XXII euro FOOD chem

BOOK OF ABSTRACTS

XXII Congress

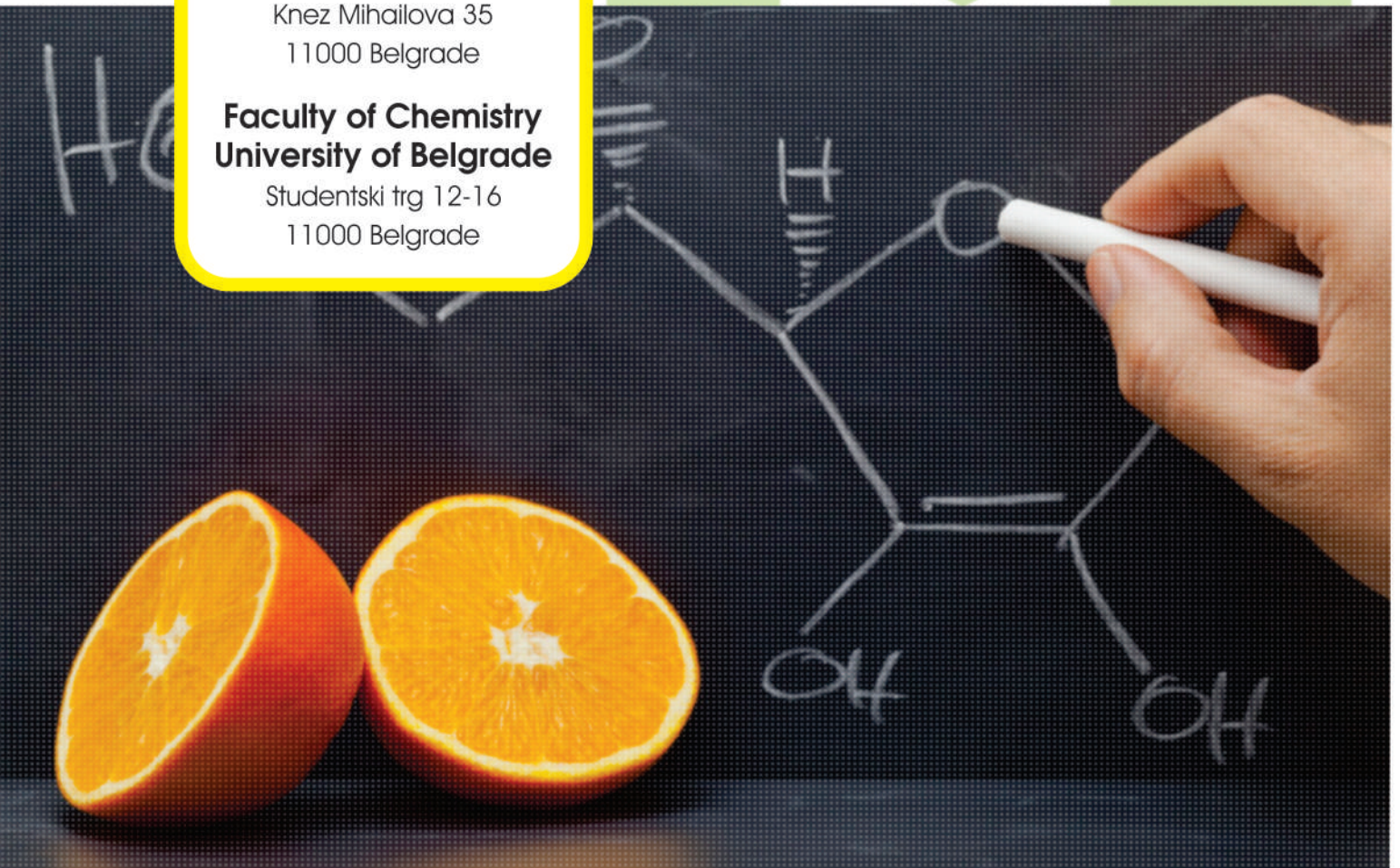
EuroFoodChem

June 14-16, 2023 | Belgrade, Serbia

https://xxiieurofoodchem.com
congress2023@xxiieurofoodchem.com

Serbian Academy of Sciences and Arts
Knez Mihailova 35
11000 Belgrade

Faculty of Chemistry University of Belgrade
Studentski trg 12-16
11000 Belgrade



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- Food adulteration, authenticity and profiling
- Food contaminants: POPs (dioxins, PCBs, etc.), pesticide residues, food contact materials, mycotoxins and other biotoxins, vet drug residues, microplastics and nanoplastics
- Food safety, composition and quality
- Inspecting chemical changes in food under processing and storage
- Food development and processing applications through rheology and extrusion
- Food imaging data analysis for gaining insights into the relation of microstructure to properties needed in food engineering, chemistry, microbiology, and safety

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XXII euro FOOD chem

XXII Congress EuroFoodChem

June 14-16, 2023
Belgrade, Serbia

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Under the auspices

Ministry of Science, Technological Development and Innovations, Republic of Serbia

Serbian Academy of Sciences and Arts

Organizers

EuChemS, Division of Food Chemistry

Serbian Chemical Society

WELCOME ADDRESSES

Dear Colleagues and Friends,

On behalf of the Food Chemistry Division of EuChemS and Serbian Chemical Society with support of the Serbian Academy of Sciences and Arts, I am delighted to welcome all the experts from different countries to Belgrade, Serbia to XXII EuroFoodChem.

Following the previous successful meetings of EuroFoodChem since 1981, Belgrade is for the first time honored to host this important international gathering in the field of food chemistry.

After a very successful virtual edition in 2021, we are thrilled to organize a face-to-face meeting again.

The Congress program offers both exciting recent trends in food chemistry research and engaging networking opportunities that we all have missed over the last couple of years. In addition to abstract presentations and lectures by world renowned speakers, we will be offering a variety of networking options. The EuroFoodChem is an excellent opportunity for initiating or strengthening cooperations and knowledge.

For centuries Serbia has been strategically the most important region in the Balkans; many conquerors fought for this piece of land and left their own traces in time and space. We can only hope that the rich and tightly packed scientific program will allow you to explore the capital of Serbia and historical places nearby.

Serbia is a country of diversities and the city of Belgrade, as a place of intersection of different cultures and history, is the most beautiful example of it. Wine making has a long tradition in Serbia and it is now experiencing its renaissance. Vineyards have been a part of the diverse Serbian landscape since before the times of Romans. Belgrade is also a new hot spot on the European gastronomical map. In a city with so many historical influences, tradition intertwines with innovation.

I would also like to thank all of you who have worked with devotion on putting up this meeting together. On behalf of all of us involved in the event preparation, I wish you a great time at EuroFoodChem, and thank you for your participation and contribution to the high scientific quality of the event.

Hope that you will find the Congress and your stay in Belgrade valuable, enjoyable, and memorable!

Congress Chairman

Tanja Ćirković Veličković

COMMITTEES

Chair: Tanja Ćirković Veličković, Corresponding Member of Serbian Academy of Sciences and Arts

SCIENTIFIC COMMITTEE

Joana Amaral (Portugal) – Chair, EuChemS-FCD
Marco Arlorio (Italy) – Past-Chair, EuChemS-FCD
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Jelena Mutić (University of Belgrade – Faculty of Chemistry, Serbia)
Jelena Trifković (University of Belgrade – Faculty of Chemistry, Serbia)
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Elizabet Janić Hajnal (University of Novi Sad, Institute of Food Technology in Novi Sad, Serbia)
Danijela Kostić (University of Niš, Faculty of Sciences and Mathematics)

CONGRESS TOPICS

- **Food composition, quality, and safety**
- **Food sustainability, including byproducts valorization**
- **Novel foods**
- **Food and health, functional foods, and ingredients**
- **Chemical reactions and interactions of food components**
- **Chemical changes in food under processing and storage**
- **Food adulteration, authenticity, and traceability**
- **Novel methods for food chemistry**
- **Food contaminants**

GENERAL INFORMATION

Official Language:
English. No simultaneous translation will be provided:

Registration Desk opening times.

Day 1: June 14, 2023, 8:30-10:30h
Day 2: June 15, 2023, 8:30-10:30h
Day 3: June 16, 2023, 8:30-10:30h

The Registration Desk is situated in Serbian Academy of Sciences and Arts
Knez Mihailova 35, 11000 Belgrade

LOCAL ORGANIZER

PCO – ARIA Conference & Events doo
Karadjordjev trg 34 – 11080 Zemun – Belgrade, Serbia
Office: + 381 11 2600 978
Mail: office@aria.co.rs
www. ariaconference.com

Liability and Insurance: Neither the Food Chemistry Division of EuChemS nor the local organizers will assume any responsibility whatsoever for damage or injury to persons or property during the Congress. Participants are recommended to arrange for their personal travel and health insurance.
Certificate of Attendance: Will be given at the registration desk and sent by email after the end of the Congress.

ABOUT BELGRADE

With a 7.000-year history, Belgrade is one of the oldest cities in Europe and the meeting point for centuries among different civilizations. Dynamic and vibrant two million people city it is the capital of Serbia, located at the confluence of the rivers Sava and Danube, where the Pannonian Plain meets the Balkan. Discover magnificent nature, monasteries, archaeological sites, wineries, ethno villages, and spas.

BELGRADE FORTRESS

Kalemegdan is Belgrade's central park and fortress complex lying on a hill overlooking the Sava and Danube confluence, on the eastern side of the river Sava. This has been the sight of the ancient Roman city of Singidunum, the medieval and Turkish era Belgrade and was converted into a park in the mid-19th century. It is home to several galleries and museums, restaurants, sports courts, and the Belgrade Zoo.

SKADARLIJA

It is in the heart of Belgrade, the old, romantic and bohemian, known in history and legend, and it lives more than a century in many songs, interesting stories, and anecdotes as well as many literary and journalistic writings. The history of Skadarlija began in the 1830s with the settlement of Gypsies in the abandoned trenches in front of the ramparts. The 1854 town plan of Belgrade reveals that the Gypsy hovels had been replaced by brick buildings into which artisans, caterers, petty clerks, and others moved. The whole locality was referred to as the Gypsy Quarter until 1872 when it was named Skadarlija and it kept that name until the present day. Skadarlija changed its name once in the meantime, during the Austro-Hungarian occupation, when it was changed to The Rose Street. And that's the only time the name of the street was changed for a short period of time. Today Skadalija is one of the key tourist attractions of Belgrade. It is under state protection as a cultural monument. It has its own code and its own flag, with the symbols of a walking stick, carnation, and a hat. In this neighborhood there are a handful of restaurants where you'll be able to really experience the traditional Serbian cuisine. There are also galleries, antique shops, and souvenir shops.

ROYAL PALACE

The Royal Palace was built between 1924 and 1929 with the private funds of His Majesty King Alexander I (the grandfather of HRH Crown Prince Alexander). The architects were Zivojin Nikolic and Nikolay Krassnoff of the Royal Academy. The palace is made of white stone in the Serbian-Byzantine style. Attached to The Royal Palace there is a Royal Chapel dedicated to Saint Apostle Andrew the First-Called, the Patron Saint of The Royal Family. The Royal Palace was the home of King Alexander I and King Peter. Today The Royal Palace is the home of Crown Prince Alexander II and his family.

TEMPLE OF SAINT SAVA

It is located on the Vračar plateau, on the eastern part of the Svetosavski Trg square in Belgrade which is believed to be the location where the remains of Saint Sava were burned in 1595 by Ottoman Grand Vizier Sinan Pasha in order to break the Serbian spirit. The Church of Saint Sava is not only the largest Serbian Orthodox church, it is the largest Orthodox place of worship in the Balkans and one of the largest Orthodox churches in the world. It holds a special place in the hearts of Belgraders. It is a gathering spot, a safe refuge, and an important place of support for all those in need. In between its walls the residents of Serbia's capital share their joy and their sorrow, and celebrating important holidays in front of it has become a tradition a long time ago.

PRACTICAL INFORMATION

Climate: Weather in June
The weather in Belgrade in June is quite pleasant this month. The thermometer averaged maximum of 28° C. In the morning the temperature drops to 17° C. Thus, the mean temperature average in June in Belgrade is 22° C. On this month of June, day length in Belgrade is generally 15:35. The sun rises at 04:51 and sunset is at 20:26
Time Zone: Belgrade and Serbia are in the Central European time zone region – GMT +1
Water: Water in Belgrade is safe to drink.
Currency
RSD – Serbian Dinar
1 EUR ≈ 120 RSD

What additional to see in Belgrade in brief
The National Museum, Residences of Princess Ljubica and Prince Miloš, Nikola Tesla Museum, House of Flowers, Cathedral, St Sava's Church, St Mark's Church, Church of the Holy Virgin Mary – Ružica, Church of Christ the King, Synagogue and Bayrakli Mosque, Ada Ciganlija, Zemun and Gardos, Kalemegdan.
For any other information visit following websites:
Tourist Organization of Belgrade: http://www.tob.rs/

How to get from Belgrade Nikola Tesla airport to the city center
Transfer Options from Belgrade Airport to the City Center:
There are 3 transportation options to get from Belgrade Nikola Tesla Airport (BEG) to the City Center: **private car transfer, taxi, public bus**. Distance from Belgrade Nikola Tesla Airport to the city center is about 20 km. The fastest option is taking a car transfer. Trip takes around 30 minutes by car and cost between 25-30 Euros. The Cheapest option is the Bus and takes around 60 minutes to reach the city center.
Near airport exit gate there is bus station for "Mini Bus A1". Mini bus A1 operate between airport and Slavija Square. Bus stop also in New Belgrade (Fontana stop) and near Belgrade main train station.

Important telephone numbers:
Emergencies
Police - 192,112
Fire department - 193
Ambulance -194

Information for oral and flash presentations
Plenary lectures - 45 minutes / Serbian Academy of Sciences and Arts
Knez Mihailova 35, 11000 Belgrade
Keynote lectures - 30 minutes / Serbian Academy of Sciences and Arts
Knez Mihailova 35, 11000 Belgrade
Oral presentations - 15 minutes / Faculty of Chemistry, University of Belgrade
Studentski trg 12-16, 11000 Belgrade

Flash presentations - 5 minutes / Faculty of Chemistry, University of Belgrade
Studentski trg 12-16, 11000 Belgrade

Technical Equipment is available for all presentations.
Preview Desk: All speakers should give their presentation 15 minutes before their session at the presentation hall. Please make sure that your presentation is fully operational. Only presentations on USB Memory devices will be accepted.

Information for Poster Presenters
Please note that only paper posters will be accepted.
Presenting authors are requested to be available for short presentations and discussion of their posters during the allocated Poster Walk. Poster board is 95 cm width & 238 cm height. Recommended dimensions for posters 80 x 120 cm.

