrochemical sensor development. Usually, an improved electrochemical response, repeatability and selectivity are achieved by modifying the surface of a working electrode [2].

In this abstract, we address the modification of commercial screen-printed carbon electrode (SPCE) with heat-treated graphene oxide (htGO) and metal oxides, as well as the study and optimization of experimental parameters, such as pH, accumulation time, temperature and scan rate. Dispersions of htGO nanoparticles, several metal oxide microparticles (Co_3O_4, MnO_2, Mn_2O_3, Bi_2O_3 and Fe_2O_3) and Nafion were prepared in different volume ratios. Working electrode's surface was modified by drop-casting prepared dispersion.

Electrochemical characterization was mostly performed using cyclic voltammetry (CV). Dispersion of htGO, MnO_2 and Nafion proved to be optimal for the determination of thiamethoxam and clothianidin, whereas dispersion of htGO, Mn_2O_3 and Nafion proved to be optimal for the determination of imidacloprid.

Modified electrodes were used and CV was performed in solutions of imidacloprid, clothianidin and thiamethoxam at different scan rates. Experiments showed that the process is diffusion controlled. The electrochemical response of NNI was tested in the solutions at different pH values. Electrochemical reduction of analyte proved to be proton dependent, showing an equal number of electrons and protons involved in the reaction. Optimal conditions for the determination of NNI were found at neutral pH. Based on these results, a four proton and four electron mechanism for a two-step reduction of the nitro group was proposed. The CVs were measured for every analyte in the temperature range from 10 to 35 °C. In general, the electrochemical response increased with the temperature, as expected due to faster difussion. Lastly, the effect of accumulation time was studied. The response increased for the first 8-12 min and either decreased or remained the same thereafter, which showed the saturation of the working electrode's surface.

Future research will use this knowledge when testing other nanomaterials for the modification of SPCE for the determination of NNI in honey with the aim of achieving higher current response relative to the background.

References
[1] V. Strobl, M. Albrecht, L. Villamar-Bouza, S. Tosi, P. Neumann, L. Straub (2021) *Environmental Pollution*, 284, 117106.
[2] J. Ganesamurthi, M. Keerthi, S. M. Chen, R. Shanmugam (2020) *Ecotoxicology & Environmental Safety*, 189, 110035.

Bio-Active Nanofibrous Nonwovens as in-situ Producers of Hexanal

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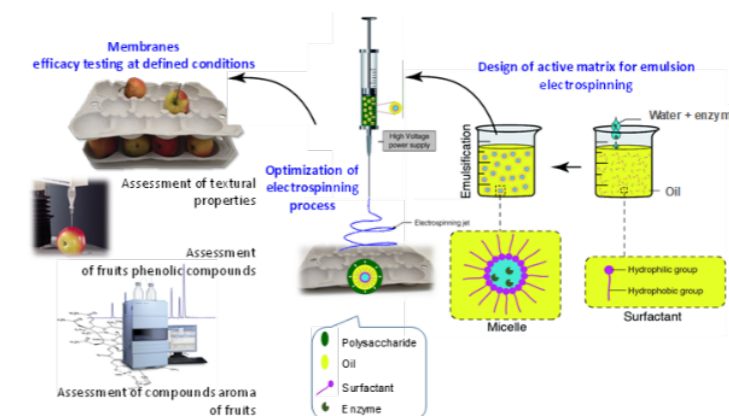
Horticultural products such as fresh fruits and vegetables are very perishable by nature and susceptible to spoilage due to fungi and bacteria infection. The fruit storage involves physical and chemical changes that negatively modify fruit quality. This leads not only to substantial fruit and vegetable losses representing financial losses for the farmers, packers, and distributors, but also to increased solid waste that can have a negative environmental impact in terms of greenhouse emissions as well as increased pressure on land and water resources [1]. The current potential post-harvest treatment solutions are based on biological control and physical methods such as irradiation with high-energy electrons, heat treatment, storage in a controlled and modified atmosphere or gaseous treatments, the application of supplementary chemical treatments as fungicides, bactericides etc. [2]. Lately, there has been an increase in the recognition of volatile organic compounds (VOCs), synthesized by plants including aldehydes such as acetaldehyde, hexanal and benzaldehyde, alcohols such as ethanol and acetic acid that have been shown to suppress the growth of plant pathogenic microorganisms indicating that these compounds could be one of the important mechanisms for biological control of plant diseases [3]. Hexanal inhibited activity of phospholipase D enzyme, which is a key enzyme causing degradation of membrane phospholipids.

In recent years, electrospun nanofiber mats with high surface area-to-volume ratio were intensively studied as a matrix for shelf-life extension of different fresh fruits and vegetables [4]. Emulsion electrospinning is a relatively novel and simple technique that allows the encapsulation of bio-active compounds (e.g., flavonoids, vitamins, peptides, enzymes, proteins, etc.) in the core section using solidified polymers as a barrier and minimizes the quantity of organic solvents used in the food systems [5].

By appropriate design of electrospun membranes, using emulsion electrospinning technique, bio-active nanofibrous nonwoven as a carrier of enzyme-initiated hexanal for packaging or storage of fresh fruits can be achieved. In our study we used individually lipase (LIP) or lipoxydase (LOX), and combination thereof in ratio of 1:1. GC-MS measurements revealed the highest release of hexanal from as prepared emulsions with combination of both enzymes at two pre-defined temperatures (1°C and 25°C). As prepared emulsions were fabricated in nanofibrous mats, with *in-situ* release properties of enzyme-initiated hexanal.

References
[1] FAO (2019) *The state of food and agriculture*, http://www.fao.org/3/ca6122en/ca6122en.pdf.
[2] L. -Z. Deng et al. (2019) *Critical Reviews in Food Science and Nutrition*, 59, 9, 1408-1432.
[3] M. Mari et al. (2016) *Postharvest Biology and Technology*, 122, 70-81.
[4] C. Shi, A. Jash, L. T. Lim (2021) SN Applied Sciences, 3, 385.
[5] F. Zhang et al. (2021) *Trends in Food Science & Technology*, 337, 127763.

Session 4: NANOMATERIALS AND NANOTECHNOLOGY

2nd ISO-FOOD Symposium FROM FOOD SOURCE TO HEALTH

Studying the Uptake and Distribution of Metal-Doped Nanoplastics in Hydroponically Grown Tomato Plants

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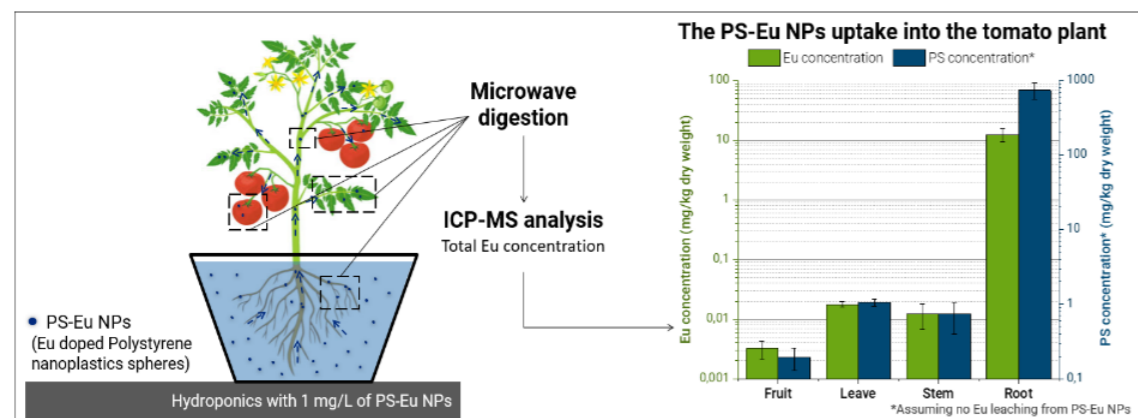
The ubiquitous pollution of microplastics (MPs; <5 mm) and nanoplastics (NPs; <1 μm) in the environment is a raising global concern. The smaller these persistent contaminants are, the greater the threat to the agricultural (eco)systems, food chain and human health [1].

Understanding the risks posed by NPs in the human food chain is very scarce due to limited quantitative information on their potential uptake and translocation in edible plants. Analytical challenges contributing to these knowledge gaps are related to difficulties in the characterisation and quantification of NPs in complex biological matrices. Recently, the use of metal-doped NPs and their quantification by ICP-MS analysis of the corresponding metal has been proposed for studying the fate, transport and biological uptake of NPs in natural environments [2]. Doping NPs with a metal tracer can effectively overcome analytical challenges related to NPs low concentrations, high natural backgrounds and not sufficiently sensitive and selective analytical techniques. The use of metal-doped NPs was already proven to be a promising approach for studying the uptake and transport of NPs in different plants [3].

The UPTAKE project aims to study the uptake and distribution of 30 selected contaminants of emerging concern (CEC) into tomatoes as a model plant under different growing regimes. The objective of the project is the assessment of potential health risks associated with human exposure to hazards present in the tomato fruit as a result of the agricultural reuse of treated wastewater and solids for plant cultivation. In the framework of the UPTAKE project, an independent hydroponics experiment was conducted, where the uptake of NPs into the tomato plant was studied for the first time.

Hydroponically grown tomato plants were exposed to polystyrene (PS) NPs with a diameter of 200 nm doped with rare earth element europium (PS-Eu NPs) at a concentration of 1 mg·L^{-1} for the 5-week period. After the harvest, the plant samples (roots, stems, leaves, fruits) were digested and analysed with the ICP-MS method to determine the total Eu concentration. By knowing the stability of PS-Eu NPs during the experiment, we could correlate the total Eu concentrations with the concentration of NPs.

The first results show that PS-Eu NPs were detected in all parts of plant, with the highest accumulation in the roots and the lowest but still quantifiable concentrations in the fruits. The number concentration and size of PS-Eu NPs accumulated in tomato plant will be further studied by single particle ICP-MS analysis of the extracted PS-Eu NPs.

References

[1] *Nanoplastics: state of knowledge and environmental and human health impacts.* European Commission, Directorate-General for Environment, Publications Office of the European Union, 2023.

[2] D. M. Mitrano et al. (2019) *Nature Nanotechnology*, 14, 362–368.

[3] Y. Luo et al. (2022) *Nature Nanotechnology*, 17, 424–431.

Two-dimensional Magnetic Nanomaterial: Versatility and Detection Performance Capabilities for Toxic Metal Ions

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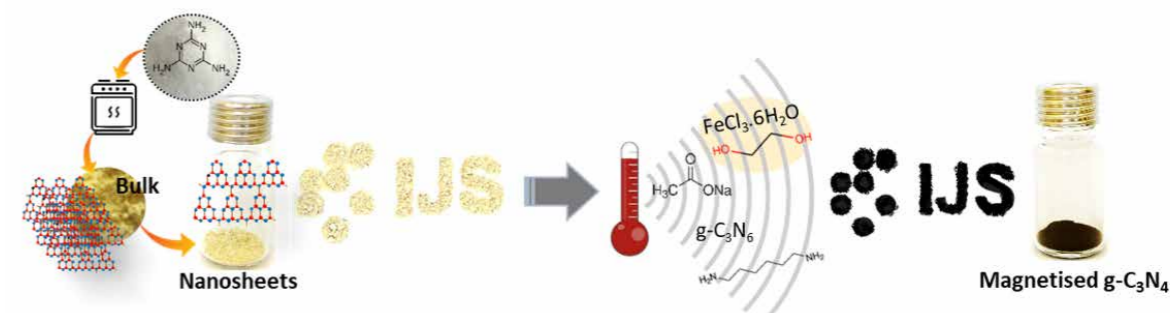
Heavy metals have harmful effects on human health, and exposure to these metals has been increased through industrial and anthropogenic activities and modern industrialization. Therefore, their detection in food samples at low concentrations is vital [1[a,b]]. Exposure to toxic metal ions arises from the effects of pollution and bioaccumulation throughout the food chain [2]. In the field of smart and greener adsorbent material for sample concentration and detection, various nanosystems have been utilized as emerging solutions. In addition, magnetic nanomaterials have become one of the most interesting materials in the development of adsorbents for heavy metals [3]. Magnetic nanomaterials have attracted high attention in the development of novel magnetic sorbents for environmental monitoring, bioanalysis, and food analysis [4]. In addition, they have a wide application as sensing materials in the development of biosensors, electrochemical sensors, surface-enhanced Raman spectroscopy sensors, resonance light scattering sensors, and colorimetric sensors [5].

Based on the above properties, a novel de novo de novo tris-s-triazine carbon nitride nanosheet decorated magnetite nanoparticles (M-g-CN) was fabricated and tested. A thermal-triggered intermittent sonication technique was developed to prepare a cost-effective, eco-friendly, and non-toxic nanocomposite for the efficient removal of inorganic mercury (Hg^{2+}) in diverse aqueous matrices. The novel synthesis technique provides a high yield (97.7%) of M-g-CN which is reusable, stable under various conditions, and highly selective towards Hg^{2+} species. The nanosheets allow and serve as the binding constraints for Hg^{2+} with decorated magnetite particles used to pull the material towards magnetic fields in the aqueous matrices. The wider application of as-synthesized M-g-CN can easily be adopted as a green adsorbent in food industries, and remediation of toxic effluents.

References

[1] (a) M. Zhang, W. Guo (2023) *Food Chemistry*, 406, 135034. (b) Z. Chen, Z. Zhang, J. Qi, J. You, J. Ma, L. Chen (2023) *Journal of Hazardous Materials*, 441, 129889.

[2] M. Haddad, D. Nassar, M. Shtaya (2023) *Scientific Reports*, 13, 4121.

[3] R. Singh Chouhan, G. Žitko, V. Fajon, I. Živković, M. Pavlin, S. Berisha, I. Jerman, A. Vesel, M. Horvat (2019) *Sensors*, 19, 3432.

[4] R. Singh Chouhan, I. Jerman, D. Heath, S. Bohm, S. Gandhi, V. Sadhu, S. Baker, M. Horvat (2021) *Nano Select*, 2021, 2, 712–743.

[5] R. Singh Chouhan, M. Horvat, J. Ahmed, N. Alhokbany, S. M. Alshehri, S. Gandhi (2021) *Cancers*, 13, 2213.