SOCIAL PROGRAM

WELCOME RECEPTION, June 14, 2023
THE ILIJA M. KOLARAC ENDOWMENT
Studentski trg 5, 11000 Belgrade

CONGRESS DINNER
June 15th, 2023 at restaurant Velika Skadarlija, Skadarska 40d
Dress code: Casual

PROGRAM AT A GLANCE

Day 1: June 14, 2023

8:30-10:30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **OPENING CEREMONY**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

FOOD CHEMISTRY DIVISION AWARDS SESSION
Moderators: Joana Amaral and Michael Murkovic

9:30-10:15 **Plenary Lecture 1**
Czedik-Eysenberg-Lecture
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Vitamins – food chemistry research vs information needed in nutrition research
Prof. Vieno Piironen

10:15-10:45 **Keynote Lecture 1**
Junior award talk
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Improvement of olive oil flavor and bioactive composition by optimizing industrial extraction using taste sensor devices
Itala Marx

10:45-11:30 **Coffee break**

POSTER PRESENTATIONS (T1 and T9)
Faculty of Chemistry, Studentski trg 12-16

11:30-12:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T1-Food composition, quality and safety
Hall 1-Main Chemical Amphitheater
T9-Food contaminants
Hall 2-Small Chemical Amphitheather Dräger

12:30-13:30 **Lunch**
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

13:30-14:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T1-Food composition, quality and safety
Hall 1-Main Chemical Amphitheater
T9-Food contaminants
Hall 2-Small Chemical Amphitheather Dräger

14:30-15:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T8-Novel methods for food chemistry
Hall 1-Main Chemical Amphitheater
T5-Chemical reactions and interactions of food components
Hall 2-Small Chemical Amphitheather Dräger

15:30-16:15 **Coffee break**

POSTER PRESENTATIONS (T1 and T5)
Faculty of Chemistry, Studentski trg 12-16

TOPIC: EMERGING CONTAMINANTS
Moderators: Tanja Ćirković Veličković and Irena Vovk

16:15-17:00 **Plenary Lecture 2**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Food and packaging – a symbiosis?
Prof. Thomas Gude

17:00-17:30 **Keynote Lecture 2**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Micro- and nanoplastics in food current analytical methods and challenges
Prof. Ralf Greiner

18:00 **WELCOME RECEPTION**
The Ilija M. Kolarac Endowment, Studentski trg 5

Day 2: June 15, 2023

8:30-10.30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **Vendors slot**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

PROANALYTICA
How do we overcome challenges between sampling and analysis?
Goran Đokić

SHIMADZU
Analytical Methods for determination of Contaminants in Food and Food Packaging
Uwe Oppermann
Shimadzu Europa GmbH, Duisburg, Germany

TOPIC: FOOD AND HEALTH
Moderators: Hans-Jacob Skarpeid and Vieno Piironen

9:30-10:15 **Plenary Lecture 3**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

A Foodomics study on the activity of bioactive compounds from plants, algae and agrifood by-products against Alzheimer disease
Prof. Alejandro Cifuentes

10:15-10:45 **Keynote Lecture 3**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Overview on analytical methods for allergen control in foods and compliance with the proposed reference doses
Prof. Linda Monaci

10:45-11:30 Coffee break

POSTER PRESENTATIONS (T4 and T8)
Faculty of Chemistry, Studentski trg 12-16

11:30-12:30 **ORAL PRESENTATION**
Faculty of Chemistry, Studentski trg 12-16

T4-Food and health, functional foods and ingredients
Hall 1-Main Chemical Amphitheater
T5-Chemical reactions and interactions of food components
Hall 2-Small Chemical Amphitheather Dräger

12:30-13:30 Lunch
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

13:30-14:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T4-Food and health, functional foods and ingredients
Hall 1-Main Chemical Amphitheater
T5-Chemical reactions and interactions of food components
Hall 2-Small Chemical Amphitheather Dräger

14:30-15:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T6-Chemical changes in food under processing and storage
Hall 1-Main Chemical Amphitheater
T7-Food adulteration, authenticity and traceability
Hall 2-Small Chemical Amphitheather Dräger

15:30-16:15 Coffee break

POSTER PRESENTATIONS (T6, T7 and T8)
Faculty of Chemistry, Studentski trg 12-16

TOPIC: CHEMICAL CHANGES IN FOOD UNDER PROCESSING AND STORAGE
Moderators: Thomas Gude and Cristina Todasca

16:15-17:00 **Plenary Lecture 4**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Process-induced reactions in food: Glycation, lipation and beyond
Prof. Thomas Henle

TOPIC: FOOD SAFETY

17:00-17:30 **Keynote Lecture 4**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Essential oils antimicrobial activity and their role in food safety
Prof. Miroslava Kacaniova

20:00 **CONFERENCE DINNER**
Restaurant Velika Skadarlija, Skadarska 40d

Day 3: June 16, 2023

8:30-10.30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **Vendors slot**
ANALYSIS
Proteomics in food - from farm to fork
Luka Mihajlović, PhD

TOPIC: FOOD SUSTAINABILITY AND BYPRODUCTS VALORIZATION
Moderators: Livia Simon Sarkadi and Zuzana Ciesarová

9:30-10:15 **Plenary Lecture 5**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Food Sustainability and byproducts valorization
Prof. Manuela Pintado

10:15-10:45 **Keynote Lecture 5**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

We know how to extract proteins from rapeseed – NapiFeryn BioTech for food sustainability
Prof. Anna Chmielewska

10:45-11:30 **Coffee break**

POSTER PRESENTATIONS (T3)
Faculty of Chemistry, Studentski trg 12-16

11:30-12:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16
T2-Food sustainability, including byproducts valorization
Hall 1-Main Chemical Amphitheater
T3-Novel foods
Hall 2-Small Chemical Amphitheather Dräger

12:30-13:30 **Lunch**
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

Vendors slot
Hallway, ground flor, Faculty for Chemistry, Studentski trg 12-16
ANALYSIS
High resolution mass spectrometry in food analysis
Luka Mihajlović, PhD

TOPIC: NOVEL METHODS FOR FOOD CHEMISTRY
Moderators: Slavica Ražić and Joana Amaral

13:30-14:15 **Joint Session DAC-DFC EuChemS / Plenary 6**
Faculty of Chemistry, Studentski trg 12-16
New analytical solutions for the global challenges of food safety
Prof. Rudolf Krska

14:15-14:45 **Joint Session DAC-DFC EuChemS/ Keynote 6**
Faculty of Chemistry, Studentski trg 12-16
Understanding food aromas: in the frontier between sensorial and analytical data
Prof. Silvia Rocha

14:45-15:45 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16
T8-Novel methods for food chemistry
Hall 1-Main Chemical Amphitheater
Flash presentations
Hall 2-Small Chemical Amphitheather Dräger

15:45-16:45 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16
T8-Novel methods for food chemistry
Hall 1-Main Chemical Amphitheater
Flash presentations
Hall 2-Small Chemical Amphitheather Dräger

16:45-17:30 **Coffee break**

POSTER PRESENTATIONS (T2, T8 and T3)
Faculty of Chemistry, Studentski trg 12-16

17:30 – 18:00 **CLOSING CEREMONY**
Faculty of Chemistry, Studentski trg 12-16

Day 4, June 17, 2023

POST-CONGRESS TOUR:
Archaeological Park Viminacium

SCIENTIFIC PROGRAM

Day 1: June 14, 2023

8:30-10:30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **OPENING CEREMONY**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Academician Zoran Popović, Vice-President of SASA
Prof. Dušan Sladić, President of Serbian Chemical Society
Dr Marina Soković, Ministry of Science, Technological Development and Innovations, Republic of Serbia
Prof. Tanja Ćirković Veličković, Congress Chair, corresponding member of SASA

FOOD CHEMISTRY DIVISION AWARDS SESSION
Moderators: Joana Amaral and Michael Murkovic

9:30-10:15 **Plenary Lecture 1**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Czedik-Eysenberg-Lecture
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Vitamins – food chemistry research vs information needed in nutrition research
Prof. Vieno Piironen

10:15-10:45 **Keynote Lecture 1**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Junior award talk

Improvement of olive oil flavor and bioactive composition by optimizing industrial extraction using taste senior devices
Itala Marx

10:45-11:30 *Coffee break*

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

T1 - Food composition, quality and safety
Moderators: Ivana Prodić and Petar Ristivojević

PP 1 **Khalfalla Maha, Zoltan Győri, Zsombik Laszlo**
A comparative study of Ca and Fe concentrations in Hungarian proso millet grains (*Panicum miliaceum L.*) varieties

PP 2 **Valentina Nikolić, Slađana Žilić, Marijana Simić, Vesna Kandić, Beka Sarić, Danka Milovanović, Snežana M. Jovanović**
Chemical composition, antioxidant properties, and in vitro digestibility of flour and ground hulls of differently coloured oat varieties

PP 3 **Alžbeta Hegedűsová, Ivana Mezeyová, Marcel Golian**
Carotenoid profile of the pulp of selected musk gourd varieties

PP 4 **Hiroko Seki**
Quality differences among tomato varieties

PP 5 **Grünwald Monna, Francis G, Esatbeyoglu T.**
Comparison of the nutritional composition of different cultivars of the edible variety of *Jatropha curcas* (L.) - An untapped protein source

PP 6 **Gracia Patricia Blanch, Sonia de Pascual-Teresa, María Luisa Ruiz del Castillo**
Study on the phenolic composition and antioxidant properties of white, yellow and black corn (*Zea mays* L.) foodstuffs.

PP 7 **Alena Vollmannova, Monika Norbova, Judita Lidikova**
Rediscovered fruit quince (*Cydonia oblonga* Mill.) as a food source of biologically valuable substances

PP 8 **Kristi Kerner, Viive Sarv, Ivi Jõudu, Alo Tänavots, Petras Rimantas Venskutonis**
Effect of blackcurrant skin ingredients on the physicochemical properties of pork meatballs

PP 9 **Jadranka Odović**
Determination of total acidity of fruit juice

T9 - Food contaminants
Moderators: Ivana Prodić and Petar Ristivojević

PP 10 **Janette Musilová, Lýdia Karvaš Kemiačová, Silvia Fedorková, Hana Franková**
The risk of garden crops contamination grown in soils with an increased content of heavy metals

PP 11 **Eva Ivanišova**
Uptake of heavy metals by selected species of the genus *Allium*

PP 12 **Olívia Pinho, Sümeyra Sevim, Olga Viegas, Miguel A. Faria, Sara C. Cunha, Isabel M.P.L.V.O. Ferreira**
Inhibition of acrylamide in biscuits by fibre supplementation

PP 13 **Sarić Beka, Gürsul Aktağ Işıl, Žilić Slađana, Simić Marijana, Nikolić Valentina, Gökmen Vural**
Effect of dry-heat treatment on acrylamide and HMF formation in maize flour

PP 14 **Žilić Slađana, Gürsul Aktağ Işıl, Gökmen Vural**
Relation of free asparagine content in small-grain cereals and the generation of acrylamide in the cookies

PP 15 **Miloš Ilić, Jelena Mutić, Jelena Aćimović, Boban Anđelković, Dalibor Stanković, Tamara Mutić, Tanja Ćirković Veličković**
Sample preparation for isolation of microplastic particles from mussel samples

PP 16 **Tamara Mutić, Boban Anđelković, Dragana Stanić-Vučinić, Miloš Ilić, Tanja Ćirković Veličković**
Chemical characterization and quantification of microplastics particles from mussel samples based on Micro-FTIR spectroscopy

PP 17 **Andreea Lavinia Mocanu, Fulvia Manolache, Gabriel Mustatea, Cristina Todasca**
Investigation of acrylamide and HMF formation in biscuits produced by different ingredients

PP 18 **Małgorzata Starowicz, Nigel Halford, Neil Buck, Marianna Rakszegi, Viktor Korzun, Jane K. Parker, Christine Nowakowski, Zuzana Ciesarová, Kristína Kukurová**
Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine (ACRYRED) - COST ACTION 21149

11:30-12:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T1-Food composition, quality and safety - Hall 1, Main Chemical Amphitheater
Moderators: Jelena Trifković and Petras Rimantas Venskutonis

11:30-11:45 **OP 1** **Luisa Calcinai, Barbara Prandi, Ilaria Puxeddu, Stefano Sforza, Tullia Tedeschi**
Assessment of chemical modifications on the reduction of the allergenic potential of legume based proteins

11:45-12:00 **OP 2** **Dias A.L.S., Fenger J-A., Meudec E., Verbaere A., Costet P., Hue C., Coste F., Lair S., Cheynier V., Boulet J-C., Sommerer N.**
Dark matter revealed: the brown or black color of fine chocolates probed by polyphenol metabolomics and molecular networking

12:00-12:15 **OP 3** **Naz Erdem, Neslihan Göncüoğlu Taş, Tolgahan Kocadağlı, Vural Gökmen**
An approach on how to modulate recipe to reduce sugar in biscuits

12:15-12:30 **OP 4** **Marlene Walczak, Farah Gutsche, Michael Hellwig**
Methylated lysine derivates in food

T9-Food contaminants - Hall 2, Small Chemical Amphitheather Dräger
Moderators: Thomas Gude and Jelena Mutić

11:30-11:45 **OP 5** **Maja Krstić Ristivojević, Vesna Jovanović, Mirjana Radomirović, Olga Trifunović, Dragana Stanić-Vučinić, Tanja Ćirković Veličković**
Tropomyosin quantification in seafood samples-right choice of standard makes a difference

11:45-12:00 **OP 6** **Bram Miserez, Graciele Necchi Rohers, Jet Van De Steene, Liesbeth Jacxsens, Bruno De Meulenaer**
Detection of food contact chemicals from inks and adhesives in food

12:00-12:15 **OP 7** **Zuzana Ciesarová, Kristína Kukurová, Viera Jelemenská, Miona Belovic, Aleksandra Torbica**
Asparaginase treatment of fruit additives enriching biscuits

12:15-12:30 **OP 8** **Jane K. Parker, Alex Gilbert, Dimitris P. Balagiannis, M. Jose Oruna Concha**
Acrylamide formation in protein fortified flour and bread

12:30-13:30 ***Lunch***
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

T1-Food composition, quality and safety - Hall 1, Main Chemical Amphitheater
Moderators: Zuzana Ciesarová and Dušanka Milojković-Opsenica

13:30-13:45 **OP 9** **Sabrina Stranig, Erich Leitner, Dorothea Leis, Barbara Siegmund**
Corky off-flavour in garlic? The presence of haloanisoles in garlic as serious problem for food industry

13:45-14:00 **OP 10** **Custódio Lobo Roriz, Carlos S. H. Shiraishi, Maria Inês Dias, Ricardo C. Calhelha, Maria José Alves, Márcio Carocho, Vasco da Cunha Mendes, Rui M. V. Abreu, Miguel A. Prieto, Sandrina A. Heleno and Lillian Barros**
Chemical and bioactive profile of five fig fruit varieties

14:00-14:15 **OP 11** **Andreia S. Ferreira, Elisabete Coelho, Cláudia Nunes, Manuel A. Coimbra**
Alternatives to the titanium dioxide (E171) whitening colorant in foods

14:15-14:30 **OP 12** **Mihajlo Jakanovski, Aleksandra Dramićanin, Nikola Horvacki, Jelena Trifković, Dubravka Rašić, Irena Brčić Karačonji, Dušanka Milojković-Opsenica**
Microgreens and germs: The gleam of next-generation super foods - manipulations in production technologies and future strategies for maintaining the shelf life and quality of products

T9-Food contaminants - Hall 2, Small Chemical Amphitheather Dräger
Moderators: Jelena Radosavljević and Jane Parker

13:30-13:45 **OP 13** **Zita E. Martins, Marta Silva, Armindo Melo, Catarina Mansilha, Miguel A. Faria, Isabel M.P.L.V.O. Ferreira**
From Data mining to Meta-analysis: Presence of mycotoxins in food

13:45-14:00 **OP 14** **Nikola Gligorijevic, Dragana Stanic-Vucinic, Tamara Mutic, Tamara Lujic, Tanja Cirkovic Velickovic**
Binding and corona formation of ovalbumin to polystyrene and polyethylene terephthalate microplastics under neutral and acidic conditions

14:00-14:15 **OP 15** **Ana Sánchez-Arroyo, Laura Plaza-Vinuesa, Blanca de las Rivas, José Miguel Mancheño, Rosario Muñoz**
Enzymatic detoxification of ochratoxin A: Aspergillus niger vs. Alcaligenes faecalis ochratoxinases

14:15-14:30 **OP 16** **Miguel A. Faria, Carolina Monteiro, Helena Ramos, Soraia Sá, Eugénia Pinto, José O. Fernandes, Sara Cunha, Isabel M. P. L. V. O. Ferreira**
Combined synergic exposure to food contaminants: A matter of concern?