Antimicrobial Active Polymer Nanocomposite PVDF-HFP/PVP/MoO3 as Nanostructured Coating for Potential Application on Food Contact Surfaces

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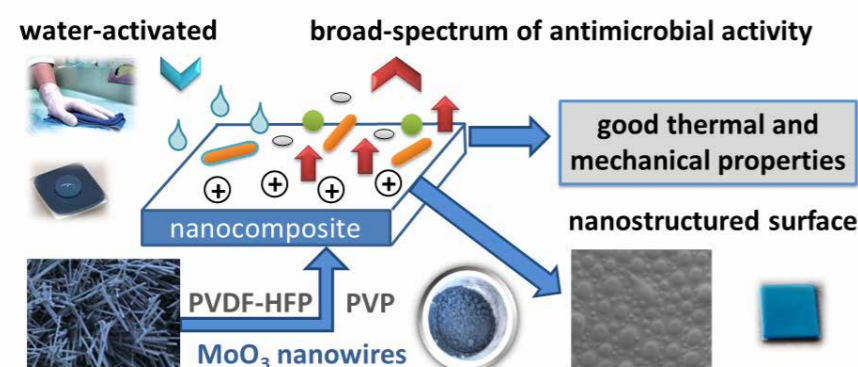
Contact surfaces are key areas for the transmission of microbial contamination in various environments, such as public transportation, shopping centres, hospitals, and food industry. Microorganisms colonise the surfaces, where they grow and form a biofilm that protects them from negative environmental influences. However, bacterial biofilms on the surfaces in the food industry present the possibility of food contamination, which in turn leads to food spoilage and cross contamination. The current high consumption of disinfectants and biocides can be replaced by environmentally friendly and durable antimicrobial coatings.

The polymer nanocomposite PVDF-HFP/PVP/MoO$_3$ has numerous surface properties that are unfavourable for microorganism attachment while releasing the combination of several biocidal agents [1]. The nanostructured surface of nanocomposite has a hydrophilic character. The MoO$_3$ nanowires with high specific surface area slowly dissolve on the surface in the presence of water, causing a decrease in pH and hydrolysis of the PVP polymer. A relatively small amount (0.09 mg/ml) of MoO$_3$ was dissolved out of the nanocomposite within 90 minutes, which corresponded to a drop in pH to about 4. Molybdic acid is formed first, followed by the formation of carboxylic acid and ammonium salts. Therefore, on the surface has been detected the positive charge.

The combination of these antimicrobial agents provides a broad spectrum of antimicrobial activity (inactivation of bacteria and fungi). The antimicrobial activity has been demonstrated in bacterial strains *S. aureus, L. monocytogenes, E. coli, and P. aeruginosa, in yeasts P. anomala* and *C. albicans*, and in moulds *P. verrucosum* and *A. flavus* [1].

In addition, the surface of the nanocomposite should have good mechanical (high modulus of elasticity and hardness) and thermal properties for industrial use. It is known that the addition of MoO$_3$ nanowires increases the thermal stability and mechanical strength compared to PVDF-HFP/PVP polymer composite [2].

Acknowledgements

References

[1] U. Gradišar Centa, M. Sterniša, B. Višić, Ž. Federl, S. Smole Možina, M. Remškar (2020) *Surface Innovations*, 9 (5), 256-266.

[2] U. Gradišar Centa, M. Mihelčič, V. Bobnar, M. Remškar, L. Slemenik Perše (2022) *Coatings*, 12 (9), 1241.

Foodomics Study on the Activity of Bioactive Compounds from Plants, Algae and Agrifood By-products Against Alzheimer Disease

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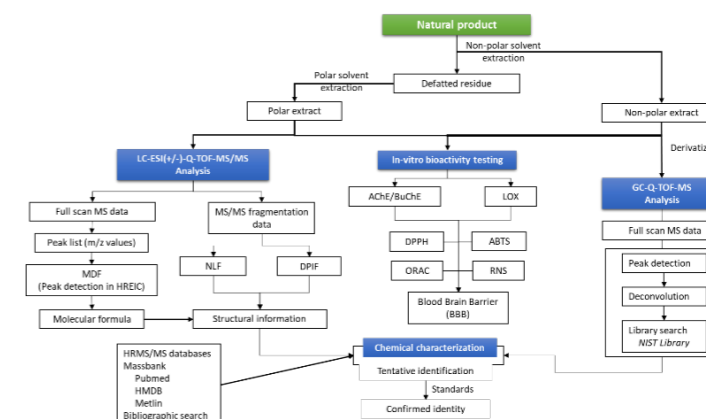
Today, Alzheimer's disease (AD) is considered a progressive and irreversible disease for which there is no effective cure, mainly due to their complex and multifactorial nature. The existing treatments focus solely on the symptoms of the disease.

Due to this problematic situation, the scientific community has promoted the search for an effective treatment for neurodegenerative diseases such as AD. In this context, natural products are gaining importance as a source of bioactive molecules with high pharmacological and/or nutraceutical value and neuroprotective potential. In particular, plants, algae and agrifood industry by-products have shown great potential for revalorization and generation of high value-added products, due to their content of bioactive molecules with beneficial effects on health.

We will present here a multi-analytical approach to study the neuroprotective effect of different extracts enriched in bioactive compounds from different sources. For this purpose, we employed green extraction processes based on compressed fluids such as pressurized liquid extraction (PLE) and supercritical fluids (SFE), making use of Generally Recognized As Safe (GRAS) solvents. On the other hand, using a battery of *in vitro* bioactivity assays, the neuroprotective potential of the extracted bioactives has been evaluated through inhibition assays of the cholinesterase enzymes (acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE)), as well as of the lipoxygenase enzyme (LOX), involved in cholinergic neurotransmission processes and neuroinflammation, respectively. In addition, the antioxidant capacity of the obtained extracts has been studied by free radicals (ABTS), and reactive oxygen and nitrogen species (ROS and RNS) scavenging assays. In parallel, the phytochemical composition of the evaluated extracts has been characterized making use of advanced analytical techniques such as gas chromatography and liquid chromatography coupled to high-resolution mass spectrometry (GC-Q-TOF MS/MS and LC-Q-TOF MS/MS).

The ability of the studied bioactive compounds to cross the blood-brain barrier (BBB) to exert their neuroprotective function in the brain was evaluated using two complementary methodologies, an *in vitro* methodology, based on parallel artificial membrane (PAMPA-BBB) and a more advanced cellular model based on human brain microvascular endothelial cells (HBMEC-BBB), considered the anatomical and functional basis of the BBB. The possible cytotoxic effect of the extracts was also evaluated in four different human cell lines: human kidney cells HK-2, THP-1 monocytes, HBMEC endothelial cells and SH-SY5Y neuroblastoma.

The extracts with the highest *in vitro* neuroprotective effect and positive results in BBB permeability assays were selected to be tested in an in vivo model of AD, using the CL4176 transgenic strain of the nematode Caenorhabditis elegans (C. elegans), a well-known AD model.

The use of Foodomics (including transcriptomics, lipidomics and metabolomics), allowed the identification and quantification of a large number of metabolites and genes that can explain the observed neuroprotective effect.

SMA®t-EVOO-Tools: Providing Insights into Greek Extra Virgin Olive Oil Authenticity and Geographical Origin

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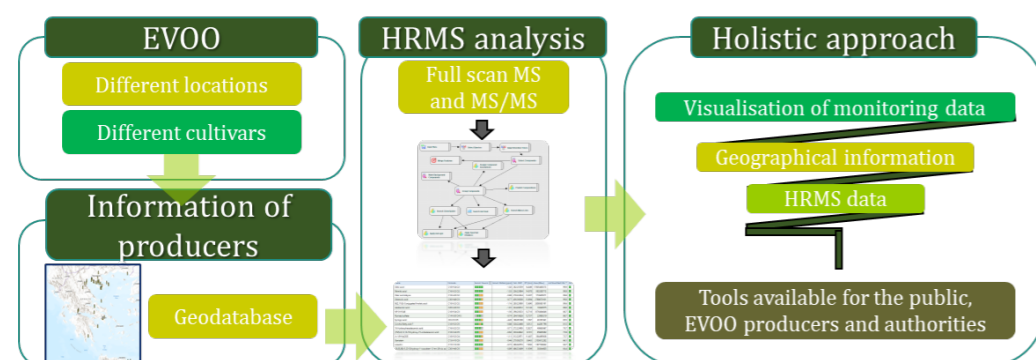
Extra virgin olive oil (EVOO) is one of the most important components of the Mediterranean diet, and its consumption has been continuously increasing due to its high nutritional value [1]. In Greece, more than thirty olive varieties are produced. Albeit Greece presenting with high rates of EVOO production, the quality potential of Greek EVOOs is rarely investigated and such information is scarcely available to the public or the producers [2].

Identifying these gaps, both in the scientific and EVOO producing sector, the project SMA®t-EVOO-Tools aims to map the olive oil authenticity characteristics in regions of interest including local Greek EVOO varieties. At the same time, a reliable geodatabase in a Geographic Information System (GIS) environment enables efficient multi-layer data querying and analytics, creating a powerful tool to assess and correlate data in retrospective analysis. Additionally, a representative library of the qualitative/ quantitative characteristics of more than 300 Greek EVOO samples from at least 5 different geographical areas of Greece is developed. Furthermore, the use of chemometric techniques for the correlation of the different types of olive oil profiles enriched with the qualitative/quantitative characteristics, result in a faster identification of bioactive components. What is more, development of innovative High Resolution Mass Spectrometry (HRMS) methodologies using specialized tools, metabolomics and chemometrics are employed for the identification of the geographical origin of EVOOs, providing links between bioactive compounds such as oleuropein, oleocanthal and hydroxytyrosol and geographical origin or specific cultivars. In order to attract both producers and management and decision-making authorities, an integrated approach for the monitoring-based geographical mapping of data concerning the key factors affecting the quality characteristics of EVOO is exploited, by visualization of collected the monitoring data.

Finally, although Greek EVOO is held in high regard in the realm of nutrition, SMA®t-EVOO-Tools identifies and highlights burning issues regarding the quality characteristics of EVOOs. Concludingly. monitoring techniques integrated with public-reported data provide not only a scientific basis for product management and assistance in policy formulations at the national and international levels but also a powerful tool for the direct dissemination of information to the public.

Acknowledgements

References

[1] N. P. Kalogiouri, R. Aalizadeh, M. E. Dasenaki, N. S. Thomaidis (2020) *Analytica Chimica Acta*, 1134, 150.

[2] A. Lioupi, N. Nenadis, G. Theodoridis (2020) *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1150, 122161.

"Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" and the Fate of EC Reg. 432/2012: Are the Basic Terms and the Figures Any Clearer Now?

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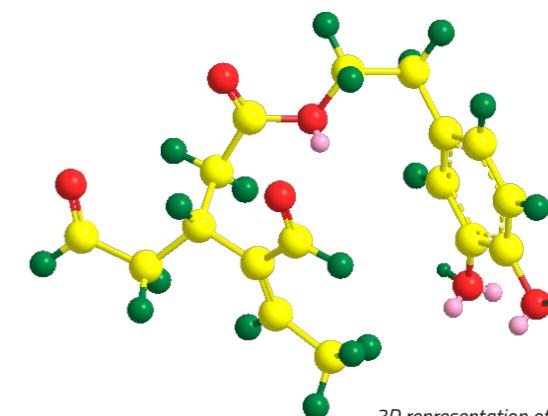
3D representation of oleacein from the phenol fraction of the VOOs.

More than ten years have passed since the European Commission issued Regulation 432/2012, which endorsed the health claim in the title. It did so on the basis of a scientific opinion issued by EFSA - the European Food Safety Authority. Conditions of use of the claim are: *"The claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil"* [1].

However, in clarifying or describing the conditions under which the claim may be used, there have been some inconsistencies and ambiguities both in terminology and in the lack of a prescribed analytical methodology. The term 'olive polyphenols' is not the most accurate, since only virgin olive oils (VOO) of higher quality contain significant amounts of oleuropein and ligstroside aglycones and their conversion products. Also, the term 'olive oil' is generic and does not correspond to any dietary category, not to mention the inappropriate term 'polyphenols', which does not correlate with any of the secoiridoids in VOO and which are the main reason for the health claim [2, 3]. In recent years, several attempts have been made to correct this error, as the Regulation does not prescribe any method of analysis [4]. Unfortunately, these attempts have been largely unsuccessful, precisely because of the rigidity and vagueness of the basic Regulation, as each analytical protocol has interpreted the Regulation in its own way. The basic problem is still the insufficient chromatographic separation which prevents the exact calculation of those 5 mg per 20 g of VOO. This deficiency led to the introduction of hydrolytic protocols to express and quantify in a more unambiguous way the still insufficiently clarified syntagm 'hydroxytyrosol and its derivatives.' In the light of all mentioned facts, it cannot be overlooked that, despite the initial enthusiasm in the years before the publication of the Regulation, the International Olive Council has only deepened the analytical gap with the implementation of its 2009 (2017) method [5].

Acknowledgements

References

[1] European Commission (2012) Regulation (EU) No. 432/2012 of 16th May 2012, *Official Journal of the European Union*, L136, 1-40.

[2] A. Mastralexi, N. Nenadis, M. Z. Tsimidou (2014) *Journal of Agricultural and Food Chemistry*, 62, 2459-2461.

[3] M. Z. Tsimidou, N. Nenadis, M. Servili, D. L. García-González, T. G Toschi (2018) *European Journal of Lipid Science and Technology*, 120, 1800098-1800104.

[4] M. Z. Tsimidou, N. Nenadis, A. Mastralexi, M. Servili, B. Butinar, S. Vichi, O. Winkelmann, D. L. García-González, T. G Toschi (2019) *Molecules*, 24, 2429-2445.
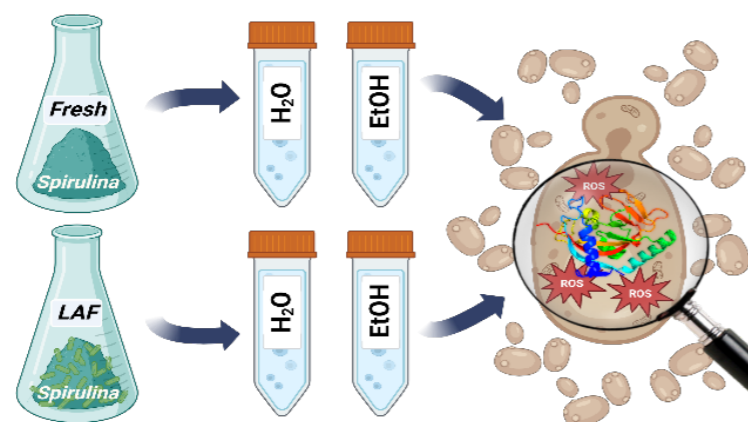
[5] M. Z. Tsimidou (2022) *Separations*, 9, 351-359.

Fermented and Non-Fermented *Spirulina* Water and Ethanol Extracts Antioxidant Activity *in vitro* and at the Cellular and Proteome Level

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Spirulina is rich in bioactive compounds with high antioxidative potential [1]. To further enhance its antioxidative efficiency, lactic acid fermentation (LAF) can be employed, as it has been previously shown to improve the nutritional and functional properties of food [2,3]. This study assesses the role of LAF in the antioxidant activity of fermented and non-fermented *Spirulina* biomass extracts *in vitro* and at the cellular and proteome level, using the yeast *Saccharomyces cerevisiae* as a model organism.

Fresh *Spirulina* biomass was subjected to a 24-hour LAF process using a *Lactobacillus plantarum* culture and extracted with both water and ethanol. The extracts were then tested for their DPPH radical scavenging activity and further used to treat yeast cells ((1) non-fermented (NFV) and (2) fermented (SV) *Spirulina* water extract, and (3) non-fermented (NFE) and (4) fermented (SE) *Spirulina* ethanol extract treated yeast) to determine their cellular antioxidant activity.

The results showed that ethanol extracts of fermented *Spirulina* biomass had higher radical (DPPH) scavenging activity than non-fermented *Spirulina* biomass ethanol extracts. Also, the fermented *Spirulina* ethanol extracts caused a 40% decrease in intracellular oxidation levels and reduced oxidative lipid damage in the yeast cells, making it a potential source of antioxidants. In addition, yeast cell exposure to fermented or non-fermented *Spirulina* ethanol or water extracts resulted in changed protein expression. There were significant differences found between yeast that were treated with non-fermented and fermented *Spirulina* extracts, as well as yeast that was treated with water and ethanol *Spirulina* extracts. In SV samples, most of the differentially expressed proteins were upregulated, compared to NFV. In contrast, most of the examined proteins involved in

various cellular processes were significantly downregulated in SE samples compared to NFE samples. The expression of stress response-related proteins increased in SV samples compared to NFV samples, while it was mainly decreased in SE samples compared to NFE samples. These results suggest that the ethanol extract of fermented *Spirulina* has a higher antioxidant efficiency than the water extract. Additionally, LAF appears to play an essential role in reducing the expression of cell stress response-related proteins.

This study showed how cells react to exogenous antioxidants at the proteome level and how they adjust their cellular processes to maintain their redox and metabolic balance. It also suggests that adding fermented *Spirulina* to food could lead to added-value foods production based on microalgae.

References

[1] K. Chopra, M. Bishnoi, in *Spirulina in Human Nutrition and Health*, M. E. Gershwin, A. Belay (Eds.), Boca Raton (USA), CRC Press, 2008, 101–118.

[2] J.-G. Liu, C.-W. Hou, S.-Y. Lee, Y. Chuang, C.-C. Lin (2011) *Process Biochemistry*, 46, 7, 1405–1410.

[3] R. Di Cagno, R. Coda, M. De Angelis, M. Gobbetti (2013) *Food Microbiology*, 33, 1-10.

Characterization of the Neuroactive Compound Profile in Organic, Conventional and Processed Tomatoes

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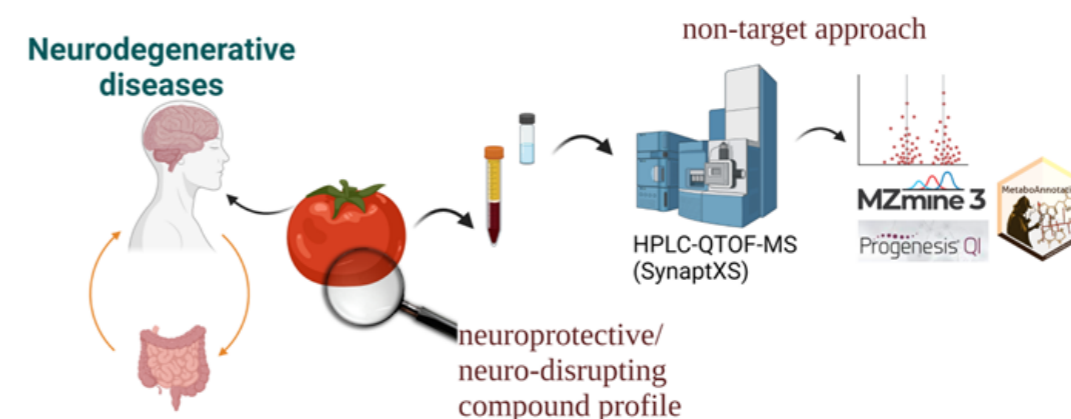
Neurodegenerative (ND) diseases are debilitating and largely untreatable conditions that affect 55+ millions of people worldwide. Despite the growing evidence of a correlation between diet, gut, and ND diseases, their connection is still unclear [1]. Tomato is one of the most widely produced and consumed vegetables in the world and has the potential to contain neuroactive compounds. To get insights into the metabolomic profile of diverse tomatoes and characterize the exposure to neuroactive compounds when consuming tomatoes, a non-target methodology was developed using UHPLC-QToF (Synapt XS). Sample preparation, instrumental analysis, and data processing were optimized based on 27 neuroprotective and 15 neuro-disrupting chemicals. The experimental design includes four groups of tomatoes, namely organic and conventional "datterini", conventional plump, and processed tomatoes. Samples were randomly analyzed in full scan and data dependent acquisition modes. The obtained recoveries of tested compounds were between 67-157% at higher (1.6 µg g^{-1} d.w.) and 41-130% at lower (0.1 µg g^{-1} d.w) concentration. Method and instrumental repeatability (relative standard deviation of triplicates) was <20% and <15%, respectively. For most of compounds, the R^2 of linear calibration curve was >0.9 and matrix effect ±60%. Data-processing was performed using Mzmine3 and Progenesis QI. Compound identification was performed using the "MetaboAnnotation" R package and was firstly based on the group of tested compounds matching the retention time, *m/z*, and MS^2 spectra with the corresponding analytical

standard. Among the still un-annotated features, those with MS^2 spectrum were considered for further annotation by matching their m/z values against a suspect list of 2,079 compounds. The corresponding MS^2 spectrums of potentially annotated features were compared with the information available in on-line databases or using *in-silico* fragmentation tools. The preliminary identification suggests the presence of group and non-group specific neuroactive compounds and identified several neuroprotective, e.g., polyphenols, vitamins, as well as neuro-disrupting compounds, e.g., industrial residues, and mycotoxins. In parallel, principal component analysis suggests a bigger difference between different types and differently processed tomatoes than differently produced tomatoes. Next step will be to find the most relevant neuroprotective/disrupting compounds that will be further investigated in the *in vitro* batch colon model to extend the knowledge of diet-gut microbiome-brain interactions and contribute towards preventing the burden of ND disease.
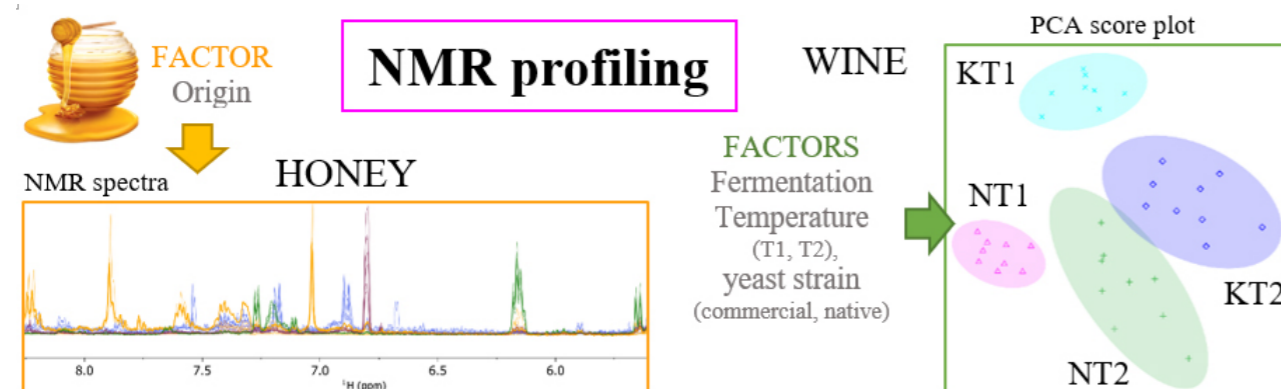
References

[1] F. A. Ceppa et al. (2020) *Frontiers in cellular and infection microbiology*, 10, 297.

NMR Profiling of Slovenian Honey and Wine

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Recently there has been a rise in awareness and attention to food traceability, origin and quality. Foods are generally extremely heterogeneous matrices consisting of various molecules, such as lipids, proteins, carbohydrates, vitamins, amino acids, phenolic compounds, and minerals. The presence of these compounds in food systems covers a wide range of concentrations. Furthermore, many dynamic and kinetic processes often occur in food systems due to molecular motions, reactions and the presence of microorganisms. The degree of heterogeneity is increased due to numerous factors, such as origin, climatic conditions, storage and processing practices that influence chemical composition of food.

Nuclear magnetic resonance (NMR) spectroscopy is a robust method, which can rapidly analyse complex mixtures at the molecular level without requiring separation or purification. The advantages of NMR spectroscopy are straight forward sample preparation, non-targeted analysis, dynamic detection, qualitative analysis, the possibility of changing the recording conditions and determining the isotopic ratio at a single site in the molecules. NMR with multivariate statistical analysis is a powerful approach for studying the effects of previously described factors on chemical composition of samples and classification of samples. We performed two different NMR profiling studies. In the NMR study on Slovenian honey samples, we achieved classification of samples based on botanical origin.

The NMR study performed on wine samples showed differences in NMR profile of wine samples produced by alcohol fermentation at two temperatures and with wild and commercial yeast strain. PCA loading plots revealed the most influential bins of NMR spectra in the distribution of the samples. Molecules characteristic for specific groups present in these bins were identified using 2D NMR spectra (^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-TOCSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H COSY).

It has been shown that different factors and processes can influence the chemical composition of foods, which can be tracked by NMR profiling. NMR is a useful tool because it can reveal and identify all the known and unknown characteristic components present in the specific group of samples. Using additional 2D and selective NMR spectra (^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-TOCSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H COSY, selective TOCSY, selective TOCSY-NOESY) the chemical structure of unknown molecules can be determined. This allows us to achieve comprehensive chemical analysis of food.

References

[1] E. Hatzakis (2019) *Comprehensive Reviews in Food Science and Food Safety*, 18, 189-220.

Future Perspectives in Using Earth Observation Data Technologies for a Sustainable Agriculture in Europe: The HE Partnership on Agriculture of Data

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In line with the European Green Deal and the EU's Farm to Fork Strategies, agricultural production in Europe needs to become more sustainable. This requires the environmental and climate performance of agriculture to increase; profitability, efficiency and competitiveness of the farming sector to be maintained; and operational risks (e.g., due to climate change) to be managed effectively. At the same time, EU policies, including the Common Agricultural Policy (CAP), are becoming more performance-oriented, requiring efficient and effective policy monitoring. This includes subsequent evaluation of the impact achieved and generating new knowledge for policy formation. Data and digital technologies offer the potential to achieve both these ambitious goals (sustainable agricultural production and more evidence-based and tailored policy) and to make the "twin" green and digital transition a reality. An increasing amount of high-quality data is required to achieve this as reflected in the European Strategy for Data. Applying data technologies to Earth observation (EO), environmental, agricultural and other data offers a wide range of opportunities for new and innovative data-based solutions and subsequent actions, such as input to farm management systems, which can increase the sector's performance and strengthen policy-making, implementation and monitoring capacities. Capitalizing on this potential requires that the solutions account for the needs and conditions on the ground and are accepted and deemed useful by farmers, advisors, the political and administrative systems and other key stakeholders. The HE Partnership on Agriculture of Data aims to support the transition to a sustainable agriculture in Europe as well as to strengthen policy monitoring and evaluation capacities by harnessing the potential that data technologies, in combination with EO, environmental, agricultural and other data, offer.

The partnership will contribute to, among others, the objectives of the European Green Deal and the Headline ambition of "A Europe fit for the Digital Age", the Farm-to Fork Strategy, the Europe Strategy for Data and the Coordinated Plan for Artificial Intelligence (AI) in particular, as well as to the objectives of the CAP, the United Nations' Sustainable Development Goals (SDGs) and to the ambitions for better policy-making.

Towards AI-driven Food and Nutrition Science and Society: Opportunities and Challenges

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In the last decades, a great amount of work has been done in the predictive modelling of issues related to human and environmental health. Resolution of issues related to healthcare is made possible by the existence of several available biomedical vocabularies and standards, which play a crucial role for understanding health information, together with a large amount of health data. However, focusing solely on healthcare data limits the potential benefits that our lives and societies could have from the rapid development of artificial intelligence (AI) and its enormous capabilities. As such, Lancet Planetary Health in 2019 noted that the focus of future improvements in our well-being and societies will depend on investigating the links between food systems, human health, and the environment. However, despite a large number of available resources and work done in the health and environmental domains, there is a lack of resources that can be utilized in the food and nutrition domain, as well as their interconnections. In particular, this is important during the current pandemics of COVID-19, when food provision and security, as well as healthy nutrition and environment, are tremendously needed for quick recovery and long-term sustainable development of our societies.

For the purpose of attaining human and societal well-being through advances in the field of AI, the talk will focus on opportunities for utilizing big data from food and nutrition and their interrelations with biomedicine and the environment. Huge amounts of data containing valuable information are now available in various datasets, registries, and scientific and grey literature, which makes it possible to use advanced AI methods. However, before applying AI methods to real-life data, that is heterogeneous (i.e., of different types and formats), unstructured (textual) data needs to be structured and normalized with other structured data. In this talk, we will explain AI methods and resources that can be used on different levels in the modelling process [1], starting from raw data to discovered knowledge. Finally, the existence of such methods and resources will be linked to several application scenarios of utilizing food and nutrition data in predicting food-disease interactions [2], COVID-19 mortality rate [3], and food chain traceability [4].

Acknowledgements

References

[1] G. Cenikj et al. (2022) *Database*, 2022, baac107, 1-12.
[2] G. Cenikj, T. Eftimov, B. Koroušić Seljak, *Proceedings of the 20th Workshop on Biomedical Language Processing*, Online, 2021, 30-40.
[3] M. Trajanoska, R. Trajanov, T. Eftimov (2022) *Expert Systems with Applications*, 209, 118377.
[4] D. Potočnik et al. (2020) *Food chemistry*, 326, 126958.