14:30-15:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T8-Novel methods for food chemistry - Hall 1, Main Chemical Amphitheater
Moderators: Malgorzata Starowicz and Irena Vovk

14:30-14:45 **OP 17** **Mónica M. Costa, Maria P. Spínola, José A. M. Prates**
Improving protein extraction from Chlorella vulgaris using combined mechanical/physical and enzymatic pre-treatments

14:45-15:00 **OP 18** **Joana Costa, Caterina Villa, Isabel Mafra**
Single-tube nested real-time PCR as an efficient tool to quantify allergenic tree nuts in processed foods

15:00-15:15 **OP 19** **María Vergara-Barberán, Hiba Salim, Laura Pont, Estela Giménez, and Fernando Benavente**
Determination of protein biomarkers by on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry. From biomedicine to food science

T5-Chemical reactions and interactions of food components - Hall 2, Small chemical amphitheater Dräger
Moderators: Maja Krstić Ristivojević and Livia Simon Sarkadi

14:30-14:45 **OP 20** **Alicja Tymczewska, Magdalena Gierszewska, Aleksandra Szydłowska-Czerniak**
Chitosan films enriched with rapeseed cake extract obtained using a deep eutectic solvent

14:45-15:00 **OP 21** **Franziska Hanschen, Lars Andernach**
The role of amines in glucosinolate hydrolysis in Brassica vegetables

15:00-15:15 **OP 22** **Leon V. Bork, Sascha Rohn, Clemens Kanzler**
Non-Enzymatic Browning Reactions of Phenolic Compounds – Formation of Melanin-like Colorants

15:15-15:30 **OP 23** **Friederike Manig, Franziska Pietz, Louisa Schueth, Theresa Strunz, Thomas Henle**
Saliva – a suitable matrix to study the metabolic transit of food components and metabolites

15:30-16:15 *Coffee break*

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

T1-Food composition, quality and safety - Hall 1, Main Chemical Amphitheater
Moderators: Jelena Trifković and Petras Rimantas Venskutonis

PP 19 **Rafaela Santos, Mariana Mota, Anabela Raymundo, Catarina Prista**
Characterization of physicochemical properties of a Portuguese miso

PP 20 **Milena Šenk, Dušanka Milojković-Opsenica, Milena Simić, Milan Brankov, Igor Kodranov, Vesna Dragičević**
Role of soybean - millet intercropping and bio-fertilizer in managing potential bio-availability of essential elements

PP 21 **Mladen Rajaković, Uroš Gašić, Danka Bukvički, Mirjana Pešić, Ana Čirić, Danijel Milinčić, Dejan Stojković**
Phytochemical composition of hydro-ethanolic extracts from *Cucumis metuliferus* E. Mey. fruit peels

PP 22 **Veronika Barišić, Małgorzata Starowicz, Urszula Krupa-Kozak, Ivana Flanjak, Małgorzata Wronkowska**
Changes of volatiles, free fatty acids and antioxidant profiles in gluten-free sponge cakes with the powdered cocoa bean shell (CBS)

PP 23 **Vojin Cvijanović, Aleksandra Dramićanin, Beka Sarić, Mihajlo Jakanovski, Nevena Momirović, Nebojša Momirović, Dušanka Milojković-Opsenica**
The influence of integral and organic growing systems on sugar content in selected tomato types and cultivars

PP 24 **Steva M. Lević, Milica Lučić, Ivana Sredović Ignjatović, Antonije Onjia**
Experimental design and the desirability function in the estimation of overall food quality

PP 25 **Nevena Momirović, Dragan Nikolić, Mihajlo Jakanovski, Aleksandra Dramićanin, Mirjana Mosić, Nebojša Momirović, Dušana Milojković-Opsenica**
The sugars content of parental and new perspective descendant strawberry genotypes – potential approach for the future selection process

PP 26 **Teodora Cvanić, Vanja Travičić, Olja Šovljanski, Ana Tomić, Jelena Vulić, Anja Saveljić, Jasna Čanadanović-Brunet, Gordana Ćetković**
Phytochemical composition and antioxidant activity peel crude extract of *Cucumis metuliferus* (E. Mey. Ex. Naudin) from Fruška gora

PP 27 **Katalin Patonay, Helga Szalontai, Péter Radácsi and Éva Zámboriné-Németh**
Mints producing thymol isomers – New chemotypes in five Hungarian *Mentha longifolia* (L.) accessions involved to experimental cultivation

PP 28 **Vesna Glavnik, Irena Vovk, Nisa Beril Sen, Etil Guzelmeric, Erdem Yesilada**
HPTLC-MS/MS analyses of phenolic compounds in bee pollen botanically originated from *Hedera helix*

PP 29 **Danijel D. Milinčić, Jovana Petrović, Jasmina Glamočlija, Uroš Gašić, Ana Doroški, Aleksandar Kostić, Slađana Stanojević, Mirjana B. Pešić**
Biocompounds from mushroom aqueous and polysaccharide extracts

PP 30 **Vesna Dragičević, Vesna Kandić, Margarita Dodevska, Milan Brankov, Dejan Dodig†**
Some antioxidants and dietary fibre in various small grains (cereals)

T5 - Chemical reactions and interactions of food components
Moderators: Ivana Prodić and Petar Ristivojević

PP 31 **Fernanda Cosme, Inma Arenas, Miguel Ribeiro, Luís Filipe-Ribeiro, Rafael Vilamarim, Elisa Costa, João Siopa, Fernando M. Nunes**
Effect of Fining with Chitosan and k-Carrageenan on Protein Stability, Macromolecular, and Phenolic Composition of White Wines

PP 32 **Sophie Poppe, Michael Hellwig**
Oxidative stability of selenomethionine

TOPIC: EMERGING CONTAMINANTS
Moderators: Tanja Ćirković Veličković and Irena Vovk

16:15-17:00 **Plenary Lecture 2**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Food and packaging – a symbiosis?
Prof. Thomas Gude

17:00-17:30 **Keynote Lecture 2**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Micro- and nanoplastics in food current analytical methods and challenges
Prof. Ralf Greiner

18:00 **WELCOME RECEPTION**
The Ilija M. Kolarac Endowment Studentski trg 5

Day 2: June 15, 2023

8:30-10.30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **Vendors slot**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
PROANALYTICA
How do we overcome challenges between sampling and analysis?
Goran Đokić

SHIMADZU
Analytical Methods for determination of Contaminants in Food and Food Packaging
Uwe Oppermann
Shimadzu Europa GmbH, Duisburg, Germany

TOPIC: FOOD AND HEALTH
Moderators: Hans-Jacob Skarpeid and Vieno Piironen

9:30-10:15 **Plenary Lecture 3**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
A Foodomics study on the activity of bioactive compounds from plants, algae and agrifood by-products against Alzheimer disease
Prof. Alejandro Cifuentes

10:15-10:45 **Keynote Lecture 3**
Serbian Academy of Sciences and Arts, Knez Mihailova 35
Overview on analytical methods for allergen control in foods and compliance with the proposed reference doses
Prof. Linda Monaci

10:45-11:30 Coffee break

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

T4 - Food and health, functional foods and ingredients
Moderators: Maja Krstić Ristivojević and Danijela Kostić

PP 33 **Bartosz Fotschki, Katarzyna Ognik, Joanna Fotschki, Dorota Napiórkowska, Ewelina Cholewińska, Magdalena Krauze, Jerzy Juśkiewicz**
Chromium Nanoparticles Support the Pro-Healthy Regulation of Liver Lipid Metabolism and Inflammation in Obese Rats when Combined with the Abandonment of High-Fat/Low-Fiber Diet

PP 34 **Natália Čmiková, Simona Kunová, Miroslava Kačániová**
Mentha × piperita L. var. *officinalis* forma *rubescens* Camus antimicrobial activity *in vitro and in situ*

PP 35 **Cristina Martínez-Villaluenga, Irene Tomé-Sánchez, Ana Belén Martín-Diana, Daniel Rico, Iván Jiménez-Pulido, Juana Frias, Vermont P. Dia**
Anticancer activity of hydrolysed wheat bran mediated through macrophages stimulation

PP 36 **Bartosz Fotschki, Jerzy Juśkiewicz, Dorota Napórkowska**
Diet with raspberry polyphenols and prebiotic enhances liver lipid metabolism and regulates synthesis of bile acids in obese rats

PP 37 **Xiangrong Fang, Yuqing Zhang, Marika Kalpio**
Health effects and bioavailability of omega-3 fatty acids

PP 38 **Natalia Płatosz, Natalia Bączek, Joanna Topolska, Dorota Szawara-Nowak, Wiesław Wiczkowski**
Chokeberry and red cabbage anthocyanins selectively cross the blood-cerebrospinal fluid barrier

PP 39 **Lukáš Kolarič, Michaela Lauková, Lucia Minarovičová, Peter Šimko**
The use of β-cyclodextrin for decreasing the cholesterol content in cereal products

PP 40 **Ana Sousa, Luis Patarata, Fernando Nunes, Fernanda Cosme**
Antimicrobial activity of red wine phenolic and non-phenolic fractions

PP 41 **Zhihua Wua, Ying Zhanga, Hongbing Chena**
IgE recognition and structural analysis on disulfide bonds crosslinked allergens aggregation in roasted peanut

PP 42 **Ana Cristina Ferrão, Raquel Guiné, Marco Silva, Paula Correia**
Microbiological and chemical composition of some Portuguese hazelnuts

PP 43 **Ítala M.G Marx, Elizandra Ardohain, Paula Rodrigues, Andreia Afonso, Tânia C.S.P. Pires, Maria Inês Dias, Lillian Barros, Sandrina A. Heleno**
Chemical characterization of *Cistus ladanifer* L. phenolic extract and its antifungal activity against *Botrytis cinerea*

PP 44 **Cristina Caleja, Matilde Rodrigues, André Almeida, Maria Inês Dias, José Pinela, Lillian Barros**
Fatty acid profile and indices of atherogenicity and thrombogenicity of fish by-product oil for pet food formulations

PP 45 **Zuzanna Gralak, Kamil Brzuzy, Aneta Jastrzębska**
Determination of selected parameters and compounds in fermented food

T8 - Novel methods for food chemistry
Moderators: Ivana Prodić and Petar Ristivojević

PP 46 **Rocío Galindo-Luján, Laura Pont, Victoria Sanz-Nebot, Fernando Benavente**
MALDI-TOF-MS for protein profiling and classification of food. Application to quinoa grains

PP 47 **Paula Dominguez-Lacueva, María J. Cantalejo-Diez, Nerea Iturmendi-Vizcay**
Spectrofluorimetric characterization of ozonated olive oils

PP 48 **Sandra Vuković, Đorđe Moravčević, Ilinka Pećinar, Sofija Kilibarda, Ana Vujošević, Danijel D. Milinčić, Maja Sudimac, Dragoljub Pavlović, Aleksandar Ž. Kostić**
Determination of antioxidant properties of turmeric spice extracts prepared by ultrasound assisted (UAE) and classical solvent extraction (CSE)

PP 49 **De Ancos Begona, Ojeda GA, Sgroppo SC, Rodríguez-Rodríguez E, Olmedilla-Alonso B, Sánchez-Moreno C.**
Green extraction of phenolic compounds and carotenoids from pulp and peel of mango criollo by ultrasound assisted extraction with deep eutectic solvents

PP 50 **Tuba Esatbeyoglu, Sherif M. Afifi, Eman M. Kabbash, Ralf G. Berger, Ulrich Krings**
Citrus sinensis: Comparative Untargeted Metabolic Profiling Using Liquid Chromatography-Mass Spectrometry and Multivariate Data Analysis to Uncover Authenticity

PP 51 **Dunja Malenica, Marko Kass, Rajev Bhat**
Optimization of ultra-sound assisted extraction and conventional solvent extraction of TPC and DPPH from sea buckthorn pomace and hempseed hull

11:30-12:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T4-Food and health, functional foods and ingredients - Hall 1, Main chemical amphitheater
Moderators: Jelena Radosavljević and Hans-Jacob Skarpeid

11:30-11:45 **OP 24** **Monika Pischetsrieder, Thomas Sommer, Julia Saller, Yan Li1, Harald Hübner, Liubov Kalinichenko, Christian Müller**
Neurobiological regulation of food intake: Unbiased identification of food bioactives by virtual screening

11:45-12:00 **OP 25** **Petras Rimantas Venskutonis, Rolana Gužauskaitė, Rita Kazernavičiūtė**
Development of functional ingredients from guelder rose (Viburnum opulus L.*) fruit pomace and their application for increasing nutritional value of bread*

12:00-12:15 **OP 26** **Disca Vincenzo, Capuano Edoardo, Arlorio Marco**
Prebiotic effect of enzymatic treated cocoa bean shells (CBS): a static in-vitro digestion

12:15-12:30 **OP 27** **Michael Murkovic**
Recent results on heat induced carcinogens – formation of furfuryl alcohol during roasting of coffee

T5-Chemical reactions and interactions of food components - Hall 2, Small chemical amphitheater Dräger
Moderators: Michael Murkovic and Elizabet Janić Hajnal

11:30-11:45 **OP 28** **Sandra Grebenteuch, Lothar W. Kroh, Sascha Rohn**
Formation of volatile methyl ketones during lipid oxidation

11:45-12:00 **OP 29** **Clemens Kanzler, Tatjana Rüger, Johann Falkenhagen, Leon V. Bork, Sascha Rohn**
Colorants of the Maillard reaction: formation and structure of food melanoidins

12:00-12:15 **OP 30** **Anna Širová, Anna Průšová, Zuzana Procházková, Karel Cejpek**
Development of α-dicarbonyl compounds from oligosaccharides

12:30-13:30 Lunch
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

T4-Food and health, functional foods and ingredients - Hall 1, Main chemical amphitheater
Moderators: Jane Parker and Vieno Piironen

13:30-13:45 **OP 31** **Juana Frias, Antonio Del Casale , Zuzana Ciesarova, Marta Laranjo, Photis Papademas, Effie Tsakalidou, Guy Vergères, Smilja Pracer, Marie Christine Champomier Vergès, Vittorio Capozzi, Christophe Chassard1**
Promoting Innovation Of Fermented Foods (Pimento) - Cost Action CA20128

13:45-14:00 **OP 32** **Fernanda Machado, Helena Laronha, Elisabete Coelho, Cláudia Nunes, Manuel A. Coimbra, Filipe Coreta-Gomes**
Polysaccharides hypocholesterolemic potential: from structure to function

14:00-14:15 **OP 33** **<u>Laura Quintieri</u>, Linda Monaci1**
Widening potentials of whey proteins and a look towards unexplored application fields

14:15-14:30 **OP 34** **Lucía González-Mulero, <u>Marta Mesías</u>, Francisco J Morales, Cristina Delgado-Andrade**
Acrylamide bioaccessibility in cereals, potatoes and chips. Effect of the food matrix and colonic fermentation

<u>T6-Food sustainability, including byproducts valorization - Hall 2, Small Chemical Amphitheather Dräger</u>
Moderators: Ivana Prodić and Małgorzata Starowicz

13:30-13:45 **OP 35** **<u>Rafael Mascoloti Spréa</u>, Tiane C. Finimundy, Ricardo C. Calhelha, Tânia C.S.P. Pires, Joana S. Amaral, Miguel A. Prieto, Lillian Barros**
Chemical characterization and bioactive properties of industrial residues from walnut oil production (Juglans regia L.*)*

13:45-14:00 **OP 36** **<u>Andrea Fuso,</u> Pio Viscusi, Laura Righetti, Clara Pedrazzani, Ginevra Rosso, Ileana Manera, Franco Rosso, Augusta Caligiani**
Hydrothermal treatment for hemicellulose extraction: investigation of temperature effect on fibre structure and study of degrading compounds in hazelnut shells

14:00-14:15 **OP 37** **<u>Leichtweis, M.G.</u>, Molina, A.K., Pereira, C., Pires, T.C.S., Calhelha, R.C., Mohamed, M.H., Oliveira, M.B., Ferreira, I.C.F.R., Barros, L.**
Sustainable use of pumpkin: characterization of the pulp and valorisation of by-products in obtaining preservative extracts

14:15-14:30 **OP 38** **<u>Sofia F. Reis</u>, Pedro A. R. Fernandes, Beatriz Barrosa, Elena Cassin, Vítor J. Martins, Sara Gonçalves, Diogo Figueira, Diogo Castelo-Branco, Manuel A. Coimbra, Elisabete Coelho**
Brewer's Spent Yeast as a source of Vegan and Clean Label Additives for Mayonnaise Formulations

14:30-15:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

<u>T6-Chemical changes in food under processing and storage - Hall 1, Main Chemical Amphitheater</u>
Moderators: Cristina Todasca and Jelena Radosavljević

14:30-14:45 **OP 39** **Lars Störmer, Susanne Siebeneicher, Martin Globisch, Thomas Henle**
Formation of the Lipation product 2-Amino-6-(3-methylpyridin-1-ium-1-yl)-hexanoic Acid (MP-Lysine) during Roasting of Peanuts

14:45-15:00 **OP 40** **<u>Zongyao Huyan</u>, Nicoletta Pellegrini, Josep Rubert, Wilma Steegenga, Edoardo Capuano**
Different Levels and Pattern of Lipid-Derived Gut Microbial Metabolites after Fermentation of Different Lipid-rich Foods

15:00-15:15 **OP 41** **Xiyu Jiang, Jinfeng Bi1, Xuan Liu, Meng Liu, Dazhi Liu, Jianing Liu, Ruud Verkerk, Matthijs Dekker**
Retention of anthocyanin and antioxidant activity in the presence of pectin in mixed juices for different processing methods

15:15-15:30 **OP 42** **<u>Simone Angeloni</u>, Maria Alessia Schouten, Laura Acquaticci, Giovanni Caprioli1, Massimo Ricciutelli1, Gianni Sagratini, Santina Romani, Sauro Vittori**
UHPLC-MS/MS quantification of acrylamide in various foodstuffs: formation and strategies of mitigation

<u>T7-Food adulteration, authenticity and traceability - Hall 2, Small chemical amphitheater Dräger</u>
Moderators: Irena Vovk and Petar Ristivojević

14:30-14:45 **OP 43** **Carla Egido, Claudia Martínez-Alfaro, Víctor García-Seval1, Javier Saurina, Sònia Sentellas, <u>Oscar Núñez</u>**
Classification, characterization, and authentication of honey by HPLC-UV fingerprinting and chemometrics. Application to the detection of honeys adulterated with syrups.