Cold Plasma Pre-Treatment and Grain Priming: Zinc Enhancement in Buckwheat Grains and Sprouts

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Zinc biofortification of cold plasma pre-treated buckwheat (*Fagopyrum esculentum* Moench) grains
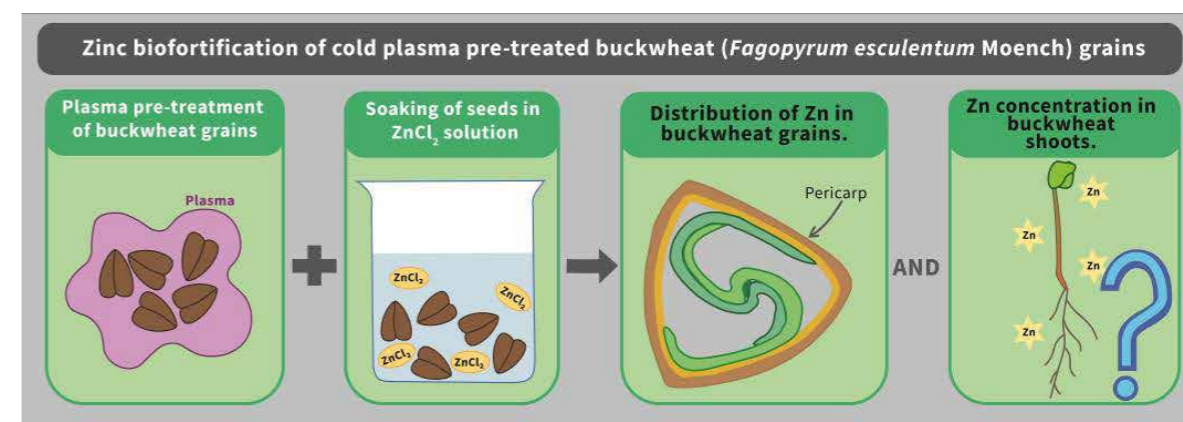
To alleviate essential element deficiency in humans, different ways to increase their available concentration in edible produce (*i.e.* bio-fortification) can be applied [1]. Sprouts are a popular part of healthy diets, providing important vitamins and essential elements throughout the year, due to its simple production process [2]. Element enhancement in sprouts can be easily achieved by soaking grains in element-rich solutions before germination. To improve the translocation of the element from the solution into the grain, additional pre-treatment techniques can be applied. One of such potential techniques is cold plasma treatment of grains. Cold plasma is ionised gas [3] and treating different materials with cold plasma results in chemical modification of the surface (mostly oxidation) and in some cases even morphological changes caused by plasma etching [4]. In grains, cold plasma treatment increases hydrophilicity of the grain surface and increases water uptake [5].

The aim of the study was to test if cold plasma pre-treatment promotes zinc (Zn) biofortification of common buckwheat (*Fagopyrum esculentum* Moench) sprouts, for which five-fold increase in Zn concentration was shown when grain was soaked (primed) in $ZnCl_2$ for 16 h. Cold plasma pre-treatment increased wettability and water uptake of common buckwheat grains. However, a combination of cold plasma pre-treatment and grain priming did not increase Zn concentration in the sprouts more than priming alone; rather there was a decrease in Zn concentration in sprout shoots. When the fresh weight portion of whole sprouts (i.e. of roots and shoots) was considered, comparable average requirements of Zn, namely 24.5 % and 35 % for adult men and women would be satisfied by consuming sprouts of cold-plasma pre-treated and not pre-treated grain.

This was the first study to combine plasma pre-treatment of grains and sprout biofortification to enhance Zn composition of buckwheat sprouts. Further potential advantages of cold plasma pre-treatment need to be evaluated, for example shortening the duration of soaking required to produce Zn-enriched sprouts.

Acknowledgements

References

[1] H. E Bouis, A. Saltzman (2016) *Global Food Security,* 12, 49-58.
[2] S. L. Kim, Y. K. Son, J. J. Hwang, S. K. Kim, H. S. Hur, C. H. Park (2001) *Fagopyrum, 18,* 4.
[3] D. A. Frank-Kamenetskii (1972) *Springer US,* 1-8.
[4] P. Starič, J. Mravlje, M. Mozetič, R. Zaplotnik, B. Š. Batič, I. Junkar, K. Vogel-Mikuš (2022) *International Journal of Molecular Sciences,* 23, 7369.
[5] A. Zahoranová, L. Hoppanová, J. Šimončicová, Z. Tučeková, V. Medvecká, D. Hudecová, B. Kaliňáková, D. Kováčik, M. Černák (2018) *Plasma Chemistry and Plasma Processes,* 38, 969-988.

Supplementing Cheese with Polyphenol Rich Fruits to Increase Content of Beneficial Bioactive Polyphenols

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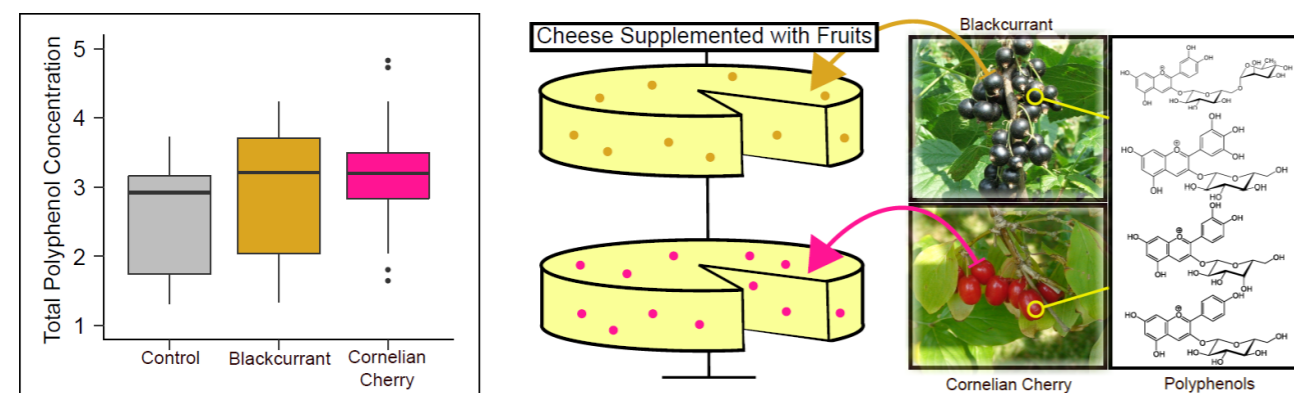
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Polyphenols are phytochemicals potentially beneficial for human health [1]. By adding polyphenol rich fruits to a product normally poor in these bioactive compounds could therefore improve it nutritionally. In this study, we tested this process with Caciotta-like cheese.

However, polyphenols are antimicrobial and therefore potentially disruptive of cheese fermentation [2]. Disruption of essential lactic acid bacteria (LAB) could negatively affect cheese nutrition and/or organoleptic properties.

Model-cheeses had been manufactured with a supplementation of cornelian cherry (Cornus mas) or blackcurrant (Ribes nigrum) due to their high polyphenol content [3]. Two doses (0.3 and 0.6%) and two farming methods (organic and conventional) of both berries had been tested.

The model cheeses were analysed for polyphenols concentration by means of Folin–Ciocalteu method and microbiota by plate counts using selective media targeting either LAB, coliforms or total aerobic bacteria.

The total polyphenol content of cheeses increased when supplemented with fruits, in particular with higher dose and with conventionally farmed fruits.

In plants, polyphenols are commonly produced as defense against various hazards. The difference between farming methods could be due to the plants producing different polyphenol profiles based on farming method and/or location.

When compared to control cheeses the microbial community of experimental cheese did not differ. We observed higher counts mesophilic lactocci in cheeses supplemented with blackcurrant compared to with cornelian cherry. This difference between fruits could be caused by a difference in polyphenol profile.

The cheeses added with blackcurrant and cornelians cherries were successful with increased concentration of total polyphenols, without modifying fermentation of the products.

Works are in progress to increase total polyphenol content and investigate polyphenol profile of fruits and cheeses.

Acknowledgements

References

[1] C. G. Fraga, K. D. Croft, D. O. Kennedy, F. A. Tomás-Barberán (2019) Food & Function, 10 (2), 514-528.

[2] R. Tabasco, F. Sánchez-Patán, M. Monagas et al (2011) Food Microbiology, 28 (7), 1345-1352.

[3] S. Cyboran, D. Bonarska-Kujawa, H. Pruchnik, R. Zyłka, J. Oszmiański, H. Kleszczyńska (2014) Food Research International, 65, 47-58.

Cave Bear Diet Revealed by Stable Isotopes Analysis

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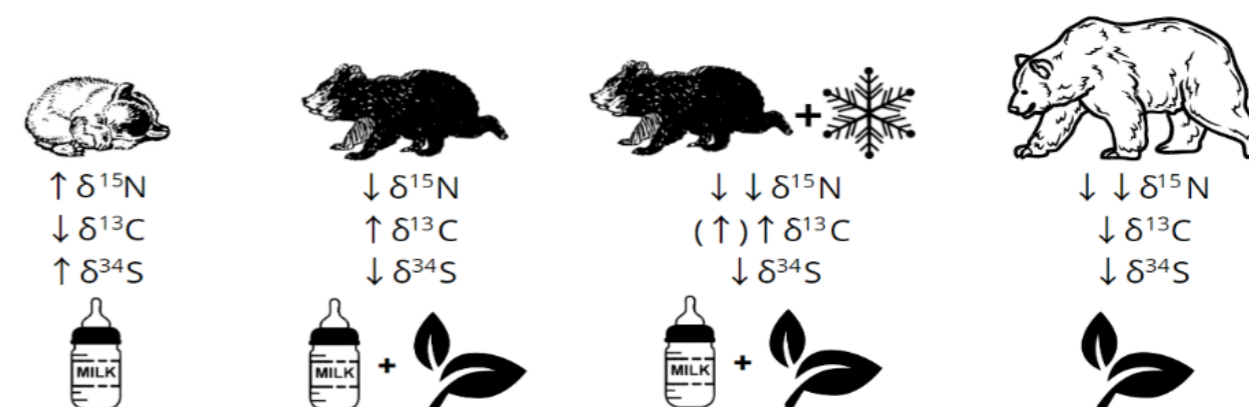
Cave bears lived in the late Pleistocene and became extinct about 24,000 years ago. Despite the large number of studies, many questions about their life and their diet remain opened. Observed $\delta^{15}N$ values are equal to or lower than those of herbivores from the same site, suggesting that cave bears had a predominantly plant-based diet [1]. However, some other studies show an omnivorous [2] or even carnivorous diet [3]. In addition to the diet, there are also metabolic effects of hibernation influencing the carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratio in the body [4].

Based on ontogenetic development, we sampled dental tissues formed at a specific life period of interest for stable isotope analysis of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulphur ($\delta^{34}S$) in collagen as well as sequentially sampled dental enamel to obtained $\delta^{13}C$ and $\delta^{18}O$ values.

The $\delta^{15}N$ values (on average 6.1‰) show that the 1-3 months old cubs feed exclusively on milk. In the age group of 5-10 months, the values are slightly lower (5.1‰), indicating a milk diet with gradual intake of plant food. Additional winter months in 5-15 months' group give even lower $\delta^{15}N$ values (3.4‰). All subsequent groups show low $\delta^{15}N$ values (2.8‰) and indicate a plant-based diet.

In general, $\delta^{13}C$ values of adult bears (-22.4‰) indicate a diet of C3 plants. Very low $\delta^{13}C$ values in 1-3 months old cubs (-22.6‰) correspond well with lower $\delta^{13}C$-values in milk from the hibernating mother. In 5-10 months old cubs, $\delta^{13}C$ values are higher (-22.3‰), reflecting consumption of milk from the non-hibernating mother and ingestion of solid food. In yearlings (5-15m), we assume that the highest $\delta^{13}C$ values (-21.3‰) belong to cubs that failed to hibernate and starved, and the lowest (-21.9‰) to cubs that succeeded in hibernating and successfully metabolized fat stores depleted in ^{13}C. In older bears there is a decreasing trend of $\delta^{13}C$ values (-22.4‰).

Highest $\delta^{34}S$ values (9.9‰) are observed in 1-3 months old cubs that consumed only mother's milk, while all other age groups that consumed plants, generally have similar $\delta^{34}S$ values (6.5‰).

The analysis of $\delta^{13}C$ and $\delta^{18}O$ obtained on enamel, showed highest $\delta^{13}C$ (-13.2‰) and $\delta^{18}O$ values (-6.9‰) in enamel formed from late first winter to autumn. In enamel formed in the second winter $\delta^{18}O$ values remain similar (-7.0‰), whereas $\delta^{13}C$ values slightly decrease (-15.0‰). In enamel formed in the second year of life $\delta^{13}C$ (16.6‰) and $\delta^{18}O$ (-7.3‰) values are the lowest.

Acknowledgements

References

[1] H. Bocherens (2015) Quaternary Science Reviews, 117, 42-71.

[2] M. Robu, J. Fortin-Noreus, M. Richards, C. C. Schwartz, J. G. Wynn, C. T. Robbins, E. Trinkaus (2013) Canadian Journal of Zoology, 91, 227-234.

[3] I. Ramírez-Pedraza, C. Tornero, S. Pappa, S. Talamo, D. C. Salazar-García, R. Blasco, J. Rosell, F. Rivals (2019) Scientific Reports, 9, 5716.

[4] S. G. Jenkins, S. T. Partridge, T. R. Stephenson, S. D. Farley, C. T. Robbins (2001) Oecologia, 129, 336-341.

Can Ionizing Radiation Cause Isotopic Fractionation of Organic Compounds

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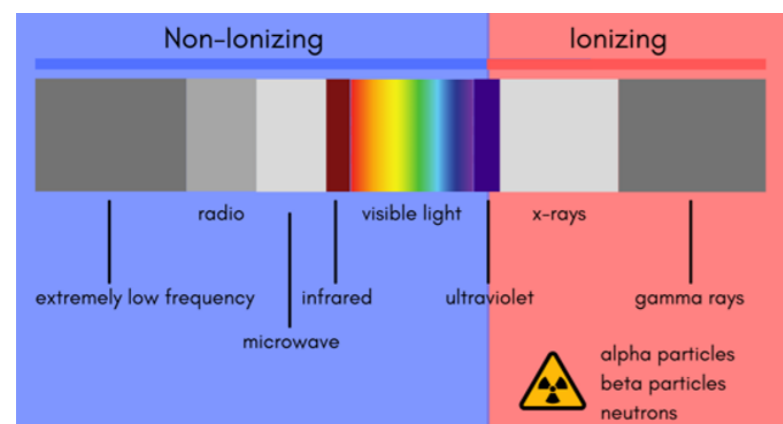
Spectrum of Ionizing and Non-Ionizing Radiation

(adapted from sciencenotes.org)

The chemical and isotopic compositions of food are often used to constrain food authenticity, traceability, safety and quality. Ionizing radiation in the form of high doses of energetic γ radiation over short periods of time is being widely used to sterilize food and equipment [e.g., 1,2]. Moreover, biosafety regulations in various countries mandate the sterilization of imported biological materials, including organic stable isotope reference materials, which is typically performed via γ irradiation. Few have considered that γ irradiation, or radioactivity in general, can provide sufficient chemical activation energies to trigger reactions that cumulatively lead to chemical and isotopic shifts on a molecular level. The possible consequences of γ irradiation for compound-specific stable isotope ratios in organics have never been properly evaluated. Shortcomings of the few previous experimental studies include (i) reliance on stable isotope measurements of bulk organic matter, or fractions thereof, that are far less sensitive or informative than compound-specific data on the molecular level; and (ii) examining only carbon isotopes ($\delta^{13}C$), whereas the expected reactions are more likely to be detected via hydrogen isotopes (δ^2H) due to radiation-induced exchange with hydrogen atoms in water.