14:45-15:00 **OP 44** **<u>Kristína Kukurová</u>, Zuzana Ciesarová, Viera Jelemenská, Janka Kubincová, Zuzana Dubová, Jana Horváthová, Blanka Tobolková, Filip Dimitrov, Michael Murkovic, Barbara Siegmund, Martina Orolínová, Nikola Sitárová**
Slovak-Austrian cooperation in honey quality assessment

15:00-15:15 **OP 45** **Liliana Grazina, Joana S. Amaral, Joana Costa, <u>Isabel Mafra</u>**
Authentication of ginkgo-containing foods: real-time PCR detection of Styphnolobium japonicum as a potential adulterant

15:15-15:30 **OP 46** **<u>Alba Tres</u>, Beatriz Quintanilla-Casas, Berta Torres-Cobos, Francesc Guardiola, Stefania Vichi**
Sesquiterpene chromatographic fingerprinting: lights and shadows for the geographical and varietal authentication of olive oils

15:30-16:15 Coffee break

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

<u>T6 - Chemical changes in food under processing and storage</u>
Moderators: Maja Krstić Ristivojević and Danijela Kostić

PP 52 **<u>Sara Marçal</u>, Helena Araújo-Rodrigues, Ana A. Vilas-Boas, Débora A. Campos, Manuela Pintado**
Functional mango peel powders: what is the impact of different drying methods on their phytochemical composition and antioxidant activity?

PP 53 **Albert Gashi, Kaltrina Berisha, György Kenesei, Zsuzsanna Mednyánszky, <u>Livia Simon-Sarkadi</u>**
Effect of High Hydrostatic Pressure on Free Amino Acid and Biogenic Amines in Sausages During Storage

PP 54 **<u>Latife Betül Gül</u>, Osman Gül, Abdullah Akgün**
Effect of different drying techniques on the physicochemical and techno-functional properties of sesame protein isolate

PP 55 **<u>Vesna Jovanović</u>, Mirjana Radomirović, Maja Krstić Ristivojević, Dragana Stanić-Vučinić, Tanja Ćirković Veličković**
The effect of food processing and packaging of clams on the content of tropomyosin

PP 56 **<u>Aleksandra Stojićević</u>, Mališa Antić, Biljana Rabrenović, Igor Tomašević, Bojana Milovanović Savić**
Chemical and colour changes of flavored cold-pressed sunflower oil during long-term storage conditions

PP 57 **<u>Zhang Meili</u>, Wang Peiyao**
Effect of stir-frying on the flavor characteristics of oat flour and dough formation

PP 58 **<u>Degenek Jovana</u>, Iličić Mirela, Kanurić Katarina, Vukić Vladimir, Vukić Dajana, Zorica Tomičić**
The effect of kombucha as a non-conventional starter culture on the chemical composition and free amino acid profile of fresh cheese

PP 59 **<u>Lama Ismaiel</u>, Ancuta Nartea, Benedetta Fanesi, Deborah Pacetti, Paolo Lucci, Henry Jaeger**
Impact of high-pressure processing technology on lipid oxidation and antioxidants in Sardines (*Sardina pilchardus*)

PP 60 **<u>Teresa Kaeubler</u>, F. Manig, T. Henle**
Canned versus home-made: Maillard reaction products in complex food samples

PP 61 **<u>Dana Urminská</u>, Tatiana Bojňanská, Nora Haring**
The effect of enzymatic oxidation, so called tea fermentation, on the antioxidant activity of commertial bagged teas

PP 62 **<u>Yosra Zbiss</u>, Filipe Lema, Luana Fernandes, Inês Braga, Alexandre Gonçalves, Maria do Céu Fidalgo, Jorge Moreira, Elsa Ramalhosa**
Effect of variety on physicochemical properties of coated chestnuts along short storage

PP 63 **<u>Jihong Wu</u>, Xin Pan, Fei Lao**
A critical insight into the changes of aroma-active compounds during fruits and vegetables processing

PP 64 **<u>Anna Průšová</u>, Zuzana Procházková, Anna Šírová, Karel Cejpek**
α-Dicarbonyl compounds in honeys and honey-related products

<u>T7 - Food adulteration, authenticity and traceability</u>
Moderators: Maja Krstić Ristivojević and Danijela Kostić

PP 65 **<u>Marko Ilić</u>, Kristian Pastor, Aleksandra Savić, Mirjana Vasić, Đura Vujić, Marijana Ačanski**
Legume authentication method based on free amino acid composition

PP 66 **<u>Maja Bensa</u>, Mojca Jevšnik, Irena Vovk**
Food fraud in Europe - a 5 year overview

<u>T8 - Novel methods for food chemistry</u>
Moderators: Ivana Prodić and Petar Ristivojević

PP 67 **<u>María del Pozo</u>, Lara García, Luis Vázquez, María Dolores Petit Domínguez, Elena Casero, Carmen Quintana**
Synthesis and characterization of ReS2-CB[n]-0D nanodots Development of an analytical method for Ponceau-4R determination.

PP 68 **<u>Mihajlo Kulizić</u>, Milan Stanković, Rada Baošić, Svetlana Đogo Mračević, Aleksandar Lolić**
Application of a flow injection system with carbon paste/copper Schiff base composite electrode on the ascorbic acid determination

PP 69 **Dušan Vasić, Ilinka Pećinar, Nenad Mićanović, Luiz Fernando Cappa de Oliveira, Jelena Popović-Đorđević**
Raman spectroscopy as a tool for chemical characterisation of 12 Serbian fruits

PP 70 **Liliana Grazina, Paula Paíga, Isabel Mafra, Joana Costa, Manuela Moreira, Cristina Delerue-Matos, <u>Joana S. Amaral</u>**
Targeted UHPLC-MS/MS and DNA-based methods: a complementary approach for the botanical authentication of ginkgo-containing food supplements

TOPIC: CHEMICAL CHANGES IN FOOD UNDER PROCESSING AND STORAGE

Moderators: Thomas Gude and Cristina Todasca

16:15-17:00 **Plenary Lecture 4**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Process-induced reactions in food: Glycation, lipation and beyond
Prof. Thomas Henle

TOPIC: FOOD SAFETY

17:00-17:30 **Keynote Lecture 4**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Essential oils antimicrobial activity and their role in food safety
Prof. Miroslava Kacaniova

20:00 **CONFERENCE DINNER**
Restaurant Velika Skadarlija, Skadarska 40d

Day 3: June 16, 2023

8:30-10.30 **Registration**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

9:00-9:30 **Vendors slot**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

ANALYSIS
Luka Mihajlović, PhD
Proteomics in food - from farm to fork

TOPIC: FOOD SUSTAINABILITY AND BYPRODUCTS VALORIZATION

Moderators: Livia Simon Sarkadi and Zuzana Ciesarová

9:30-10:15 **Plenary Lecture 5**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

Food Sustainability and byproducts valorization
Prof. Manuela Pintado

TOPIC: NOVEL FOODS

10:15-10:45 **Keynote Lecture 5**
Serbian Academy of Sciences and Arts, Knez Mihailova 35

We know how to extract proteins from rapeseed – NapiFeryn BioTech for food sustainability
Prof. Anna Chmielewska

10:45-11:30 Coffee break

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

T3 - Novel Foods
Moderators: Jelena Mutić and Mirjana Radomirović

PP 71 **Maria P. Spínola, Mónica M. Costa, José M. Pestana, Cristina M. Alfaia, Beatriz Tavares, Madalena M. Lordelo, José A. M. Prates**
Impact of 15% *Arthrospira platensis* (Spirulina) inclusion combined with/without enzymes on breast's meat quality in broilers

PP 72 **Nuno Mateus, Diana Melo Ferreira, Zélia Azevedo, Eliana Pereira, Cristina Caleja, Victor de Freitas, Lillian Barros, Carla Barbosa, Maria Beatriz Oliveira**
Effect of alginate-based edible coatings enriched with *Origanum vulgare* L. essential oil on the shelf-life of biological apples

PP 73 **Aistė Galinskaitė, Rūta Gruškienė, Tatjana Kavleiskaja, Ramunė Stanevičienė, Elena Servienė, Jolanta Sereikaitė**
Nisin-Loaded Fucoidan Particles: Preparation and Characterization

PP 74 **Yu Peng, Mo Li, Xin Wen, Yuanying Ni**
Production and functional characteristics of low-sodium high-potassium soy protein for the development of healthy soy-based foods

PP 75 **Ivana Buljeta, Josipa Vukoja, Anita Pichler, Josip Šimunović, Mirela Kopjar**
Cellulose/saccharide delivery systems of raspberry phenolics

PP 76 **Ina Ćorković, Anita Pichler, Ivana Ivić, Josip Šimunović, Mirela Kopjar**
Microencapsulation of volatile compounds from chokeberry juice into alginate/pullulan hydrogel beads

PP 77 **Tatiana Bojňanská, Alena Vollmannová, Dana Urminská, Janette Mussilová, Alžbeta Hegedüsová, Judita Lidiková**
Application of organic sunflower cake to composite flour and effect on the properties of the dough and the fiber content of the bread

PP 78 **Sanja Stojanović, Aleksandra Margetić, Marinela Šokarda-Slavić, Nataša Božić, Zoran Vujčić, Biljana Dojnov**
Obtaining of FOS by controlled hydrolysis of inulin with *Aspergillus welwitschiae* FAW1 endoinulinase

PP 79 **Sana Ben-Othman, Reelika Rätsep, Uko Bleive, Hedi Kaldmäe, Andres Sats, Toonika Rinken**
Use of oil-seed proteins for the microencapsulation of chokeberry and sea-buckthorn pomace extracts

PP 80 **José M. Pestana, Maria P. Spínola, Paula A. Lopes, Mónica M. Costa, Madalena M. Lordelo, José A. M. Prates**
Effect of 15% Spirulina incorporation with commercial peptidases supplementation on colour and sensory breast meat profile in broilers

PP 81 **Marija Pavlović, Marinela Šokarda Slavić, Marina Ristović, Aleksandra Margetić, Sanja Stojanović, Miloš Momčilović, Zoran Vujčić**
Highly active endo-pectinase from *Aspergillus tubingensis*: A novel enzyme for fruit processing

PP 82 **Marina Ristović, Sanja Stojanović, Marija Pavlović, Aleksandra Margetić, Marinela Šokarda-Slavić, Zoran Vujčić, Biljana Dojnov**
Highly active xylanase used in juice clarification

PP 83 **Liene Jansone, Zanda Kruma, Solvita Kampuse**
Valorisation of sauerkraut processing by-products

PP 84 **Mila Č. Lazović, Marko D. Jović, Ivica Z. Dimkić, Dušanka M. Milojković Opsenica, Petar M. Ristivojević, Jelena Đ. Trifković**
Potential application of green extracts rich in phenolics for innovative functional foods: Natural deep eutectic solvents as medium for isolation of biocompounds from berries

PP 85 **Soraia Santos, Cristiana Breda, Ana Abraão, Rui Dias Costa, Irene Gouvinhas, Ana Barros**
Edible flowers: a novel antioxidant source to enhance food stabilit

PP 86 **Višnja Kosić, Nataša Božić, Biljana Dojnov, Gordana Stevanović, Predrag Banković, Aleksandra Milutinović-Nikolić, Zorica Knežević-Jugović**
Stable, environmentally friendly and inexpensive biocatalysts for obtaining important ingredients applicable in the food industry

PP 87 **Andreia Ribeiro, Isabel M. Martins, Maria Eduarda Relvas, Larissa C. Ghirro, José Carlos B. Lopes, Maria Filomena Barreiro, Madalena M. Dias**
Hydroxyapatite Pickering emulsions loaded with olive leaf extract as an innovative alternative to traditional mayonnaise-like food sauces

PP 88 **Ana Catarina Costa, Maria Gerö, Maria Ramos, Daniela Correia, Mayumi Delgado, Diogo Castelo Branco, Diogo Figueira, Anabela Raymundo, Catarina Prista**
FermentedVegAlgae: Development and characterization of fermented seaweed products through lactic fermentation and ultrasounds approach

11:30-12:30 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T2-Food sustainability, including byproducts valorization - Hall 1, Main Chemical Amphitheater
Moderators: Livia Simon Sarkadii and Dušanka Milojković-Opsenica

11:30-11:45 **OP 47** **Giovanni Caprioli, Simone Angeloni1, Massimo Ricciutelli, Gianni Sagratini, Sauro Vittori**
Spent coffee ground and coffee silverskin: possible use as nutraceuticals and ingredients for fertilizer products

11:45-12:00 **OP 48** **Ana Catarina Costa, Sheyma Khemiri, Maria Ramos, Margarida Próspero, Maria Gerö, Daniela Correia, Mayumi Delgado, Diogo Castelo Branco, Diogo Figueira, Anabela Raymundo, Catarina Prista**
Chemical and functional characterization of innovative and healthy fermented plant-based products from sustainable land and sea vegetables

12:00-12:15 **OP 49** **Cristina N. Duarte, Oludemi Taofiq, Maria Inês Dias, Sandrina Heleno, Celestino Santos-Buelga, Rolando C. S. Dias, Lillian Barros, Joana S. Amaral1**
Chemical characterization and bioactive properties of winemaking residues towards their possible exploitation

12:15-12:30 **OP 50** **Elizandra Ardohain, Ítala M.G Marx, Andreia Afonso, Vanessa Vieira, Cláudia Barros, Natacha Fontes, António Graça, Maria do Carmo Val, Mateus Nicolau de Almeida, Lillian Barros, Sandrina A. Heleno**
PreVineGrape: Development of a biofungicide against grapevine pathogens using plants and food agro-industry bio residues

T3-Novel foods - Hall 2, Small Chemical Amphitheather Dräger
Moderators: Jane Parker and Elizabet Janić Hajnal

11:30-11:45 **OP 51** **Andreia Ferreira, Bernardo Ferreira, Rita Bastos, Angélica M. Rocha, Norton Komora, Maria João Alegria, Elisabete Coelho, Cláudia Nunes, Manuel A. Coimbra**
Food industry clean label strategies provided by carbohydrates

11:45-12:00 **OP 52** **Soraia P. Silva, Abigail Gonzalez, Dalila Roupar, Andreia F. Salvador, Clarisse Nobre, Manuel A. Coimbra, Elisabete Coelho**
Development of a novel food ingredient from pine nut skin with prebiotic activity

12:00-12:15 **OP 53** **Carla S.S. Teixeira, Caterina Villa, Sérgio F. Sousa, Joana Costa, Isabel M.P.L.V.O. Ferreira and Isabel Mafra**
Identification of potentially bioactive peptides from house cricket (Acheta domesticus) by in silico gastrointestinal digestion

12:15-12:30 **OP 54** **Zsanett Bodor**
Utilisation of lentils as valuable raw materials in gluten-free products

12:30-13:30 ***Lunch***
Faculty of Chemistry - Lunch rooms 1 & 2, 1st floor, Studentski trg 12-16

Vendors slot
Hallway, ground flor, Faculty for Chemistry, Studentski trg 12-16

ANALYSIS
High resolution mass spectrometry in food analysis
Luka Mihajlović, PhD

TOPIC: NOVEL METHODS FOR FOOD CHEMISTRY;
Moderators: Slavica Ražić and Joana Amaral

13:30-14:15 **Joint Session DAC-DFC EuChemS/ Plenary 6**
Faculty for Chemistry, Studentski trg 12-16

New analytical solutions for the global challenges of food safety
Prof. Rudolf Krska

14:15-14:45 **Joint Session DAC-DFC EuChemS/ Keynote 6**
Faculty for Chemistry, Studentski trg 12-16

Understanding food aromas: in the frontier between sensorial and analytical data
Prof. Silvia Rocha

14:45-15:45 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T8-Novel methods for food chemistry - Hall 1, Main Chemical Amphitheater
Moderators: Joana Amaral and Slavica Ražić

14:45-15:00 **OP 55** **Cemile Yılmaz, Tolgahan Kocadağlı, Vural Gökmen**
Method development for endocannabinoid analysis in fermented foods

15:00-15:15 **OP 56** **Arlorio Marco, Bordiga Matteo, Rossini Cesare, Luciano Navarini, Manfredi Marcello, Elettra Barberis, Thomas Wortelmann**
HS-GC-IMS: a new rapid strategy to fingerprint volatile compounds in foods: quality and integrity applications

15:15-15:30 **OP 57** **Caterina Villa, Joana Costa, Isabel Mafra**
An innovative nanoplate digital PCR approach for the quantification of allergenic sesame in foods

Flash presentations I - Hall 2, Small Chemical Amphitheather Dräger
Moderators: Danijela Kostic and Zuzana Cieasarova

14:50-14:55 **FP 1** **Ancuta Nartea, Benedetta Fanesi1, Paolo Lucci, Simona Casavecchia, Lucia Aquilanti1, Deborah Pacetti1**
Profiling of carotenoids and tocopherols of a new food ingredient based on sea fennel industrial by-product

14:55-15:00 **FP 2** **Fernando Gonçalves, Andreia Almeida, Miguel Oliveira**
Impact of wood sticks on phenolic compounds and sensorial quality of Touriga National Portuguese red wine

15:00-15:05 **FP 3** **Katarzyna Włodarczyk, Aleksandra Szydłowska-Czerniak**
Microwave treatment as a promising strategy to develop functional milk alternatives obtained from by-products of the oil industy

15:05-15:10 **FP 4** **Carlos S. H. Shiraishi, Yosra Zbiss, Custódio Lobo Roriz, Maria Inês Dias, Márcio Carocho, Ricardo C. Calhelha, Maria José Alves, Vasco da Cunha Mendes, Rui M. V. Abreu, Miguel A. Prieto, Sandrina Heleno and Lillian Barros**
Fig (Ficus carica L.*) leaves as a source of bioactive compounds: A sustainable approach to valorization of fig bioresidues*

15:10-15:15 **FP 5** **Andreia S. Ferreira, Sónia S. Ferreira, Ana S. P. Moreira, Filipe C. Gomes, Alexandra Correia, Manuel Vilanova, Manuel A. Coimbra, Cláudia Nunes**
Marine polysaccharides valorisation as functional ingredients in food products

15:15-15:20 **FP 6** **Diana Melo Ferreira, Susana Machado, Juliana Peixoto, Joana C. Lobo, Nelson Andrade, Maria Antónia Nunes, Cláudia Silva, Gerardo Álvarez-Rivera, Alejandro Cifuentes, Elena Ibáñez, Fátima Martel, Maria Beatriz Oliveira, Rita C. Alves**
Innovative olive pomace extract as a source of phenolic compounds with antitumoral activity for functional foods

15:20-15:25 **FP 7** **Stanislav Baxa, Marcela Blažková, Martin Polovka, Janka Kubincová**
MERS-SARS-COVID-NEXT- can food help?

15:25-15:30 **FP 8** **Wiem Chtioui, Ítala M.G Marx, Andreia Afonso, Tânia Pires, Lillian Barros, Sandrina A. Heleno, Paula Rodrigues**
Plant extracts as natural agents against Botrytis cinerea infection

15:45-16:45 **ORAL PRESENTATIONS**
Faculty of Chemistry, Studentski trg 12-16

T8-Novel methods for food chemistry - Hall 1, Main Chemical Amphitheater
Moderators: Joana Amaral and Slavica Ražić

15:45-16:00 **OP 58** **João Siopa, Miguel Ribeiro, Fernanda Cosme, Fernando M. Nunes1,4**
Chemical characterisation of biscuits melanoidins using shotgun proteomics

16:00-16:15 **OP 59** **Victoria Samanidou, Abuzar Kabir**
Capsule phase microextraction: A green sample preparation strategy for food matrices

16:15-16:30 **OP 60** **Marcela Segundo**
Contributions of analytical methods to unveil bioactive compounds in food matrices

16:30-16:45 **OP 61** **I.M.P.L.V.O. Ferreira, M. Silva, M. Ribeiro, H. Ramos, A.M. Araújo, A. Melo, C. Mansilha, O. Viegas, M.A. Faria, Z. Martins**
Data mining and machine learning methods to predict food contaminants exposure

Flash presentations II - Hall 2, Small Chemical Amphitheather Dräger
Moderators: Maja Krstic-Ristivojevic and Małgorzata Starowicz

15:45-15:50 **FP 9** **Tolgahan Kocadağlı, Cemile Yılmaz, Ecem Evrim Çelik, Merve Canlı, Evrim Özkaynak Kanmaz, Vural Gökmen**
Design of Functional Food Ingredients through Bioprocessing to Address Food and Mood

15:50-15:55 **FP 10** **Sónia S. Ferreira, Cláudia Nunes, Manuel A. Coimbra**
Salt pan waters can be exploited as a source of functional food and feed ingredients

15:55-16:00 **FP 11** **<u>Carlos Guerreiro</u>, Elsa Brandão, Mónica de Jesus, Leonor Gonçalve, Nuno Mateus, Victor de Freitas, Susana Soares**
Understanding polyphenol adsorption to oral models as a secondary mechanism for astringency

16:00-16:05 **FP 12** **<u>Tamara Lujić</u>, Nikola Gligorijević, Dragana Stanić-Vučinić, Tanja Ćirković Veličković**
Investigation of structural changes in ovalbumin induced by two types of MPs and its impact on protein digestibility

16:05-16:10 **FP 13** **<u>Kamil Brzuzy</u>, Zuzanna Gralak, Aneta Jastrzębska**
Optimization of sample preparation conditions for determination of free amino acids in fermented food

16:10-16:15 **FP 14** **<u>Kahina Slimani</u>, Robin Bofinger, Yvette Pirotais, Dominique Hurtaud-Pessel**
Sampling method and quantification of quaternary ammoniums biocides on agri-food surfaces

16:15-16:20 **FP 15** **Süleyman Yıltırak, Tolgahan Kocadağlı, <u>Ecem Evrim Çelik</u>, Evrim Özkaynak Kanmaz, Vural Gökmen**
Effects of sprouting and fermentation on the formation ofacrylamide and 5-hydroxymethyl formation in relation to free asparagine and reducing sugar concentrations in wholemeals

16:45-17:30 Coffee break

POSTER PRESENTATIONS
Faculty of Chemistry, Studentski trg 12-16

<u>T2 - Food Sustainability, including byproducts valorization</u>
Moderators: Jelena Mutić and Mirjana Radomirović

PP 89 **<u>Elena Peñas</u>, Fohan Agahi, Cristina Martínez-Villaluenga, Rosana Chiva, Mercedes Tamame, Juana Frias**
Chickpea flours as a high nutritional quality ingredient for healthy bakery innovation

PP 90 **<u>Joaquín Gómez-Estaca</u>, David Ibarra, María E. Eugenio, Luisa García-Fuenteilla, Raquel Martín-Sampedro, M. Dolores del Castillo**
On the valorization of coffee by-products: functionality of lignin from silverskin and parchment for nanoparticle production

PP 91 **<u>Felipe Alves, Liege A. Pascoalino</u>, Márcio Carocho, Alexandre Gonçalves, Luana Fernandes, Jorge Moreira, Sandrina A. Heleno, Lillian Barros**
The use of natural edible coatings to preserve chestnuts

PP 92 **<u>Mafalda Alexandra Silva</u>, Tânia Gonçalves Albuquerque, Rita C. Alves, M. Beatriz P.P. Oliveira, Helena S. Costa**
Application of *Cucumis melo* L. peel flour in bakery products

PP 93 **<u>Susete Pinteus</u>, Carla Tecelão, Susana Silva, Alexandra Cruz, Susana Bernardino, Vânia Ribeiro, Daniela Vaz, Vasco Mendes, Rita Pontes, Marco Alves, Telma Orvalho, Maria Jorge Campos**
Valorization of *Ficus carica* L. orchards subproducts: evaluation of antioxidant properties of fig tree leaves obtained by different green extraction approaches

PP 94 **Susete Pinteus, Carla Tecelão, Susana Silva, Alexandra Cruz, Susana Bernardino, Vânia Ribeiro, Daniela Vaz, Vasco Mendes, Rita Pontes, Marco Alves, Telma Orvalho, <u>Maria Jorge Campos</u>**
Ficus carica L. 'Dauphine' leaves as source of antimicrobial compounds

PP 95 **<u>Isabel M. Martins</u>, Andreia Ribeiro, Yaidelin A. Manrique, Rharyne França, Lorrayne Rocha, Ricardo C. Calhelha, Cristina Caleja, Eliana Pereira, Lillian Barros, Madalena M. Dias**
Biovalorisation of agricultural by-products obtained through greenextraction methodology

PP 96 **<u>Nevena Dabetic</u>, Vanja Todorovic, Aleksandar Knežević, Ivana Lukic, Sladjana Sobajic**
Recovery of phenolic compounds from grape pomace after different defatting processes

PP 97 **Rui M. V. Abreu, Custódio Lobo Roriz, Carlos Shirashi, Márcio Carocho, Vasco da Cunha Mendes, Marta Evangelista, João Nunes, Maria Jorge Campos, Sandrina Heleno, Lillian Barros**
Fig Residue as a Novel Source of Bioactive Molecules: A Sustainable Integrated Project

PP 98 **<u>Khemiri</u> S, Simões S, Santos A.J.M., Figueira D, Sousa I., Raymundo A.**
Chemical and functional characterization of clean-label food emulsion with microalgae added to the aqueous phase

PP 99 **Matilde Rodrigues, Cristina Caleja, André Almeida, <u>Maria Inês Dias</u>, José Pinela, Lillian Barros**
Lipid profile of fish by-product oils obtained by ultrasound-assisted extraction

PP 100 **<u>Ana Peleja</u>, Cláudia Neves, Fernando Gonçalves, Susana M. Cardoso, Dulcineia F. Wessel1**
Development of an ice cream enriched in derivatives of winemaking by-products

T8 - Novel methods for food chemistry
Moderators: Ivana Prodić and Petar Ristivojević

PP 101 **Carmen Quintana, Rut Martínez-Moro, María del Pozo, Elena Casero, María Dolores Petit-Domínguez**
Mos2 Quantum Dots- based optical sensing platform for the analysis of synthetic colorants. Application To Quinoline Yellow Determination."

PP 102 **Elena Casero, Ricardo Garsed, Luis Vázquez, Maria Dolores Petit-Domínguez, Carmen Quintana, María del Pozo**
2D-ReS2 & diamond nanoparticles-based sensor for the simultaneous determination of sunset yellow and tartrazine in a multiple-pulse amperometry FIA system

T3 - Novel Foods
Moderators: Jelena Mutić and Mirjana Radomirović

PP 103 **Elena Ungureanu, Gabriel Mustatea, Andreea Mocanu, Corina Stroe, Corina Panciu**
Synergistic action of ZnO nanoparticles and essential oil on the antimicrobial and functional properties of biopolymer films

PP 104 **Cristiano Mateus, Tiane Finimundy, Daniele Bobrowski Rodrigues, Lavínia Veríssimo, Regina Soares, Carlos Shiraishi, Tiago Brandão, Isabel P. Fernandes, João Gonçalves, Lillian Barros, Sandrina A. Heleno**
Development of functional Flavoured water through the incorporation of chestnut Flower Extract: Evaluating Bioactive Potential and Stability

PP 105 **Matilde Rodrigues, Cristina Caleja, José Ignacio Alonso-Esteban, Maria Inês Dias, André Almeida, José Pinela, Manuela Pintado, Lillian Barros**
Oxidative stability of fish by-product oil added of bioactive acorn extract during accelerated storage conditions

17:30 – 18:00 **CLOSING CEREMONY**
Faculty of Chemistry, Studentski trg 12-16

Day 4, June 17, 2023

POST-CONGRESS TOUR:

Archaeological Park Viminacium

PLENARY LECTURES

Vitamins – food chemistry research vs information needed in nutrition research

Vieno Piironen
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Vitamins occur in foods as several vitamers, i.e., compounds with activity of the specific vitamin. These vitamers may be trapped in food matrix or bound to other food components. In food chemistry research we usually aim at complete extraction of the analyte followed by successful separation, identification and quantification. When the data on vitamin contents are used in nutritional research several questions, however, arise. In this presentation, some of those questions are discussed based on our long-term research on vitamins. This abstract gives a few examples of questions to be discussed.

Vitamin contents are usually given as total vitamin contents calculating the sum of vitamer contents directly or after using coefficients to take their vitamin activity differences into account. However, the activities are often poorly known. The most striking example of changes during last years is perhaps vitamin E. Earlier, total vitamin E contents were given as sums of those tocopherols and tocotrienols the researchers had determined. How many tocopherols and tocotrienols were taken into account varied greatly. After that, the aim was to determine all four tocopherols and tocotrienols if present and calculate the vitamin E content as alpha-tocopherol equivalents taking into account differences in vitamin E activities. Today, only α-tocopherol is regarded as vitamin E and other tocopherols and tocotrienols are said to have other bioactivities [1]. This change led to marked decrease in vitamin E contents of many plant-based foods.

Extraction should be efficient liberating vitamers for example from membrane structures. Aiming at complete extraction may, however, lead to overestimation of the value of a food as a vitamin source. As an example, niacin occurs in cereal grains bound to polysaccharides and polypeptides so that niacin is not liberated from those bound structures in our digestion. Alkaline treatment as done in preparation of some traditional corn-based foods is needed. Approaches to mimic digestion have been developed; extraction can be done either using enzymatic or mild acidic treatment. For extraction of total niacin alkaline treatment is added. We analyzed eight cereal products using mild extraction and total extraction and compared the obtained contents with niacin contents in five food composition data bases. The comparison indicated that the values in data bases are too high in terms of available niacin [2].