A collaborative research project among Indiana University, Caltech, the University of Notre Dame, and the Jožef Stefan Institute exposes individual, chemically diverse model organic compounds to α, β, or γ irradiation over months to two years in sealed glass tubes, followed by chemical-compositional and compound-specific isotopic analyses. By using added water with a high abundance of deuterium (^2H), we sensitively monitor any radiologically-induced H-transfer into the pool of stable, originally ^2H-depleted carbon-bound organic hydrogen.

We will test the following main hypotheses:

1: Irradiation of organic matter can cause dealkylation and liberate radiogenic small hydrocarbon and other molecules that differ in C and H isotope ratios from parental material.

2: Low dose rates of α and β irradiation over long times will produce chemical and isotopic changes that are distinct from those due to very large, quick doses of energetic γ irradiation.

3: Commercial sterilization of organic matter *via* γ irradiation at a relatively low dose of up to 25 kGy does not cause significant HCN-isotopic shifts on a compound-specific molecular level, thus supporting the use of isotopic fingerprinting of forensic samples for food authentication etc. that must cross international borders and pass through mandatory biosafety γ sterilization.

The outcomes of this study will be an improved organic-molecular understanding of which functional groups and carbon skeletal configurations lend themselves to chemical and stable isotopic changes during exposure to different kinds of radiation, and the relevant doses at which such changes become isotopically measurable.

References

[1] O. Gregoire, M. R. Cleland, J. Mittendorfer, S. Dabahneh, D. A. E. Ehlermann, X. Fan, F. Käppeler, J. Logar, J. Meissner, B. Mullier, F. Stichelbaut, D. W. Thayer (2003) *Radiation Physics and Chemistry,* 67, 169-183.

[2] J. Madureira, M. I. Dias, J. Pinela, R. C. Calhelha, L. Barros, C. Santos-Buelga, F. M. A. Margaça, I. C. F. R. Ferreira, S. Cabo Verde (2020) *Science of the Total Environment* 727, 138706.

National Representative Food Consumption Data (SI.Menu Study) in Dietary Exposure and Intake Assessments

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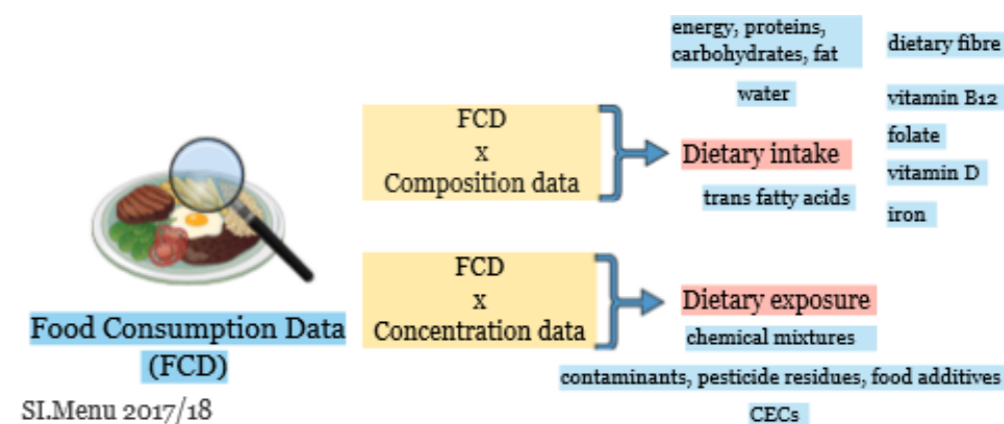
Detailed and high-quality food consumption data (FCD) are essential for scientific risk assessment process to characterise potential risks from chemicals in food. The Slovenian national food consumption survey (SI.Menu 2017/18) followed the EFSA EU Menu methodology [1,2]. Complete results were obtained for 1,956 persons (response rate 64%) including infants, toddlers, adolescents, adults and elderly, selected from the Central Register of Population of Slovenia. FCD were collected by personal interview using a general and food propensity questionnaire, and two 24h recalls with the OPEN dietary software. Collected FCD was coded according to the FoodEx2 classification [2]. Dietary intakes of the most key nutrients and micronutrients in Slovenian population have been assessed combining FCD with food composition data obtained from OPEN database. The usual dietary intake of foods and nutrients was modelled using the Multiple Source Method (MSM) [2,3]. Results showed that the diet of all population groups in Slovenia is not in accordance with healthy eating recommendations and for macronutrients, energy and water intake reference values [2]. By analysing FCD, we can also identify the main food sources of individual nutrients or contaminants in the diet, as we did for *trans* fatty acids, dietary fibre and vitamin D [3,4]. Matching FCD with compositional data for some nutrients is still challenging. The study on intake of dietary fibre showed that a compositional data for the complete set of foods are still missing [3]. By combining FCD with the data on micro-composition of the food, we estimated the dietary intake of some micronutrients. We showed inadequate average daily intake of vitamin D in adolescents, adults and older adults in Slovenia [4]. With FCD we identified the Slovenian population groups with risk regarding folate, vitamin B12 and iron intake, for which nutrient status was also assessed using blood biomarkers [5]. Dietary exposure to chemicals is estimated by combining FCD with chemical concentration data. Recently, the assessment of exposure to chemical mixtures in the diet has been a high priority. We implemented approach using the web-based Monte Carlo Risk Assessment (MCRA) platform to combine national exposure data, with hazard information to define pesticide mixtures applied to the specific health effect [6].

Acknowledgements

References

[1] National Institute of Public Health. *U. Blaznik et al. (2019) EFSA supporting publication: EN-1728, 26.*

[2] M. Gregorič et al. (2022) *Nutrients*, 14, 3618.

[3] B. Korousic Seljak et al. (2021) *Nutrients*, 13, 3826.

[4] M. Hribar et al. (2021) *Nutrients*, 13, 3528.

[5] I. Pravst et al. (2021) *Nutrients*, 13, 3860.

[6] A. Crepet et al. (2019) *International Journal of Hygiene and Environmental Health,* 222, 291-306.

Total Mercury and Methylmercury Levels in Hair, Blood, and Urine of Individuals Following Controlled Intake of Tuna Fish

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The principal source of human exposure to methylmercury (MeHg) is the consumption of fish and seafood. Fish is, nonetheless, an essential component of a healthy diet, rich in omega-3 fatty acids, vitamins, and minerals. When estimating exposure to MeHg, risk assessors typically assume that all Hg in fish is MeHg and that 95-100% of the ingested MeHg from fish is bioavailable. Recent studies however suggest that such assumptions may be incorrect and can lead to overestimations of exposure and risk, which can, in turn, hinder our ability to reasonably balance between the benefits and health risks of seafood consumption. The aforementioned assumptions are mostly based on outdated studies with significant limitations, such as unrealistic exposure routes using aqueous methylmercuric nitrate or fish tissue spiked with methylmercuric nitrate, rather than MeHg bound to fish muscle due to natural contamination [1,2]. Different cooking methods have also been proven to lower the bioaccessibility of Hg from seafood, while specific genetic polymorphisms have been associated with differing levels of Hg exposure biomarkers [3].

With the present study, we aim to reduce the uncertainties in the current human exposure and health risk assessment due to MeHg in fish and validate pharmacokinetic models which would enable us to better predict individual internal doses and potential adverse health effects. A realistic scenario of exposure of individuals to MeHg was created, through the controlled one-week consumption of tuna steaks and subsequent detailed measurements of total (THg) and MeHg in multiple biological samples over the period of 2 months. A total of 16 volunteers

(out of which 6 controls) were recruited at the Jožef Stefan Institute in Ljubljana. Each subject in the experimental group was given 5 yellowfin tuna steaks (the weight of each tuna steak was approximately 300 grams) to consume over the period of 5 consecutive days. THg and MeHg were determined in each raw steak. Subjects kept a detailed daily record of the way of preparation of tuna steaks and all foods and drinks consumed at each meal. Samples of blood, hair, and urine were taken from all volunteers prior to the start of the tuna consumption week, every other day during consumption week, and then once a week for a total of 7 post-consumption weeks. In addition, genotyping for single nucleotide polymorphisms previously found associated with Hg kinetics (e.g. APOE, CYP, GPX) was performed. Possible associations of SNPs and Hg exposure biomarkers were tested.

References

[1] B. Aberg, L. Ekman, R. Falk, et al. (1969) *Archives of Environmental Health,* 19, 478-484.

[2] J.K. Miettinen, T. Rahola, T. Hattula, K. Rissanen, M. Tillander (1971) *Annals of Clinical Research,* 3, 116-122.

[3] M.A. Bradley, B.D. Barst, N. Basu (2017) *International Journal of Environmental Research and Public Health,* 14, 169.

Unravelling the Exposure and Risk to Free Glutamate Form Food Additive and Non-Additive Sources in Belgium

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The latest evaluation of EFSA on glutamic acid and its salts (E 620-625) concluded that exposure estimates exceeded the acceptable daily intake (ADI) of 30 mg/kg bw/day as well as doses associated with adverse health effects [1]. Based on these conclusions, the EFSA panel recommended to revise maximum permitted levels of E 620-625 in food. The available data did, however, not allow to make a distinction between the exposure to free glutamate naturally present in food from that added as a food additive. Hence, we developed an approach based on careful sample selection, a sensitive analytical method and semi-probabilistic intake assessments, to perform a refined exposure and risk assessment for Belgian children, adolescents and adults to free glutamate from different sources.

A priority scheme, based on apparent major contributing food groups to exposure, expected concentrations in food products, data gaps, consumption amounts, market sales data and food labeling data, resulted in the selection and sampling of 561 food items, either labeled with E 620-625 or not. For each sample that was labeled with E620-625, at least one similar food item without E 620-625 was sampled as well. All samples were analysed by UHPLC-MS/MS. The occurrence data were linked to consumption data from the Belgian Food Consumption Survey (N = 3146, 3-64 years) [2]. The habitual intake of free glutamate was assessed using SPADE (Statistical Program to Assess Dietary Exposure [3]) for following scenarios: (1) intake from food additive and non-additive sources, with and without the assumption of brand-loyalty (2) intake from non-additive sources, (3) intake from food additive use, assessed as the difference between scenario (1) and (2). Brand-loyalty wad

determined at the food group level. The risk assessment was performed by comparing the habitual intake with the ADI.

Mean and high level exposure estimates for free glutamate were all below the ADI of 30 mg/kg bw/day. The habitual intake of free glutamate by children (3-9 y) was higher than that by adolescents (10-17 y) and adults (18-64 y), due to their higher food consumption per unit body weight. The high-level exposure of children to free glutamate used as food additive, reached 25% of the ADI. At population level, almost 80% of the mean habitual intake of free glutamate was from non-additive sources. Ripened cheese was the major contributing food group to exposure. Brand-loyalty to ripened cheese may lead to high-level exposures for children at 82% of the ADI. These results indicate that the current exposure levels in Belgium are of no health concern, but regular follow-up of the exposure by children is recommended.

References

[1] EFSA (2017) *EFSA Journal,* 15, 4910.

[2] S. Bel et. al. (2016) *Archives of Public Health,* 74, 20.

[3] A. L. M. Dekkers et. al. (2014) *The Journal of Nutrition,* 144, 2083.

Human Biomonitoring: Dietary Cadmium Exposure in Slovenian Population of Children and Adolescents

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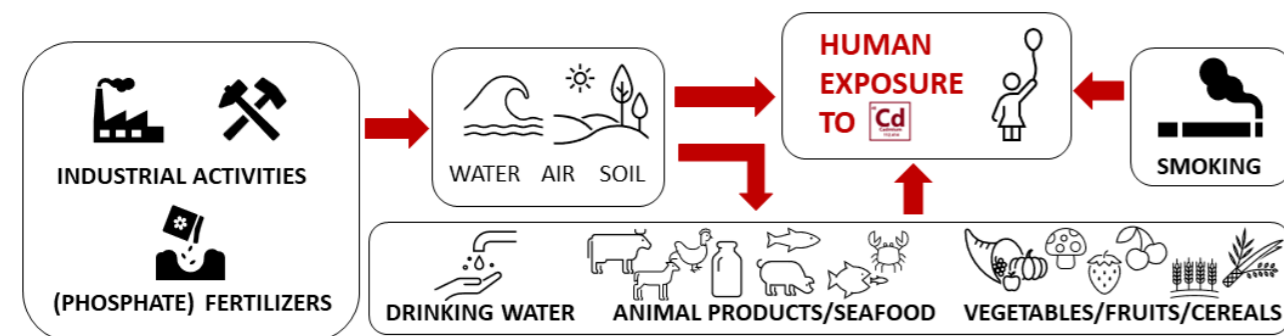
Cadmium is a toxic metal with no known biological function in human body. Industry and phosphorous fertilizers used in agriculture contribute to elevated levels of Cd in soil. The main sources of exposure in general population are drinking water and consuming food grown in contaminated land since Cd accumulates in plant life as well as in aquatic organisms in water environments [1]. The objective was to assess the exposure to Cd in the population of children aged 6-9 and adolescents aged 12-15 years residing in potentially contaminated areas of Slovenia. We measured Cd levels in blood, urine and hair samples (B-Cd, U-Cd and H-Cd) of 1099 volunteering participants.

As an analytical detector TQ-ICP-MS was used and the data about the individual's lifestyle, living environment and dietary habits were obtained by carefully constructed questionnaires.

An extensive statistical analysis using descriptive statistics, Wilcoxon rank-sum (Mann-Whitney) test, Kruskal-Walli's test, Spearman's rank correlation coefficient, Pearson's correlation, ANOVA and multiple linear regression was performed to check associations between biomarkers and different exposure determinants, as well as for assessment of spatial and time trends of exposure to Cd in Slovenian population.

Environmental data used for evaluation of Cd exposure within this research have shown that the levels in soil are the highest in the area around Jesenice which is an industrial area and the lowest in agriculturally rich Prekmurje, which reflects very well in the levels of Cd measured in the biological samples of study participants. The highest levels of B-Cd and U-Cd were detected in Jesenice (0.16 µg/L and 0.20 µg/L, respectively), highest H-Cd in Mežica Valley (17.4 ng/g) and the lowest levels of Cd in all three biomarkers in participants residing in Prekmurje (0.13 µg/L, 0.14 µg/L, 8.9 ng/g, respectively).