Recently, use of standardized *in vitro* digestion models has become popular in estimating the part of vitamin in a food that would be available for absorption in intestine, i.e., bioaccessability. Those models tell both about liberation from the food matrix and possible degradation during digestion. Both these factors have been shown to be important for example for folate bioaccessability. We showed that proportion of folate, available for absorption, could be as low as ca. 30-40% of the total folate content in breads [3,4]. The major contributor was vitamer composition. If proportion of less stable folate vitamers in a food was high, degradation took place during digestion leading to low bioaccessability whereas in folate analysis the losses could be controlled. This leads thus to overestimation of the food as a folate source.

Developing methods for vitamin analysis does thus require constant interaction of food chemists and nutritionists.

[1] European Food Safety Authority, EFSA Journal, 13 (2015) 4149.

[2] B. Chamlagain, S. Rautio, M. Edelmann, V. Ollilainen, V. Piironen, J. Food Comp. Anal., 91 (2020) 1-7.

[3] F. Liu, M. Edelmann, V. Piironen, S. Kariluoto, Food & Function, 13 (2022) 3220-3233.

[4] F. Liu, M. Edelmann, V. Piironen, S. Kariluoto, J. Agric. Food Chem., 70 (2022) 13379-13390.

Food and Packaging - a Symbiosis?

Thomas Gude

When talking about food safety there are two views on food packing. On one hand food packaging is seen technologically to help preserving the food and therefore avoiding food waste. On the other hand, food packaging is seen as evil wasting our environment and polluting our food - is this a symbiosis? Food without packaging what ever material is used does not really exist. Any food has in its lifetime from farm to fork contact to a food contact material – in production, as packaging or at home. Nevertheless, food contact materials should become more and more “chemical free”, but on the other side those materials used should be fully recyclable, residue free, environmental-friendly, and cheap – is this a symbiosys?

The current situation was created by a huge misunderstanding. The food industry always required safe food contact materials but was not willing to test the safety as they are food manufacturer and not packaging manufacturer. The packaging industry claimed always not giving any final statements about the food safety as the amount of food interactions is to numerous – they are packaging manufacturers and not food manufacturers – this is a dilemma!

The talk will highlight this misunderstanding and shows the consequences of this by giving some examples as mineral oil, PFAs, NIAS (non-intentionally added substances) etc. Furthermore, the role of legislation is also touched upon. And finally, the current analytical capabilities will be discussed as NTS (non-target screening) and bioassays (Ames, UmuC etc.) and what does it mean in the future for the symbiosis of food and packaging.

Food and health: A Foodomics study on the activity of bioactive compounds from plants, algae and agrifood by-products against Alzheimer disease

J. D. Sánchez-Martínez, A. Valdés, G. Alvarez, J.A. Mendiola, M. Herrero, E. Ibáñez, A. Cifuentes

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In the present research work, a multi-analytical approach has been employed to study the neuroprotective effect of different extracts enriched in bioactive compounds from plants, algae and agrifood by products. For this purpose, green extraction processes (e.g., based on compressed fluids such as pressurized liquid extraction (PLE) and supercritical fluids (SFE), making use of Generally Recognized As Safe (GRAS) solvents) were used. 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 butyrylcholinesterase (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 also studied by neutralization of free radicals (ABTS), and reactive oxygen and nitrogen species (ROS and RNS) scavenging assays, respectively. 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 tandem mass spectrometry (GC-Q-TOF MS/MS and LC-Q-TOF MS/MS).

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

Finally, the extracts with the highest in vitro neuroprotective effect and which that also showed favourable results in BBB permeability assays, were selected to test their neuroprotective effect in an *in vivo* model of Alzheimer’s disease, using the CL4176 transgenic strain of the nematode *Caenorhabditis elegans (C. elegans*), which under thermal induction is able to express the $A\beta_{42}$ peptide, causing paralysis in the nematode that can be quantified.

Using Foodomics, some mechanisms that explain the neuroprotective effect of the selected extracts on C. elegans were elucidated. Namely, the use of lipidomics, transcriptomics and metabolomics, allowed the identification and quantification of a large number of metabolites, genes and their linked proteins, revealing some interesting metabolic pathways and cellular processes altered.

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Process-induced reactions in food: Glycation, lipation and beyond

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Chemical reactions occurring during heating or storage are of prime importance for food safety and quality. Among these, glycation reactions (also referred to as Maillard reaction or nonenzymatic browning) have been investigated since decades, primarily aiming to understand the impact of glycation on the flavour of processed foods. Furthermore, compounds such as acrylamide reached particular importance as “process contaminants”. Despite extensive research, however, we still only partially understand the reaction process and related technological and physiological aspects. This applies in particular to the biological utilization of glycation products, whether in the human body or during biotechnological fermentation processes. In the presentation, the formation of individual glycation products during food processing will be presented on the basis of individual case studies (e.g. brewery process, peanut roasting) and a quantitative assessment of the daily uptake will be carried out.

It will be shown that the human intestinal microbiota can degrade Amadori products by deglycation processes and use them as a source of lysine. Brewer's yeasts differ in their ability to detoxify dicarbonyl compounds via oxidative or reductive pathways, possibly as a result of selective domestication. For high-fat foods such as nuts, in addition to glycation reactions, reactions of protein side chains with lipid peroxidation products are of central importance. The quantitative relevance of these reactions, which have hardly been studied so far, and the functional consequences resulting from the formation of corresponding “neolipoproteins” are demonstrated using the example of peanut roasting.

Food Sustainability and byproducts valorization

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Food industry and agriculture are recognized as one of the most prominent industry branches in Europe. The main issue in the food production chain is the amount of loss and waste which is estimated at 54% of total loss and waste as upstream processes (including production and postharvest) and 46% of total loss and waste as downstream processes (including processing, distribution, and consumption)[1]. According to the Sustainable Development Objective Target 12.3, by 2030, 50% of food waste at the retail and consumer levels and food losses along production and supply chains (including post-harvest losses) must be reduced. To respond, these problems can be turned into an advantage, if potentially they are considered by-products to be valorised. The valorisation of food by-products has been pointed by several authors as a solution to improve the economic and environmental sustainability of the food production chain. Numerous valorisation schemes have been proposed to explore food wastes and by-products as a biomass supplier to obtain different bio-based products. Between them, the integrated value chains have been identified as one of the most promising pathways to achieve the zero-waste goal and accelerate the transition of the food industry to a circular bioeconomy.

Several by-products represent a rich source of valuable compounds (fibre and pectin, proteins, polyunsaturated fatty acids and other bioactive compounds), which through biotechnological methodologies can be extracted for several industrial applications. In this context, the management of food by-products is challenged to move from a linear economy to a circular economy.

Several green technologies (polyelectrolytes precipitation, enzymatic hydrolysis, HPP, etc) may be cost-efficient technologies that allow natural production of added-value compounds, namely those with potential biological and functional properties. Several examples of extraction or conversion of components from by-products into new added value ingredients/products with application in food, feed and cosmetics have been studied recently by our research group[2,3,4,5].

During this presentation the by-product valorisation in a Circular economy context will be discussed focused on the application of green technologies and how to obtain safe and bioactive and functional ingredients towards the incorporation in food sector and synergic industries. The presentation will include examples of technologies and new ingredients obtained from key agrifood by-products valorisation encompassing research cases studies developed by our research group on plant, animal and fermentation by-products, demonstrating the main relevance of the integral valorisation using biorefinery and zero waste approach.

Yet, the obtention of several ingredients with bioactive properties (antioxidant, anti-inflammatory, antimicrobial, prebiotic, etc) from these by-products will be discussed as source o potential new food ingredients/products to respond the needs towards healthy and sustainable diets.

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New analytical solutions for the global challenges of food safety

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The COVID-19 pandemic compounded by extreme weather events have shone a spotlight on the underlying vulnerability of our global food systems [1]. Food safety management systems and analytical methods which have been established to tackle foodborne hazards, including chemical hazards need to be adapted to make them more robust and flexible towards changes affecting our global food systems, resulting in proactive risk management that can make the EU food system future-proof.

Climate-based impacts, such as heavy rainfalls, will lead to a higher contamination rate of plant food sources and extensively housed farm animals. Particularly, unintentionally present chemical contaminants in food [2], such as environmental and food process contaminants (e.g. furans) and natural toxins (esp. mycotoxins and plant toxins), can increasingly pose public health concerns. To combat emerging chemical contaminants and associated risks, a number of advanced analytical approaches will be discussed in this talk:

a) In view of climate change, prediction tools for mycotoxin and plant toxin occurrence in major food commodities are to be improved through machine learning and big data approaches (involving satellite imagery), allowing extended and more accurate forecasting.

b) Novel, (bio)analytical sensing and diagnostic tools (e.g. integrated into smartphones) would enable to determine e.g. plant toxins in herbal teas and in buck wheat.

c) Advanced analytical strategies to understand the degradation of furans and (estrogenic) mycotoxins during mechanical and thermal processing of contaminated food crops under industrial conditions are needed to produce safer (baking) products. Studies have started to study the influence of gluten-free products and the fate of tropane alkaloids during pasta production, including the identification toxicological assessment of possible thermal degradation products using isotope labelling assisted mass spectrometry.

d) To study the impact of climate change on the presence of emerging (toxic) secondary metabolites and agrochemicals, horizon scanning approaches using a combination of a targeted LC-MS/MS and untargeted LC-HRMS (liquid chromatography-high resolution mass spectrometry) metabolomics have recently been developed and applied.

e) Combatting the illegal addition of toxic chemicals to food that pose an unchecked food hazard is just another analytical challenge to be met in view of globalization

This talk will focus on the advancement of innovations to combat selected (emerging) chemical contaminants in view of climate change and globalisation based on cutting edge approaches including novel tools in analytical chemistry.

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KEYNOTE LECTURES

KN 1 KEYNOTE

Improvement of Olive Oil Flavor and Bioactive Composition by Optimizing Industrial Extraction Using Taste Sensor Devices

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Olive oil plays a key role in the Mediterranean diet, possessing unique and recognized nutritional and health properties as well as highly appreciated organoleptic characteristics. These positive attributes are related with olive oil fatty acid profile and richness in phenolic compounds that support health claims concerning olive oil protective effects on blood lipids. In addition, phenolic compounds enhance olive oil overall sensory quality, being mainly responsible for the usual olive oil pungency and bitterness. However, to ensure that oils possess a rich phenolic content, the extraction conditions can be optimized.

In this sense, the PhD thesis intended to contribute to the production of differentiated and high-value phenolic-enriched olive oil envisaging nutritional and health promotion, as well as the oils' positive sensory attributes improvement. Additionally, the feasibility of electrochemical sensor devices acting as alternative/complementary monitoring analytical tools for the olive oil industry, was also evaluated.

In order to obtain oils rich in phenolic compounds, different variables of the oil extraction process were studied, namely malaxation time/temperature and the effects of water addition during the milling step. In addition, the oils' co-extraction with olive tree leaves, as well as the olive inoculation with fungi suspension before the oil extraction, were also studied. In this study, two olive cultivars were used (*cvs.* Cobrançosa and Arbequina).

Through the different studies carried out within the scope of this PhD thesis, it was possible to obtain phenolic-enriched olive oils taking into account different extraction process conditions.

For oils industrially extracted from *cv.* Cobrançosa olives, the best malaxation temperature to obtain oils richer in phenolics and better chemical-sensory quality was 22 ºC, comparing with those extracted at 28 or 34 ºC, probably due to a reduction of the enzymatic activity of endogenous glycosidases and oxidoreductases enzymes.

Oils from *cv*. Arbequina extracted at industrial scale, without water addition during the milling step, resulted in oils richer in phenolics and higher shelf life related parameters, comparing with those extracted with 1.2, 3.5 and 6.2% (w/w, $kg_{addedwater}$/kg_{olives}), explained by the amphiphilic characteristics of secoiridoids aglycons. The increase of the water amount favors the migration of the secoiridoids compounds to the water phase, resulting in oil with lower level of this class of phenolic compounds.

It has been shown that 1% (w/w) of olive leaves incorporation during the oils' industrial extraction can increase the phenolic concentration, pigments, oxidative stability, and positive sensory attributes. However, at laboratory scale, it was observed that the oils' co-extraction with leaves reduced the phenolic content but improved their sensory characteristics (green fruity and apple sensations), due to the impact of leaves on the enzymatic pathways involved on the formation of the main oils' phenolic and volatile compounds.

The inoculation of *cv.* Arbequina olives with *Epicoccum nigrum* suspension for 24-h before the oil extraction increased oils' phenolic concentration, and their shelf life related parameters, probably due to the hydrolytic activity of the enzymes secreted by the fungus, favoring the cellular membrane hydrolysis, contributing to the phenolics hydrolysis into smaller molecules, leading to different phenolic concentrations of the obtained oils.

Furthermore, the PhD thesis showed the feasibility of using lab-made electrochemical sensor-based devices (electronic tongue and nose), in the discrimination of different samples of oils, with different phenolic, volatile, and/or sensory profiles.

Finally, these achievements constitute important advances in the development of new strategies not only to enrich oils in phenolic compounds, but also to improve their chemical-sensory characteristics. Additionally, emerging sensor-based analytical tools can contribute to the oils' quality assessment in the industrial sector, from the beginning of the process to the labeling of the final product.

Keywords: Olive oil; Phenolic compounds; Volatile compounds; Olive oil quality; Electrochemical devices; Sensory analysis.

KN 2 KEYNOTE

Micro- and Nanoplastics in Food Current Analytical Methods and Challenges

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In recent years micro- (MP: 1 µm – 5 mm) and nanoplastic (NP: <1 µm) particles [1] have been detected more and more frequently in all environmental media, with the main sources and entry paths being the improper disposal of plastic waste and from environmentally open plastic applications [2] as e.g. tyre wear and geotextiles. Through the food chain, but also upon processing, packaging and preparation, MP and NP have found their way into foods and beverages and ultimately into animals and humans. Many studies focussed on fishery products and sea salts so far, but microplastics have also been detected in for example packaged beverages, tap water, sugar, honey, packaged meat, flours, rice, fruit and vegetables [3, 4]. Inhalation and ingestion are the major human exposure routes for these tiny plastic particles. In humans, MP have been found not only in the gastrointestinal tract e.g. in feces [5], but also in blood [6], the respiratory tract e.g. in sputum [7] and lung tissue [8], in placenta and meconium [9], liver [10] and saphenous vein tissue [11].

From the large number of analytical methods for the detection of microplastics, pyr/TED-GC-MS for the determination of mass concentration and identification as well as Raman and FTIR microscopy for identification and morphological description are now most frequently used [12]. However, the comparison and interpretation of data from different studies is difficult, as there are no harmonised strategies for the analysis from sampling to evaluation. The wide variety of morphological and chemical properties to be considered in the characterisation of MP and NP pose a major challenge for method development. Standard methods, which are currently being developed by various national and international organisations, promise to remedy this situation.

In principle, the analytical requirements for the detection of MP and NP are similar to those for the analysis of other food contaminants (e.g. chemicals) with regard to sampling, sample contamination, sample processing without damage to the analyte etc. However, an additional challenge is the presence of the contaminant in particulate form and in low (number) concentrations. As an example, a processing method for these small particle concentrations in complex samples such as fish fillets is presented [13]. Unlike dissolved contaminants, homogeneous distribution is not guaranteed for particles in low concentrations, even after careful sample homogenisation.

The analysis of nanoplastics in complex matrices as food is even more challenging. In addition to the challenges of analysing microplastics, including process-related sample contamination, accumulation of small amounts of plastic and minimisation of matrix effects, the nanospecific properties of the material pose further challenges. For example, separating nanoplastics from larger matrix components by filtration is more difficult because nanoplastic particles even do not pass quantitatively through coarse filters but adsorb at the pore edges.

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KN 3 KEYNOTE

Overview On Analytical Methods For Allergen Control In Foods And Compliance With The Proposed Reference Doses

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Food allergy is considered a major safety issue due to the increasing incidence recorded throughout the European population and the life-threatening consequences that can cause in sensitive consumers [1].

To protect consumers' health, risk assesment workflows have been developed and adopted by the food industries to limit the risk of allergens contaminating foods. According to the European legislation the presence of allergens intentionally introduced into foods must be declared in the list of ingredients as prescribed by the EU regulation 1169/2011 mandating the labelling of 14 allergens, and related products, on the food backage. On the other hand, the extreme variability of individual sensitivity to allergens, the threshold levels and the absence of validated and standardised analytical methods for their quantification in foods have so far represented the main causes for the absence of a regulatory framework for the management of hidden allergens.

The need to guarantee a higher level of safety to allergic consumers and protect them in case of a cross-contamination occurring in foods expected to be allergen free, has prompted the food industries to make excessive use of Precautionary Allergen Labelling (PAL), also leading to a loss of consumer confidence and an underestimation of the risk related to the presence of the allergens declared in the food purchased. In order to overcome these drawbacks, analytical methods with challenging sensitivity basing of trustful and reproducible results have been developed over the last two decades and it has been recorded a flourishing activity of methodologies based on DNA, antibody recognition or mass spectrometry for allergen detection in different foods. Among the several efforts done over the years, the recent ThRAll project funded by EFSA [2] was focused on the development of a standardised MS-based multiple reaction monitoring method(s) capable of quantifying along the same analysis a total of six foods causing IgE-mediated food allergies namely milk (as cow's milk), egg (as hen's egg), soybean, peanut, hazelnut and almond in a complex food matrix produced at laboratory scale [3-6].

In this note it will be given a comprehensive overview on the different analytical approches developed for quantification of allergens and will be presented final results obtained within the ThRAll project [7]. Limitations and advantages of the methodologies developed will be also discussed an compared to each other in compliance with the reference doses for the different allergens as recommended by the FAO/WHO expert consultation working group and the action levels proposed as a part of the VITAL® (Voluntary Incidental Trace Allergen Labelling) program by Australia and New Zealand.

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KN 4 KEYNOTE

Essential oils Antimicrobial Activity and Their Role in Food Safety

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The contamination of food products by various microbes, including bacteria, and fungi, etc., is a significant problem for the food industry. These microorganisms, by producing various toxins during pre- and post-harvest processing, degrade food products [1]. To reduce the effect of microbial activities in food products, a variety of EO types and their individual components are used as natural antimicrobial compounds [2]. Considering this trend, our work deals with in vitro antibacterial and antifungal activity of 5 commercially available essential oils including basil, bitter orange, cedar, clove, and coriander EOs, in different concentrations against selected species of Gram-positive bacteria, Gram negative bacteria, yeasts, and microscopic filamentous fungi *in vitro* and *in situ*. Gram-positive bacteria (*Bacillus subtilis* **CCM 1999,** *Enterococcus faecalis* **CCM 4224,** *Micrococcus luteus* **CCM 732,** *Staphylococcus aureus* **subsp.** *aureus* **CCM 8223), Gram-negative bacteria (***Pseudomonas aeruginosa* **CCM 3955,** *Pseudomonas fluorescens* **CCM 1969,** *Salmonella enterica* **subsp.** *enterica* **CCM 4420,** *Serratia marcescens* **CCM 8588), and yeasts (***Candida krusei* **CCM 8271,** *Candida albicans* **CCM 8261,** *Candida tropicalis* **CCM 8223,** *Candida glabrata* **CCM 8270)** and strains of microscopic filamentous fungi of the genus *Penicillium* **(***P. expansum, P. italicum, P. aurantiogriseum, P. chrysogenum*) were used to carry out the experiment. The disk diffusion method and the minimum inhibitory concentration method were used in *in vitro* conditions. The vapor phase method was used to test antimicrobial activity *in situ*. Bread, apple, pears, carrots, and celery were used in this study as food models for the development of different microorganism species. The *in vitro* antimicrobial evaluation of EOs revealed that it was successful in preventing the growth of a variety of tested microorganisms, and that its efficacy was primarily concentration dependent. Even when used against resistant bacteria, we observed an inhibitory effect. *In situ* tests also captured its antimicrobial potential. Additionally, the findings suggest that adding EOs as a natural antimicrobial agent to the active packaging of food items, such as fruits, vegetables, and bakery goods, may extend the shelf life of these products. The simple extraction of EOs, their environmental friendliness, and their broad range of established biological functions all of which are supported by our study are the main reasons why they are so advantageous to the food industry. We intend to include sensory analyses that would disclose what EOs concentration is acceptable to product consumers to explain the usage of EOs as an antimicrobial agent. Additionally, these findings support our earlier study by giving a thorough overview of the biological actions of several commercial EOs bought from a Slovak company.

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We know how to extract proteins from rapeseed - NapiFeryn BioTech for food sustainability

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One of the key challenge facing the world today is that current food systems cannot provide adequate, healthy food for all humans. Malnutrition affects approximately one-third of the total population. In addition, food production has a negative impact on the environment – it emits greenhouse gases, is a major cause of biodiversity loss, deforestation and water pollution. At the same time, the livestock production raises justified ethical questions. Therefore, there is a strong evidence that more sustainable food production is a must [1]. It is projected that by 2050 the demand for food, especially for meat and dairy products, will more than double and the global meat production in 2029 will reach 366 Mt [2-3]. Partial replacement of animal protein with plant protein, which is not only more environmentally friendly, but also beneficial on human health, seems to be inevitable in this situation [4].

Rapeseed ranks second (after soybean) in the global production of oilseeds and the second largest rapeseed producer is the European Union [5]. Rapeseed is mainly used for oil production [6]. However, this plant is a promising source of a new protein, due to its nutritional value, well-balanced amino acid profile and functional properties. It is also an alternative to the omnipresent soy protein [7-9].

NapiFeryn BioTech developed and patented technology to obtain food grade proteins from oilseeds. The company's primary focus is rapeseed, namely the material left over after pressing oil from rapeseed. NFB mission is to improve life quality using technology to produce natural and functional plant derived proteins. The innovative technology is safe, up-to-date and efficient. It doesn't require using a harmful hexane and the conditions in which protein is isolated are mild so that it is not denatured. The NFB technology makes it possible to retrieve nearly all protein contained in rapeseed (which makes the proposal unique worldwide) as two complementary products – protein isolate (Raptein® 90) and protein-fibre concentrate (Raptein® 30). Raptein® 90 possesses above 90% of highly digestible protein. It is characterized by high solubility and ability to create stable gels, emulsions and foam, therefore it's a desirable food ingredient in such products as dairy analogues, beverages, confectionary or dressings. Raptein® 30 is a natural mix of protein and insoluble fibre with high water and oil absorption capacity and fit for applications in bakery products, meat analogues or meat extensions.

The company is scaling up the technology and delivers pre-commercial research samples from the pilot facility. The business model is to commercialize the technology package to through licensing agreements with oil processors.

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Understanding food aromas: in the frontier between sensorial and analytical data

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The human senses shape the life in several aspects, namely well-being, socialization, health status, diet, among others. However, only recently, the understanding of this highly sophisticated sensory neuronal pathway has gained new advances. Also, it is known that each olfactory receptor cell expresses only one type of odorant receptor, and each receptor can detect a limited number of odorant substances. Odorant substances are typically volatile or semi-volatile in nature, exhibit low relative molecular weight and represent a wide variety of chemical families. These molecules may be released from foods, constituting clouds surrounding them and are responsible for their aroma properties[1].

A single natural aroma may contain a huge number of volatile components, and some of them are present in trace amounts, which makes especially difficult their study. Understanding the components of food aromas has become more important than ever with the transformation of food systems and the increased innovation in the food industry. Advanced chromatographic technique such as comprehensive two-dimensional gas chromatography coupled with mass spectrometry with time-of-flight analyser (GC×GC-ToFMS) seems to be a powerful technique for the analytical coverage of the food aromas. This technique seems to fulfil the requirements of the innovative strategies in the field of the aroma chemistry such as the smell digitalization and sensomics. Indeed, innovative enhancements, such as smell digitalization and sensomics, that are multi-step analytical approaches, are used to obtain the multipart odour picture of a food, which include the identification and accurate quantitation of odorant molecules. Smell digitalization allows the measuring and chemically revealing of the smells to making them in a digital presentation, which represents cutting-edge research, usually using artificial intelligence to interpret the odour signatures. While sensomics is an approach developed to help in the mapping of both aroma and taste key active molecules, which are perceived by humans' chemosensory receptors, and then integrated by brain.

Furthermore, for a holistic understanding of the aroma of foods, it is crucial to define a broad strategy, involving diverse techniques that can assess the multiple dimensions of the aroma perception, that are intrinsically associated with the multimodal perception concept, i.e., multimodal phenomena concern stimuli that generate simultaneous (or nearly simultaneous) information in more than one sensory modality[2]. Certainly, the chemical aroma data is not enough, but crucial to move forward on this challenging. Thus, this talk will be focused on the advanced tools used to unveil the chemical nature of the food aromas, and their relevance to the quality of life and to the new challenges of society. Due to the huge significance of human olfaction in several fields, namely, to improve nutritional health, diagnose and treat diseases, understand consumer preferences and consumption, measure and chemically reveal the smells represents cutting-edge research with an increase of such a tendency to be expected in the future.

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ORAL PRESENTATIONS

T1

Food composition, quality and safety

Assessment of chemical modifications on the reduction of the allergenic potential of legume based proteins

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The world population is growing continuously, and in particular, the demand for protein ingredients has considerably grown globally in the past few years, resulting in a higher interest in the search of alternative protein sources[1]. The introduction of new protein-based foods requires a risk assessment of potential allergic responses. Nowadays, legumes are the major non-meat protein source introduced in our diet due to their high nutritional value. However, the use of large quantities of legumes poses food safety problems related to allergenicity[2]. As a matter of fact, among the 14 allergens officially listed in Annex II of Regulation EC no 1169/2011, IgE-binding proteins have also been identified in minority legumes, namely peas, beans, lentils and chickpeas, among others[4.] The modification of plant proteins, by altering their physicochemical properties, such as enzymatic hydrolysis ,provides the possibility to improve their techno-functionality and biological activities. Chemical modification[3].

The aim of this work is i) the molecular characterization of allergens from different fresh legume matrices, such a chickpea, pea, white bean and Mung bean protein isolate, ii) the setup of enzymatic hydrolysis protocols on different legume matrices, iii) the study of how this can affect the reduction of the allergenic potential of legume protein matrices.

A first characterization of allergenic proteins of the initial legume matrices by in-gel triptic digestion and High-resolution Mass Spectrometry (HR-MS) was done, followed by in silico allergenicity assessment. In vitro-allergenicity assays were then performed on, human sera selected based on evidence of allergy to legumes by clinical history, skin tests and/or positive specific IgE to different legumes. The immunoreactivity of initial legumes was tested, and in particular for Mung Bean cross-reactivity with soybean and pea known allergens was proved.

The second part of the work was mostly focused on the use of proteolytic enzymes to produce protein hydrolysates on the different legume matrices. Three commercial proteases were used, alcalase, papain and flavourzyme. The efficiency of the enzymatic reaction was studied by the determination of the protein content (Kjeldahl method) and the degree of hydrolysis (OPA/NAC analysis). The results showed that the protein content of the hydrolysates is related on the different type of enzyme and the legume matrix. Regarding the enzymes used, alcalase shows a more efficient enzymatic activity. Peptides fragments released after the enzymatic reaction were also identified by Waters Vion IMS QTof Mass Spectrometer instrument.

As enzymatic treatments are among the most common and effective processes for the reduction of food allergenicity, the allergenic potential of the enzymatic hydrolysates has been tested through immunoblotting assays with sera of patients with positive specific IgE to different legume species. The majority of hydrolysates from alcalase did not shown immunoreactivity, indicating a loss of the epitopes due to the enzymatic process. Indeed, the hydrolysates obtained with papain still showed residual immunoreactivity in some subjects for some of these matrices. The results showed in this study clearly confirm that protein hydrolysis can be a very effective strategy to reduce allergenicity, but also that the efficiency strictly depends on the nature of the enzyme and on the protein matrices used. Further studies are needed to determine the effect of different enzyme combinations and other chemical modification for the reduction of allergenic potential of legumes, leading to hypoallergenic foods production.

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Dark matter revealed: the brown or black color of fine chocolates probed by polyphenol metabolomics and molecular networking

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Fine-quality dark chocolates (70% cocoa content) have a dark brown color [1] that is partially influenced by phenolic compounds [2]. However, some of those chocolates have a light brown color, which is a challenge for manufacturers that opens new marketing strategies.

The aim of this work was to evaluate the phenolic profiles of dark chocolates having a black and brown color to reveal discriminating compounds. Among 37 fine chocolate samples provided by Valrhona and made from twenty different *Theobroma cacao* clones in years 2019 and 2020, eight dark black samples and eight light brown samples were selected. Non-targeted metabolomics approach based on UHPLC−HRMS/MS experiments, univariate and multivariate statistical analysis, as well as Feature-Based Molecular Networking analysis (FBMN) [3] was conducted. The analysis of metabolites differentially accumulated in brown and black chocolates showed an overaccumulation of 27 metabolites in black chocolates. Among them glycosylated flavanols and small glycosylated A-type procyanidins (dimers and trimers) were highly representative. On the other hand, 50 overaccumulated metabolites were found for brown chocolates. Among them, 27 larger B-type procyanidins, from trimers to nonamers, were present. *C*-glycosylated or oxidized dimers or trimers and dehydrodicatechins B also participated in the discrimination of the two sets of samples. Phenolic and color profiles were mostly related to genetic factors, *i.e.* the studied cocoa clones. However, environmental conditions may also have influenced the black chocolate color. This study provides new insights on the phenolic profiles of black and brown chocolates that may be useful to better understand the color variations of dark chocolates. Moreover, owing to their different phenolic profiles, these two types of chocolates may have different functional properties for human health.

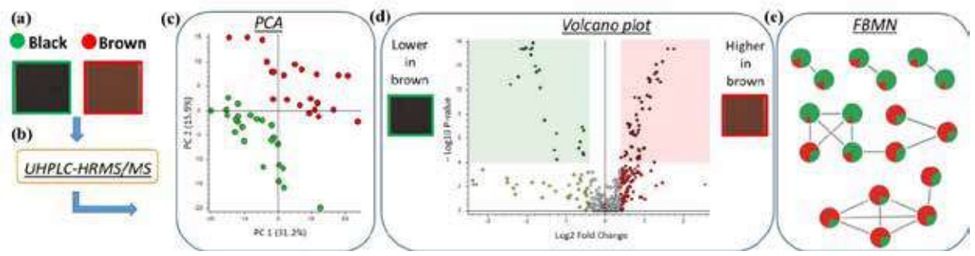

Fig.1. Chocolate samples (a), type of instrumental analysis (b), multivariate (c) and univariate (d) statistical analysis, as well as FBMN analysis (e) to reveal discriminating compounds of black and brown chocolates

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An approach on how to modulate recipe to reduce sugar in biscuits

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Biscuit is a bakery product that is frequently preferred by consumers of all ages because it is cheap, accessible, and ready for consumption. However, recently increase in diseases such as obesity, heart disease, type-2 diabetes and life trends direct the consumer, and therefore the industry and academia, to healthier options.[1] One of the most controversial ingredients from a health perspective of bakery products is the high sugar content. The most used sugar in bakery products is sucrose, as it is inexpensive and accessible, as well as giving biscuits their characteristic features.[2,3] Although the health effects of excessive consumption of sucrose are worrisome, reducing sucrose in bakery products is a major challenge, as it provides many characteristics in addition to a sweet taste.[1,2,3] Within the scope of this study, changes in sweetness perception and aroma compounds were examined by making various changes in the biscuit formulation, and the sweetness perception data were modelled for the first time using the modified Weibull model in order to explain the results mathematically. For this purpose, whole grain flour was added instead of some of the refined flour in the biscuits, protein enrichment was made to the biscuits, flavouring substances were added, the fat content of the biscuits was reduced, and the sugar type and the distribution of sugar in the dough were changed. The changes in perceived sweetness were determined by sensory analysis when the sugar concentration was changed between 6-39% for the biscuits. Volatile compounds were analyzed to explain the differences in the perceived sweetness due to the formulation changes made in biscuits. In conclusion, it was found that it is possible to obtain healthier products by adding fibre, protein, or flavouring substances to biscuits and thus reducing the amount of sugar. Moreover, by modelling the changing sweetness scores in response to sugar concentration in reformulated biscuits by using the modified Weibull model, a sensory analysis-based approach that can be used in future studies without the need for analysis has been presented. This approach can also be used by the food industry to create a catalogue for their own products for sugar reduction purposes.