Although the differences were very low, the use of multiple linear regression showed 20 % higher levels of B-Cd and U-Cd in adolescents than in children (p<0.001), and that participants consuming locally grown potato daily, had 40 % higher B-Cd and U-Cd than those consuming it less frequently (p=0.010 and 0.040, respectively). This coincides very well with some other recent studies [1,2]. Significant positive association was also found between Cd exposure and the education level of their parents (p<0.001) which is a possible indication of children's lifestyle and quality of food they consume since higher Cd levels in the body could be a result of consuming more fresh fruits and vegetables and less cheap, processed food.

Comparing the exposure data with previous studies in children from Slovenia, no significant trend in levels was observed for the last decade. Overall, in the present study the levels were higher in rural over urban areas and the highest in industrial areas. Accordingly, we can assume that for Cd exposure in the Slovenian general population, industry is a stronger determinant than use of phosphate fertilizers in agricultural lands.

References
[1] EFSA (2012) *EFSA Journal*, 10 (1).
[2] J. Jean, V. Sirot, M. Hulin, E. le Calvez, J. Zinck, L. Noël, P. Vasseur, F. Nesslany, S. Gorecki, T. Guérin, G. Rivière (2018) *Food and Chemical Toxicology*, 115, 358-364.

Integrated Research Infrastructures' Initiatives in Support to Food Quality and Safety and Sustainability of Agrifood Systems

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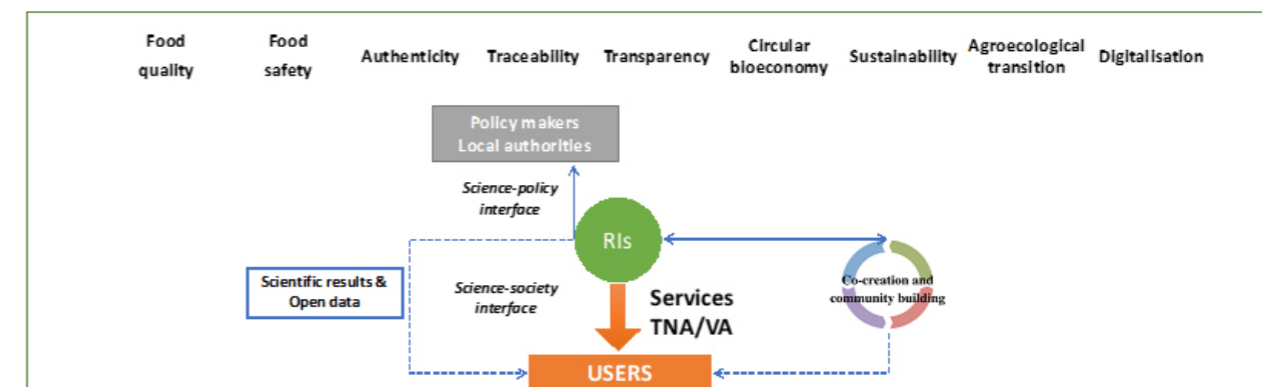
The agrifood sector is a strategic asset of all European Countries and is one of the largest and most strategic economic sectors, with particular social relevance: it is of crucial importance to ensure sufficient food production to EU citizens, and vital to ensure employment, preserve rural public goods, supply healthy and quality products, including sustainable and secure production and distribution chains. It has also to facilitate the enhancement of competitiveness and sustainability approach of SMEs into the food chain, while quality, safety, and traceability being key elements. It is essential to consider all factors affecting food quality & safety *from farm to fork*, thus making health converge into a unicum as a system by applying the "One Health" approach, which integrates human, animal, and environmental health. At the EU level, sustainable agricultural and food systems lie at the heart of the Green Deal and the Farm2Fork Strategy, with the ambition of making the European agrifood system the "global standard for sustainability" in a view of an agroecological transition. High-quality and reliable data are of utmost importance to populate the expanding data technologies with useful contents and, according to the FAIR principles, enabling advanced research supporting the agrifood sector. To address these societal challenges, the scientific community must consider the high intersectoral and interdisciplinary dimension of studying and supporting the agrifood systems. In this respect, Research Infrastructures (RIs) play a strategic role and have the capacity to assemble resources (equipment, data, knowledge) and services for the agrifood sector (including research communities) on a long-term basis both within EU and globally. In this context, RIs are the best positioned not only to address societal challenges, but also to provide turnkey solutions for citizens, policy makers and industry. In particular, initiatives focused in integrating RIs knowledge, facilities and services can boost the definition of an integrated and structured landscape, promoting more and more advanced research and highest cooperation with and within the agrifood systems' stakeholders, thus enhancing the social-economic impact. These topics are at the core of the research & innovation agenda of METROFOOD-RI (www.metrofood.eu – ESFRI domain: Health and Food). Opportunities arising from integrated initiatives involving several RIs are presented, such as: AgroServ, which aims at providing access to new integrated services for resilient and sustainable agriculture systems and the agroecological transition, and iNEXT-Discovery, which aims at enabling access to structural biology RIs also to strengthen the bridge between the "structural biology community" and the "agrifood community".

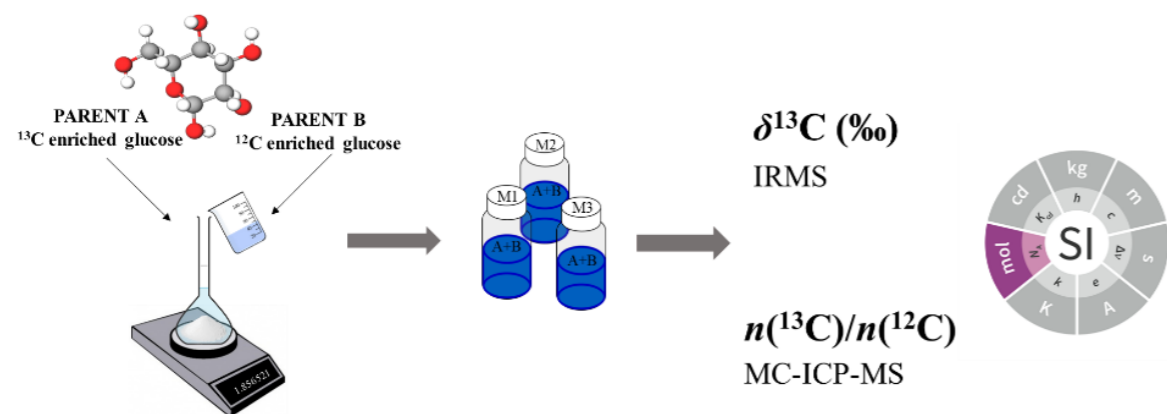# Metrology for Stable Isotope Ratio Measurements

D. Potočnik[1,*], D. Malinovskiy[2], P. Dunn[2], N. Ogrinc[1]

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Isotope delta values for H, C, N, O and S is reported relative to international standards that may be "virtual" or exhausted and have no SI traceability. Traceability of reference materials to those standards is jeopardised by poor metrology surrounding characterisation by interlaboratory comparison. Therefore, there is a need to develop novel methodology for SI traceable isotope standards and characterisation practices for reference and isotopically-enriched materials.

The EMPIR 19ENV05 (STELLAR) project has already provided opportunity to improve existing methods for determination of absolute isotope ratios of carbon in the context of atmospheric greenhouse gases. In this research the first results obtained within this activity will be presented. Measurement of absolute isotope ratios of elements typically involves preparation of gravimetric mixtures of highly isotopically-enriched materials. Characterisation of such materials for isotopic enrichment alongside purity is fundamental to this approach.

Three isotope mixtures of glucose solutions have been prepared and send to different laboratories together with naturale glucose in the form of solid and blank 2% nitric solution. Two different ways of sample preparation was tested (with and without chromosorb). The reference materials (RMs) for normalization and quality control include USGS62, USGS64, USGS66, USGS65 and USGS77. Other RMs covering different $\delta 13C$ values were also used and include: LGC1711, $\delta^{13}C$ = -42.13 ± 0.26 ‰; LGC1712, $\delta^{13}C$ = -24.62 ± 0.23 ‰; LGC1713, $\delta^{13}C$ = +12.55 ± 0.21 ‰.

The results are presented in Table 1.

	LGC	JSI			
		ISOPRIME 100 (chromosorb)	ISOPRIME 100 (no chromosorb)	EUROPA (chromosorb)	EUROPA (no chromosorb)
Sample					
M2	37.62	37.22	37.48	37.76	37.52
M3	-28.68	-29.46	-28.21	-28.69	-28.71
Natural glucose 1	-11.28	-11.48	-11.30	-11.38	-11.19

Table 1. Results of the $\delta 13C$ values (expressed in ‰-notation) in different mixed samples and natural glucose performed by two laboratories (LGC and JSI) with different instruments.

The first results indicate that there are no statistically significant differences observed between two different ways of sample preparation (with or without the chromosorb). The comparison of results between two different laboratories (LGC and JSI) and instruments are within established measurement uncertainty.

The results obtained will allow us re-calculating $n(^{13}C)/n(^{12}C)$ isotope amount ratio of the zero-point value for VPDB delta scale with lower uncertainty than in previous studies.

Acknowledgements

Aerobic Degradation of Bisphenol A Alternatives with Activated Sludge: Kinetics and Biotransformation Products

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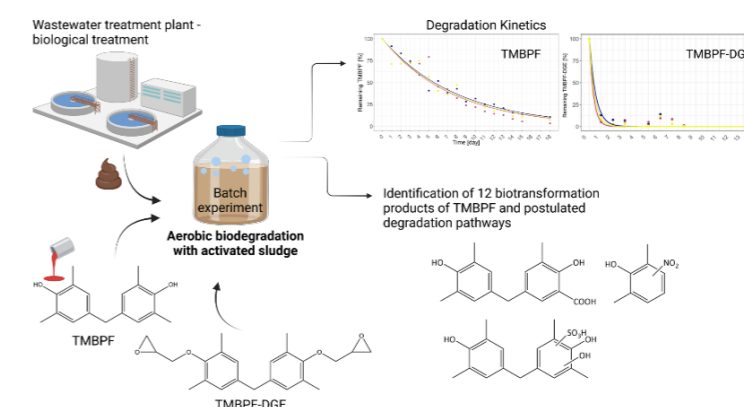
This study investigates the biodegradation kinetics of two potential bisphenol A (BPA) non-estrogenic compounds for use in food contact materials, namely tetramethyl bisphenol F (TMBPF) and tetramethyl bisphenol F diglycidyl ether (TMBPF-DGE) using GC–MS/MS and LC–MS/MS. The samples were treated with activated sludge (AS), and non-targeted analysis using LC–Orbitrap-MS was used to identify biotransformation products (BTPs) in the case of TMBPF1. A batch biotransformation experiment showed that degradation followed single first-order reaction kinetics and that the initial concentration (c_i) affected TMBPF degradation, with faster degradation observed at lower concentrations (c_i = 0.1 mg L^{-1}, $-k_t$ = 0.16, half-lives = 4.4 days *vs.* $-k_t$ =0.02, half-lives = 36.4 days at c_i = 10.0 mg L^{-1}). After 18 days, only 8% of the original TMBPF remained at the lowest tested concentration (0.1 mg L^{-1}). Similarly, the ci influenced the biodegradation kinetics of TMBPF-DGE with AS, but significantly faster, compared to TMBPF, e.g., less than 10% TMBPF-DGE remained after two days at lower ci and eight days at higher ci. The removal of TMBPF-DGE at higher ci accounts for its biodegradation and adsorption to biomass. Using the two non-target workflows, twelve TMBPF BTPs were identified, three of which were workflow and one condition-specific, respectively. The highest relative quantities of BTPs were observed in nutrient-mineral and mineral media after ten days, while after 14 days, 36% and 31% of TMBPF (c_i =1 mg L^{-1}) remained in the nutrient-mineral and mineral media, respectively. The kinetics of TMBPF and its BTPs were the same with and without an additional carbon source. The proposed biodegradation pathway of TMBPF involves cleavage of the methylene bridge, hydroxylation with further oxidation, sulphation, nitrification, nitro reduction with further oxidation, acetylation, and glycine conjugation. Overall, this research provides novel insights into the fate of TMBPF and TMBPF-DGE during biological wastewater treatment.

Acknowledgements

References

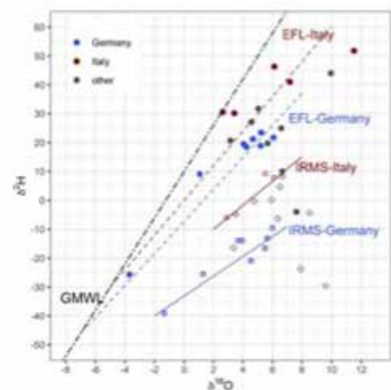[1] A. Kovačič et al. (2021) *Journal of Hazardous Materials,* 404A, 124079.

Combining Optical Spectroscopy and IRMS for the Measurement of δ^{18}O and δ^2H of Water and Ethanol Directly in Wine

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Officially certified methods for stable isotope analysis for wine authentication are limited to δ^{18}O measurements of the wine water, and to (site-specific) δ^2H of the wine ethanol using NMR. Nowadays, optical spectroscopy has become an easier alternative to quantify both δ^{18}O and δ^2H of wine water straight in the wine. We utilized an optical analyser (OA-ICOS, LGR) with Spectral Contaminant Identifier to measure the δ^{18}O and δ^2H of water in 27 wine samples without any pre-treatment. These results reveal a wealth of information about the authenticity and growth conditions of the wines, and we recommend to extend the official δ^{18}O wine water method by δ^2H. We also performed high-temperature pyrolysis and chromium reduction combined with IRMS measurements to obtain the "whole wine" isotope ratios. The δ^{18}O results of OA-ICOS and IRMS show non-significant differences, but the δ^2H results of both methods differ much more. This is expected, and the δ^2H difference between these two methods is mainly caused by the ethanol.

Therefore, we investigated the possibility to deduce the δ^2H of wine ethanol from this difference. However, the results contain large uncertainties, and also deviate from the ^{2}H-NMR results following the official method. The, rather constant, deviation is caused by the other constituents in the wine, that can be corrected for, but the uncertainty is due to the limited precision of the SCI-based correction. We recommend to improve the spectral fit of the optical method, and thereby the SCI, so one can obtain the δ^2H values of ethanol as alternative for the ^{2}H-NMR method. Whereas the site specificity of NMR can of course not be obtained this way, the average δ^2H value can serve as an indicator for possible fraudulent processes. As a side result, the SCI produces a quite accurate number for the ethanol concentration of the wine.

References

This work has been published as:

[1] X. Wang, H. G. Jansen, H. Duin, H. A. J. Meijer (2021) *European Food Research and Technology*, 247, 1899–1912.

Geographical Origin of Mineral Waters by Sr Isotope Ratio MC-ICP-MS: Long-Term Stability of The Signatures Obtained by Different Purification Procedures

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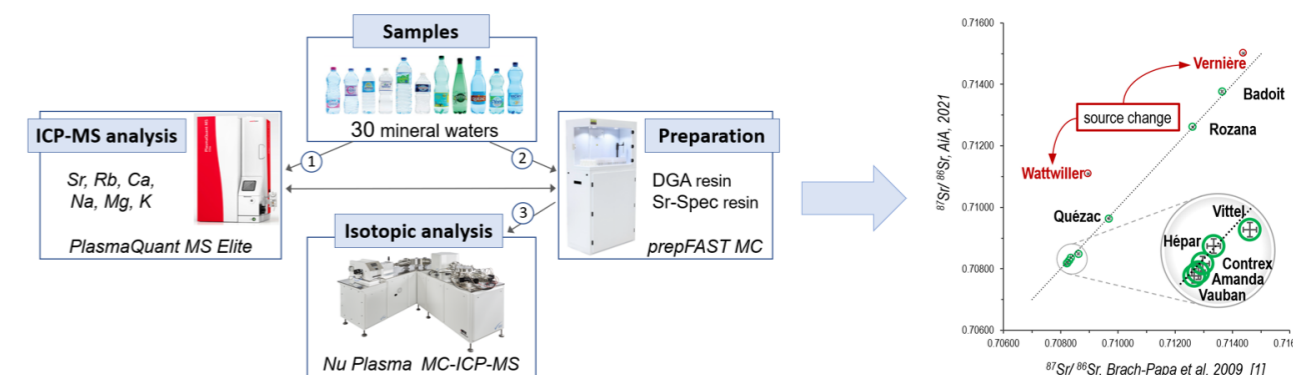
Natural mineral water is essential to life and holds an important place in the food production chain, cosmetics, pharmaceutical production, baby food, etc. For this reason, the traceability of mineral waters is an area of high interest.

Ground and spring waters move along specific flow paths and slowly react with rocks and soils and thus obtain a specific Sr isotopic composition which reflects the ^{87}Sr/^{86}Sr isotopic ratio of the soluble minerals of subsoil and bedrock, thus making a link to the geographic origin.

In this poster, we present the Sr isotope ratios determined for 30 mineral waters from France (n=24), the Spanish Basque Country (n=5) and Russia, lake Baikal (n=1). The obtained results are compared according to those previously published [1] and available isoscapes [2,3]. A clearly identified stability of the Sr isotopic composition was observed for Amanda, Vauban, Quézac, Hépar, Évian, Contrex, and Rozana (the differences between two corresponding values being less than 85 ppm over a period of 10 years) in comparison with publishes values [1,3,4]. This reinforces the fact that the Sr isotopic ratio is a reliable parameter for tracing the geographic origin of mineral-sourced waters on a relatively long-term scale.

Moreover, several protocols for separating the analyte (Sr) from alkali and alkaline earth elements using commercial columns packed with DGA resin were tested and validated with NIST SRM 987.

The objective of these protocols was to compare the differences in elution volume, eluent concentration, and resin quantity and compactness. The recovery rates observed for these protocols did not differ significantly from one another and were all above 95%, demonstrating the versatility and robustness of the prepFAST MC and DGA resin. In addition, a major advantage of this resin is its reusability - indeed, more than 50 mineral waters were run on a single column, with no significant memory effect observed.

The method described saves considerable laboratory time compared to conventional manual purification methods using Sr-Spec resin and can be successfully applied to mineral waters and potentially to other food matrices.

References

[1] C. Brach-Papa, M. Van Bocxstaele, E. Ponzevera (2009) *Spectrochimica Acta Part B: Atomic Spectroscopy*, 64 (3), 229–234.

[2] M. Willmes, C. P. Bataille, H. F. James (2009) *Applied Geochemistry*, 90, 75–86.

[3] S. Voerkelius, G. D. Lorenz, S. Rummel (2010) *Food Chemistry*, 118, 933–940.

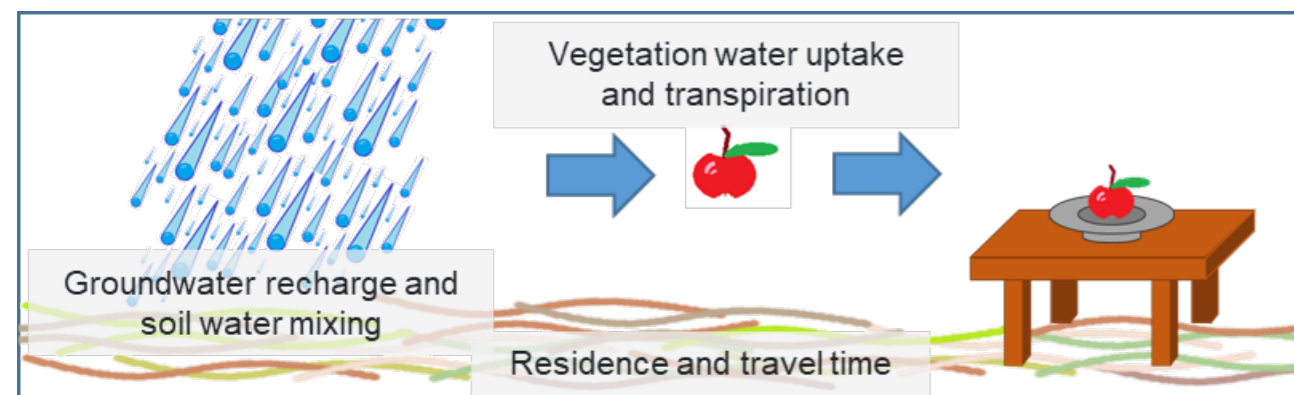[4] T. Zuliani, T. Kanduč, R. Novak (2020) *Water*, 12 (9), 2454.

The Signal of Water: Stable Isotopes from Rain to Table

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Stable isotope analysis is becoming increasingly popular to identify the geographical origin and to detect undeclared manipulation of food, beverages, and other products such as timber and cotton. The underlying rationale is that the isotope signature of plants is determined by the elements taken up from soil and atmosphere and by fractionation processes during assimilation. For example, the hydrogen and oxygen isotope signature of both plant water and biomass is mostly influenced by that of water absorbed by roots.

To strengthen the interpretation of food isotope data, it is necessary to improve our understanding of how the stable isotope signal of a product is linked to its *terroir* and its production line. Since water greatly contributes to creating this link, it is important to understand the processes that affect its isotopic composition during its journey through the soil, including the mixing and storage of different water sources.

Focusing on the partitioning and mixing of water in the Critical Zone, Earth's outer skin, WATSON aims at collecting, integrating, and synthesizing current interdisciplinary scientific knowledge based on water stable isotopes. WATSON will prepare protocols for standardized sampling and analysis procedures and will produce a critical assessment of available isotope-based models, in addition to the open-access database of isotope-based studies in the Critical Zone, which is already available online.

These outputs have the potential to benefit different sectors, including the food sector, by improving the knowledge of the processes influencing the hydrogen and oxygen stable isotope signature in plants.

WATSON is an international network of researchers and stakeholders funded by the European Cooperation in Science and Technology (COST) program. WATSON facilitates interactions between scientists and stakeholders by fostering communication and collaboration. It organizes hybrid meetings and online webinars. WATSON also organizes training schools and financially supports short-term scientific missions. We welcome the participation of European researchers and stakeholders interested in taking advantage of the stable isotope techniques. For more information, see https://watson-cost.eu/.
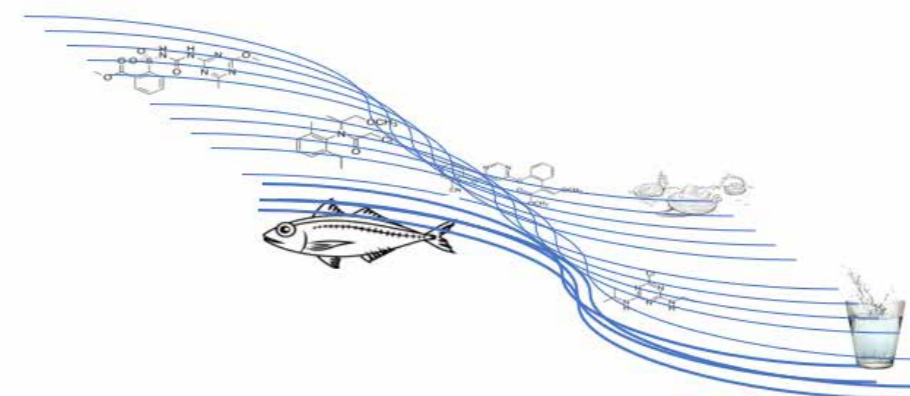
Acknowledgements

Chemical Safety of Freshwater for Human Consumption and Aquatic Life which also is Food

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Water is the most valuable resource of the XXIst century. It is crucial for life survival as we know, but droughts and contamination threaten its worldwide use. Although microbial contamination of water is a major concern, the current potabilization practices, when available, ensure a safe water usage for human activities. But what about chemical contamination? The occurrence of chemicals in water is a major concern as the use of chemically contaminated water can have unexpected consequences for life and environment. Anthropogenic water contamination occurs through the different human activities, either agricultural, urban or industrial. An enormous amount of chemicals is reaching the surface waters, fresh or marine, most of them at minute concentrations, but of unknown consequences for life. In agroecosystems, most chemicals are pesticides used in agriculture. Among them, not only the currently used pesticide as well as some banned POPs could be found. The presence of such compounds should be seen in al least two aspects: Water as food and water as the media were edible aquatic species live and could contaminate with it. For the safe use of potable water, pesticide level must be at the ng/kg level. Despite POPs and other pesticides are regulated, new compounds are added every year to the list of possible agrochemicals whose presence has not been legally considered in potable water yet. On the other hand, the presence of such compounds is not only a threat for aquatic life, but also in the case of edible fish and mussels, the residues content in the food is seldom investigated and not ruled at all.

Urban and industrial wastes thrown to freshwater sources, at molecular level contain "emerging contaminants", compounds whose effects at trace level are unknown. Pharmaceuticals, pesticides and personal care products, among other chemicals can be present. They occur as cocktails of many compounds, making the toxicological evaluation hard to perform.

The first step to assess their presence is through highly sensitive chromatography tandem mass spectrometry. techniques. Specific strategies to reach ppt levels for pesticide residues in water will be presented as well as methodologies to detect them in edible aquatic animals, focusing in the lack of global extraneous MRL (e-MRL) as proposed by the Codex Alimentarius. Finally, the presence of emerging contaminants in rivers and catchments will be presented, showing their ubiquity not only in Uruguay, but also all over the world.

Acknowledgements

**References**

[1] C. Rodriguez-Bolaña et al (2023) *Science of The Total Environment*, 874, 162310.

[2] J. L. Wilkinson et al (2022) *Proceedings of the National Academy of Sciences of the United States of America*, 119 (8), e2113947119.

[3] F. Jesús et al (2018) *Talanta*, 178, 410-418.

HRMS Strategies for the Target Analysis and Suspect Screening of Persistent and Mobile Substances in Drinking, Ground and Surface Water of Greece

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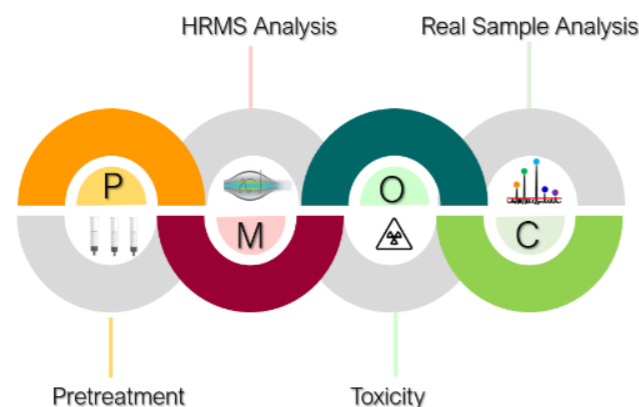
Persistent and mobile (PM) substances are being accepted as serious threats to groundwater and drinking water resources [1]. These compounds lack a single generic definition, but are identified by their resistance to environmental degradation, their affinity for water, and, when toxic, their negative effects on human health and the environment [2]. Substances like PMs are frequently challenging to analyze, tough to remove during water treatment, and eventually also tend to accumulate in aqueous media. However, there is controversy among scientists regarding the requirements that must be satisfied for a compound to be prioritized as a PM. Thus, it is considered vital to develop a method to determine as many compounds as possible (PM or noPM) in the water cycle, in order to evaluate their potential environmental hazard behavior.

In this study, the sample preparation was based on solid phase extraction, while instrumental analysis was performed by highly sensitive liquid chromatography Orbitrap–high-resolution mass spectrometry. Specifically, three different extraction materials two different columns three different mobile phases, and MS parameters had been tested. Method performance was excellent in terms of linearity ($R^2 > 0.99$), detection/quantification limits (reaching even at pg L^{-1} scale), and recoveries (70–120 %, for most of the compounds).

After optimization and evaluation, the most suitable workflow was applied to real samples to explore the putative annotation of the tested PM substances with high confidence for the first time in Greece. The strategy of suspect screening was also followed to identify PM and potentially PM substances, in previously analyzed samples. For the three studied matrices,

the maximum concentrations were estimated at 843 ng/L for diuron in surface water from river Axios and at 465 ng/ L for climbazole in groundwater from Northern Greece and 1.5 ng/L for PFOA in drinking water from the area of Thessaloniki. According to the results, several substances that are not registered to current REACH regulation as PM substances and they are not even included in the potentially revised list have alarming concentrations and detection frequency (e.g. fluconazole >1000 ng/L in groundwater samples).

The aim of the aforementioned methodology is to clarify the persistency (through their detection frequency and concentrations) of the studied compounds, and finally come to a conclusion regarding the final prioritization of them as PM substances, through actual monitoring studies and not only based on theoretical values.

References

[1] M. Scheurer et al. (2022) *Water Supply,* 22 (2), 1575-1592.

[2] T. Reemtsma et al. (2016) *Environmental Science and Technology,* 50 (19), 10308-10315.

[3] L. F. Angeles, D. S. Aga (2020) *Trends in Environmental Analytical Chemistry,* 25, e00078.

Origin, Implications, and Management Strategies for Nitrate Pollution in Vulnerable Zones in Andalusia

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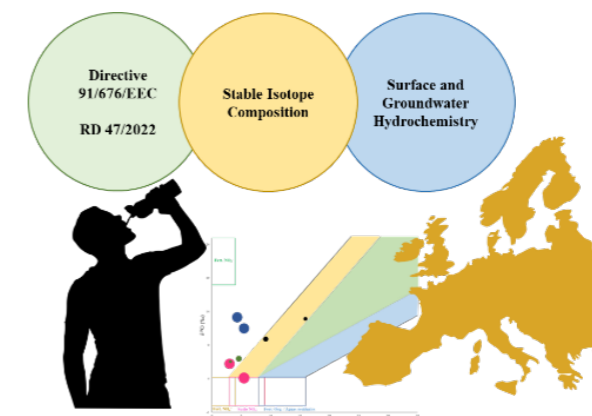
Diffuse-water pollution caused by nitrates from human activities has been identified as a major cause of the poor state of both surface and groundwater bodies. Thus, excessive amounts of nitrates in water increase the risk to human health. In an effort to protect waters against nitrates contamination from human activities sources, and act preventively against it, the European Union introduced the Directive 91/676/EEC.

Recently, the EU has claimed Spain to update the information regarding the vulnerable areas to nitrate pollution and to take the appropriate measures to reduce nitrate levels in these areas (RD 47/2022). Different vulnerable zones in Andalusia were chosen to study the contamination levels and their origin. It is essential to determine the sources of contamination, as well as the natural attenuation processes. Stable isotope ratios of nitrates and sulphates have been successfully used to trace the main origin of pollution in different contexts, overcoming the main disadvantages of other methods as indicated by other authors [1-3].

Moreover, it is important to transfer results and provide specific training to both farmers and technicians to improve decision-making in irrigation management and fertilization, for instance.

Thus, this project proposes the development of demonstrative irrigation and fertilization pilot programs in areas vulnerable

to nitrate contamination in Andalusia. Lastly, the project aims to contribute to the design, calibration, and validation of the FaST (Farming Sustainable Tool) tool, which was proposed by the European Commission to calculate the N, P, and K needs of crops.

References

[1] N. Otero, C. Torrentó, A. Soler, A. Menció, J. Mas Pla (2009) *Agriculture Ecosystems and the Environment,* V133, 103-113.

[2] L. Vitòria, A. Soler, À. Canals, N. Otero (2008) *Applied Geochemistry,* 23 (12), 3597-3611.

[3] R. Carrey, E. Balleste, R. Blanch, F. Lucena, P. Pons, J. M. López, M. Rull, J. Sola, N. Micola, J. Fraile, T. Garrido, T. Munné, A. Soler, N. Otero (2021) *Water Research,* 188, 1165437.
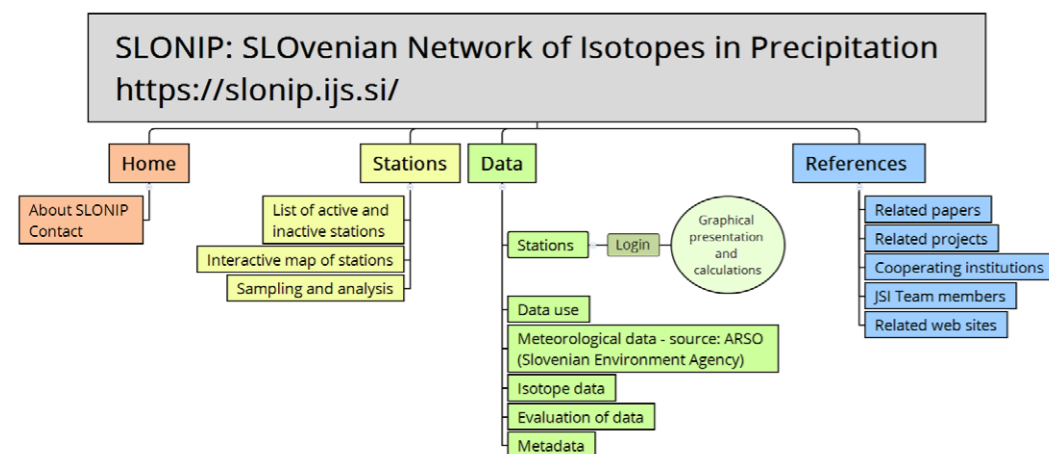
SLONIP – A Slovenian Web-based Interactive Research Platform on the Water Isotopes in Precipitation

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Water is crucial for life and moves through the Earth's four main spheres as part of the water cycle via evaporation, condensation, precipitation, interception, transpiration, infiltration, percolation, runoff and storage. The natural water cycle is also influenced by anthropogenic activities. Water molecules, as they move through the water cycle, can be traced using the hydrogen and oxygen isotopes. During the cycling, the concentrations of oxygen and hydrogen isotopes undergo changes that result according to the history and route of the molecules through the water cycle in characteristic isotopic fingerprints in particular components.

Precipitation is of major interest in the water cycle as it is the ultimate, primary source of water to catchments surface, soil and groundwater as well as to vegetation. Therefore, information about the changes in amount, mode and isotopic composition of precipitation are essential also for food and beverages authentication and traceability, particularly in geographically, climatologically and geologically diverse regions like Slovenia.

Here we present the Slovenian Network of Isotopes in Precipitation (SLONIP), a web-based interactive research platform available at https://slonip.ijs.si/ that collects existing, accessible data on the isotopic composition of precipitation in Slovenia [1], thereby improving access to the data for scientific research groups and other interested users. The SLONIP platform currently contains isotope data (i.e. δ^2H, δ^{18}O, ^{3}H and deuterium excess data) of monthly composite precipitation from eight locations obtained from various investigations performed since 1981. The platform is in line with FAIR data concepts, and the functionalities and tools

available are valuable for lay people interested in isotope precipitation data but not experienced in data treatment, analysis and visualization. Moreover, "raw" data is available to the scientific community upon request. The structure of the code for the interactive SLONIP platform is based on Django, i.e., a server-side web framework written in the programming language Python.

SLONIP provides locations, sampling and analysis (including references) information and presents the data in numerical and graphical form, including monthly, seasonal, and annual means and different local meteoric water lines, all calculated using a Python code made freely available on GitHub (https://github.com/nyuhanc/Isotopes-in-precipitation-statistics). The SLONIP platform and the offline Python script can be an excellent basis for further use of isotopes in water resources research, studying the effects of climatic factors on isotopes in precipitation, water vapour source, and inflow corridors of precipitation as well as in food and beverages authentication and traceability for interpretation of geographical origin or fraudulent practices.

References
[1] P. Vreča, A. Pavšek, D. Kocman (2022) Water, 14, 2127.

δ^{18}O and δ^2H Fingerprinting of Tap Water

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Stable water isotopes (δ^{18}O and δ^2H) are powerful tools for tracers of water cycling processes [1]. The isotopic composition of precipitation reflects climatic and geographic patterns, which are then propagated to surface water and groundwater [2, 3]. However, water in human-managed environments does not usually follow the expected variations observed in nature. Thus, there is a need for new methods and technologies requiring minimal information about the system. In this regard, monitoring stable isotopes of oxygen and hydrogen could provide water managers with a complementary approach to understanding the sources and mixing within the water supply and storage system [4].

We tested this hypothesis in Ljubljana, where the first urban-scale spatiotemporal isotope investigation of tap water was performed during three sampling campaigns in March 2019, September and December 2021. The research objective was to collect 100 tap water samples over one-hour sampling period with the help of volunteers. Based on the 273 samples collected, a spatiotemporal characterization of the isotopes in tap water was made, from which the correct positions of the water supply areas were determined.

All water samples were analyzed using a dual inlet isotope ratio mass spectrometer) with an automated H_2-H_2O and CO_2-H_2O equilibrator HDOeq48 Equilibration Unit (custom-built by M. Jaklitsch).

Tap water samples were obtained from 145 different participants during three sampling campaigns. The observed variations in δ^{18}O and δ^2H were small, but they showed a clear spatiotemporal variation, which was statistically significant. Observed seasonal changes in isotopes between aquifer and

tap mean that the residence time is low as the water moves through the system quickly with obvious mixing between sources. Also, the distinction between different water supply areas and their sources was significant for ome areas. Based on the spatial presentation of the data, the existing division among different water supply areas confirms the assumptions made by the water supply managers for most samples, while on the borders, their characterization was incorrect.

The results demonstrate the potential of water isotopes for studying and monitoring water supply systems at a finer scale and how the isotopic composition of the water can be used to distinguish multi-source tap water systems, identify the origin of the water, and trace the water's pathway through the system.

References
[1] G. J. Bowen, Z. Cai, R. P. Fiorella, A. L. Putman (2019) *Annual Reviews*, 47, 453-479.
[2] K. Nagode, T. Kanduč, S. Lojen, B. B. Železnik, B. Jamnik, P. Vreča (2020) *Geologija*, 63, 251-270.
[3] K. Nagode, T. Kanduč, S. Lojen, B. B. Železnik, B. Jamnik, P. Vreča (2022) *Water*, 13, 2064.
[4] B. Tipple idr. (2017) *Water Research*, 119, 214-224.

Incidence of Nitrate Contamination and Origin in Two Water Systems of Southern Spain

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The increasing worldwide nitrate contamination on surface and groundwater systems from the human activities (including intensive agricultural practices and animal farming) is considered a central problem in the environmental and agricultural policies of the European Union. Thus, the Directive 91/676/EEC is focused on the protection of water pollution caused by nitrates from human activities and establishing Codes of Good Agricultural Practices.

In Spain, approximately 20% of the surface has been designated as a vulnerable zone; while in Andalusia the area is even higher (25.52%). Due to this high percentage of contaminated aquifers, it has recently been established an updated legislation (RD) 47/2022, where it is established that concentrations higher than 25 mg/L and 37.5 mg/L in surface and groundwater, respectively, are affected by nitrate pollution.

In this research 80 samples were collected from two characteristics water systems located in southern Spain: the largest intensive horticultural crop zone in Europe (Almeria), and one of the main important Andalusian olive trees cultivation sited in the upper Guadalquivir river (Cordoba). In addition, the collection was carried out during two periods (May-June and October-November) of the 2021 and 2022 campaigns.

The results showed that the 56% of the water samples exceeded the limit concentrations established in the law (RD 47/2022) showing a range between 27.6 and 297.2 mg/L. The data obtained from the isotopic analysis of nitrates (δ^{15}N-NO3 and δ^{18}O-NO$_3$) and sulfates (δ^{34}S-SO$_4$ and δ^{18}O-SO$_4$), made it possible to assign the different sources of contamination from nitrates in the water samples analyzed. The wide variation range values of δ^{15}N-NO$_3$- (from +0.5 to +85.4 ‰) revealed anthropogenic source signals due to the use of fertilizers in several sampling points. These values were highly correlated with the δ^{18}O-NO$_3$- ones (from +2.1 to +54.2 ‰). The isotopic data of sulfates allowed to identify natural sources from evaporitic rocks (δ^{34}S > 12 ‰), and anthropic sources, i.e., fertilization with sulfates (δ^{34}S < 12 ‰). The results of this study demonstrated the potential of stable isotopes to determine the contamination sources in water bodies previously indicated by other authors [1,2].

Acknowledgements

References

[1] N. Otero, C. Torrentó, A. Soler, A. Mencíó, J. Mas Pla (2009) *Agriculture Ecosystems and the Environment*, V133, 103–113.

[2] R. Carrey, E. Balleste, R. Blanch, F. Lucena, P. Pons, J. M. López, M. Rull, J. Sola, N. Micola, J. Fraile, T. Garrido, T. Munné, A. Soler, N. Otero (2021) *Water Research*, 188, 1165437.

Authentication of Almond Geographical and Varietal Origin Using Triacylglycerol Fingerprinting: a Prospective Investigation

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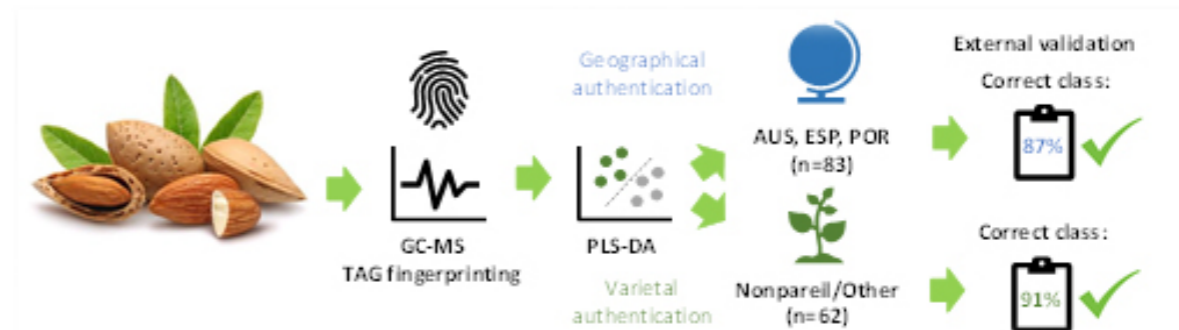
Almonds (*Prunus dulcis* (Mill.) D.A. Webb), renowned for their exceptional nutritional value and sensory attributes. Almond production is of great economic importance, especially for the leading producer countries such as the USA, Spain, and Australia, among others [1]. The price of almonds on the market fluctuates greatly based on their cultivar and provenance, rendering them vulnerable to fraudulent activities that involve counterfeiting their geographical and botanical origin. Consequently, it is of utmost importance to have reliable verification tools to ensure the authenticity of almonds, to safeguard consumer.

Recent research has shown that the triacylglycerol (TAG) composition of almonds, which is influenced by both genetic and environmental factors [2], can be an effective tool for verifying the geographical and varietal origin of almonds. In terms of data treatment and chemometric analysis, untargeted approaches such as profiling and fingerprinting methods have been utilized as an alternative to the traditional targeted approach [3]. These methods have the potential to incorporate a larger amount of analytical information than conventional targeted methods, making them valuable in the authentication process.

The aim of this prospective study was to evaluate the suitability of TAG fingerprinting by gas chromatography-mass spectrometry coupled to pattern recognition analysis for the authentication of both the geographical and varietal origin of almonds.

With this scope, the TAG chromatographic fingerprints of raw, traceable almonds of two sample sets produced in 2019-2021 were used to develop two partial least square-discriminant analysis (PLS-DA) models to classify almonds according to the country of origin or to the cultivar. For the origin model, 'Nonpareil' almonds (n=83) from Spain, Portugal and Australia were used, while in the cultivar model, Spanish almonds (n=62) belonging to the 'Nonpareil' cultivar were discriminated form almonds of other cultivars.

In each case, the sample set was randomly split into a training set (80%) and a validation set (20%). The models developed with the training sets were externally validated by classifying the samples of the validation sets. This process was performed three times (three iterations).

The results of the external validation provided overall correct classifications of 87% and 91% according to the country and the cultivar of origin, respectively.

Acknowledgements

References

[1] FAOSTAT. (n.d.). Retrieved April 13, 2023, from https://www.fao.org/faostat/en/#home

[2] A. Beltrán-Sanahuja, S.E. Maestre-Pérez, N. Grané-Teruel, A. Valdés-García, M.S. Prats-Moya (2021) *Foods*, 10, 153.

[3] N.Z. Ballin, K.H. Laursen (2019) *Trends in Food Science & Technology*, 86, 537–543.

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