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Methylated Lysine Derivates in Food

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As one of the canonical amino acids, lysine is a well-known educt for chemical modifications of food proteins. The reaction of its ε-amino group is mainly observed during processing of food. In presence of reducing sugars, lysine can be glycated. The so called Maillard reaction forms flavourings and colourants [1,2]. Not only in food but also in the human organism does lysine-modification play an important role. Enzymes can methylate the lysine-nitrogen with formation of *Nε*-mono- (MML), *Nε,Nε*-di- (DML) and *Nε,Nε,Nε*-trimethyllysine (TML). Especially in fat metabolism, TML is a relevant metabolite. If too little carnitine is consumed in the diet, it is synthesised in the cytosol from TML. The needed TML is formed from protein-bound lysine [3].

So far, free TML has been determined in noteworthy concentrations mainly in sweet pepper (8-18 mg/kg), alfalfa sprouts (2-10 mg/kg) and chickpea flour (2-5 mg/kg) [4]. In comparison, the levels of total TML in animal-based foods such as meat, fish, cheese or eggs are significantly higher (levels between 9.7-136.8 mg/kg) [5]. There are no literature data on free or protein-bound MML and DML. The background of this work is the quantification of free methylated lysine derivatives in other food groups besides vegetables.

After processing and homogenisation of the solid and liquid food samples, the derivatives were extracted and concentrated by solid phase extraction. The measurement was carried out by LC-MS/MS in the MRM mode. For quantification, the respective isotopologues were synthesised and used as internal standards.

Beyond TML, free MML and DML were qualified for the first time in different vegetables. Quantification and investigation of other food groups will follow.

Our own data as well as literature data suggest a significant daily intake of methylated lysine derivatives. Further investigations must therefore deal with their bioavailability and possible digestion by the intestinal microbiota.

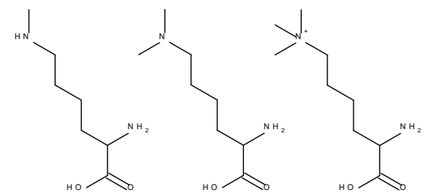

Fig.1. Structure of methylated lysine derivates

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Corky off-flavour in garlic? The presence of haloanisoles in garlic as serious problem for food industry

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Garlic (*Allium sativum*) is a vegetable and spice that is used in the kitchens throughout the world. According to the FAO, the worldwide production reached more than 20 mio metric tons in 2020, whereas China is the largest garlic producer with a relative amount of approx. 80% or the worldwide production (www.worldaltlas.com). The occurrence of corky off-flavour which is mainly caused by the presence of the haloanisole 2,4,6-trichloroanisole in garlic is of serious concern especially for food processing companies as occurrence of this off-taint may cause significant economic damage.

The reasons for off-flavour formation in garlic are not completely understood. The occurrence of dry rot of stored garlic caused by contamination with *Fusarium proliferatum* is reported as serious matter in garlic cultivation and storage, respectively [1]. Treatment of garlic with the fungicide prochloraz seems to be efficient to prevent garlic deterioration [2, 3]. Degradation of prochloraz leads to the formation of 2,4,6 trichlorophenol which was reported to be degraded in cork by filamentous fungi under the formation of 2,4,6-trichloroanisole via O-methylation [4]. This reaction might also be feasible for other microorganisms such as *Fusarium* in garlic cloves. Irrigation of garlic cultures with TCA contaminated water might be another reason for the presence of TCA in garlic [5].

In this study, we performed a survey on the presence of the haloanisoles 2,4,6 trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) in different garlic samples (i.e., fresh garlic, deep frozen garlic, garlic powder and granule obtained from different sources). Quantification of the TCA and TBA amounts was performed after enrichment of the analytes from the sample headspace via SPME “arrows” and gas chromatography triple quadrupole mass spectrometry with multi reaction monitoring. To be able to evaluate the sensory impact of the investigated haloanisoles in the garlic samples, we investigated the impact of the garlic matrix on their sensory thresholds.

With this investigation, we aim to build a basis for the evaluation of garlic quality and to demonstrate the importance of this topic for food industry.

Acknowledgments: The authors are grateful to the members of the sensory test panel for their volunteer evaluation the off-flavoured garlic samples. The authors thank Elisabeth Spenger and Sigrid Hager for technical assistance in sensory evaluation and GC-analysis. Furthermore, the authors acknowledge Compusense, Guelph, Canada for providing the opportunity of being part of the Compusense Academic Consortium.

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Chemical and bioactive profile of five fig fruit varieties

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Figs are a popular fruit enjoyed all over the world and have been consumed for thousands of years. These fruits are mentioned in various ancient texts and folklore from all around the world; in fact, the ancient Greeks considered figs as sacred fruits and believed them to be filled with life-giving properties [1]. Figs are an excellent source of vitamins, minerals, and other nutrients that are essential for a good health, due to its bioactive properties [2]. They contain antioxidants that can help protect against oxidative damage and inflammation, and also antimicrobial compounds that can stimulate the immune system and help fight infections [3]. There are many distinct fig varieties, and each variety has its own characteristics Because there is little information available in this subject, the present research was designed to carry out the chemical and bioactive profile of five different fig fruit varieties, namely Dauphine (Da), Longue d'Aout (La), Pasteliere (Pa), Marseille (Ma), and Bourjassote Noire (Bn), enabling a deeper understanding of this well appreciated fruit. The fruits' chemical profiles were evaluated using HPLC-RI for free sugars and the variety Da (87.78 g/100g dw) presented the highest level of these compounds. The variety with the greatest results for tocopherols, performed by HPLC-fluorescence, was Ma (2.40 μg/100g dw). For the profile in organic acids, performed using UFLC-DAD, Bn (34.25 mg/g dw) and Ma varieties also provided the highest contents. The fruits' phenolic compounds assessed using HPLC-DAD/ESI-MS revealed that the highest amount of total phenolic compounds belong to Bn (1.965 mg/g extract), where 7 different compounds were identified. Different methodologies were also applied to assess the bioactivities of the hydroethanolic extracts obtained by maceration of the five fig varieties. The antioxidant activity was assessed using the TBARS and CAA assays, and it was possible to confirm that the Pa (EC_{50} = 2.18 mg/mL) variety revealed the best results for TBARS assay, however none of the five varieties showed results for the CAA assay. It was possible to confirm that among all the bacterial strains tested for the study of antimicrobial activity, *S. aureus* (MIC = 10 mg/mL) was the one that was most sensitive to all types of fruits, and that *A. brasiliensis* (MIC = 5 mg/ml) was the most susceptible fungus. Only two of the three tumour cell lines were inhibited, namely CaCo2 and MCF7. For CaCo2, all varieties exhibited anti-proliferative capability, particularly Ma (GI_{50} = 118 μg/mL). For MCF7, the variety with the strongest anti-proliferative capacity was Da (GI_{50} = 156 μg/mL). None of the samples exhibited toxicity against the PLP2 normal cell line. Regarding the anti-inflammatory potential none of the varieties displayed activity. In general, it was possible to conclude that the variety that stood out in the chemical characterization was the Bn variety, whereas when it came to the results obtained in the assessment of the various bioactivities, Pa stood out, highlighting that these fruits can contribute to health maintenance by providing bioactive properties.

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Alternatives to the titanium dioxide (E171) whitening colorant in foods

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Titanium dioxide (TiO2) is a white and opacifying agent widely used in several areas, namely in pharmaceutical, cosmetic, and food industries. In Europe, TiO2 had been used as a food colour additive (E171), with the technological function of making food more visually appealing, providing a white colour to colourless foods, namely in sauces, icings, chewing gums, and candies. However, in 2022 European Food Safety Authority (EFSA) determined that TiO2 is no longer authorized as a food additive, due to its potentially harmful effects to human health [1]. Consequently, European food industries are seeking for white colorant alternatives. Thus, the aim of this study was to develop alternatives to the white TiO2 powder to be used in food stuffs. The strategy used was the consolidation of starch with an inorganic material, which consisted in swelling of the starch granules, and, consequently, decreasing the distance between inorganic particles, bringing them into contact to form a solid network [2]. Therefore, rice starch was consolidated with different inorganic additives approved by EFSA, namely calcium carbonate (E170), calcium phosphate (E341), silicon dioxide (E551), and calcium silicate (E552), by gelatinization at 90 ºC during 30 min. The resulting white powders were then milled and sieved. As a showcase, the different white powders were applied in the recipe of commercial candies. The measurement of colour by CIELAB system (L*a*b* coordinates) revealed that candies produced with the addition of rice starch consolidated with silicon dioxide are more promised (L*=69), following by the mixture of rice starch with calcium silicate (L*=61), calcium phosphate (L*=53), and calcium carbonate (L*=48). In conclusion, the consolidation of rice starch with inorganic compounds revealed to be a good strategy as white food colour additives to replace TiO2.

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Microgreens and germs: The gleam of next-generation super foods - manipulations in production technologies and future strategies for maintaining the shelf life and quality of products

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Considering the well-being cognizance of masses, microgreens and germs have emerged as potential therapeutic functional foods for improving overall health by dietary supplementation. Microgreens and germs have an exceptional volume of various nutrients accounting for higher nutritive benefits compared to their mature counterparts [1]. These plants are difficult to store in fresh, due to their high surface area to volume ratio, high respiration rate, delicate leaves that easily wilt, rapid post-harvest decay transpiration, leakage of nutrient-rich exudates, tissue damage, and early senescence [1]. Therefore, within this study, the effect of drying on the sugar content of 12 samples of different microgreens and germs was monitored. A comparative study of the content of vitamin C and sugar between samples of microgreens and germs in both dry and fresh states was also done. The content of vitamin C was determined by the HPLC method, while the sugar profile was obtained using HPAEC-PAD. The results showed that there was a difference between samples of microgreens and germs (fig. 1), as well as the contents of vitamin C and sugar in the samples are inversely proportional (fig. 2), which is in line with the fact that part of the sugar is converted into vitamin C during ripening, which then serves as an antioxidant and protects against harmful effects [2]. Analyzes also showed that dry samples have a higher sugar content compared to fresh ones (fig.2.), which could serve as an argument to promote cost-effective production and future strategies for maintaining the shelf life and quality of these products in the sense that they do not lose their nutritional value during the drying process.

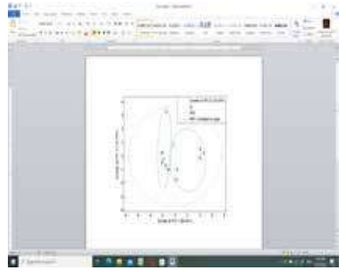
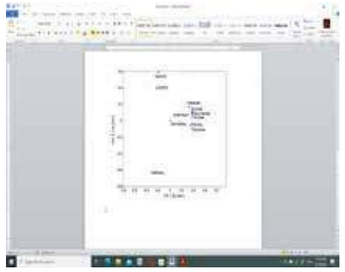
A  B 

Fig.1. Principal Component Analysis - The difference between microgreens and germs; score plot (A) – germ samples (G) 1-6, microgreen (MG) samples 7-12, and loading plot (B).

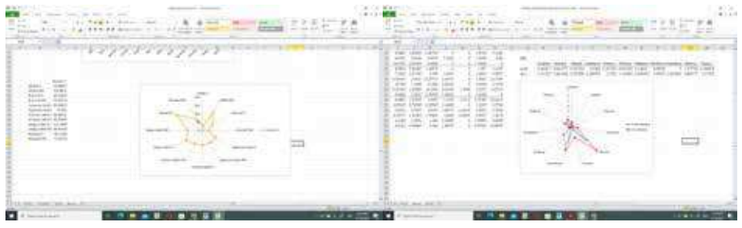


Fig.2. Content of vitamin C (A) and sugars (B) in dry and fresh samples of microgreens (MG) and germs (G).

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ORAL PRESENTATIONS

T2

Food sustainability, including byproducts valorization

Spent coffee ground and coffee silverskin: possible use as nutraceuticals and ingredients for fertilizer products

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Coffee is one of the most consumed beverages worldwide and according to the latest statistics the global consumption is more than 150 million of 60 kg coffee bags per year. Consequently, large amount of coffee residues needs to be disposed of. The main generated by-products are coffee silverskin (CS) and spent coffee grounds (SCGs): silverskin, the thin tegument that covers the coffee bean, is obtained when green coffee beans are roasted, whereas SCGs are mainly formed during the production of instant coffee. CS and SCG disposal represent an environmental problem but, at the same time, they are a potential source of valuable by-products.

Indeed, they are a good source of nutrients and bioactive compounds such as soluble dietary fibre, protein, minerals, fat, caffeine and polyphenols [1, 2]. In our research group, the two matrices have been fully chemically characterize in terms of polyphenols, fat and fatty acid profile, volatiles, cholesterol-lowering phytosterols, and vitamins [1-4]. The content of polyphenols and phytosterols have been monitored also in all the coffee production chain, starting from green coffee, to CS, roasted coffee, SCG and espresso coffee to have a complete view of theirs evolution. From a biological point of view, this research highlights that CS and SCG extracts protect cells against H2O2-induced oxidative stress by upregulating endogenous antioxidant enzymes such as thioredoxin reductase, heme-oxygenase 1, NADPH quinone oxidoreductase, and glutathione reductase. Moreover, the hydroalcoholic and methanolic SCG and CS extracts were shown to be the most active against all selected enzymes such as tyrosinase, α-glucosidase, α-amylase and α-cholinesterases. Finally, we evaluated the effect of CS/SCG-containing fertilizers on plant growth and modulation of soil microbiome. SCG and CS application as organic amendment are known to modify soil chemical-physical properties, provide macro and micronutrients fundamental for plant growth, reducing the need for inorganic fertilizer, and stimulate soil microflora exerting a beneficial effect on soil-plant systems. In this study, a novel granulated formulation of SCG and CS was developed and assayed for its potential toxicity on seed germination and root elongation of four crop plants.

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Chemical and functional characterization of innovative and healthy fermented plant-based products from sustainable land and sea vegetables

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Today, consumers look for products able to promote health, while at, the same time, valuing the sustainability and flavour of the products. So, to guarantee healthy and tasty products, while minimizing food loss and waste, are the main challenges faced by the food industry. Food fermentation has become an important mild-processing technology that takes advantages of the nutritional and health benefits resulting from microorganisms' metabolism to develop innovative, sustainable, clean-label, safe, and appealing food products [1].

Attentive to consumer demands, the ***FermentedVegAlgae*** project, a partnership between the Instituto Superior de Agronomia, Mendes Gonçalves and SONAE MC companies, blends innovation, environmental and health concerns, adding value to surplus vegetables (that would otherwise be discarded) and less-used nutritionally-rich plant sources (macroalgae), through the process of food fermentation.

Up to now eight fermented plant-based prototypes (sauerkraut-like and spreads) were developed: six from surplus vegetables (with white or red cabbage together with other vegetables like carrot, ginger, garlic and chilli), three from macroalgae (*Palmaria palmata* and *Alaria esculenta*), and three from mixtures of vegetables and macroalgae. Fermentation performance was evaluated by measuring pH, acidity and the total soluble solids, until stability.

The evolution of the fermentation process and centesimal composition of the final fermented products were performed. For the **vegetable fermentations**, 1% added NaCl and a consortium of 4 lactic acid bacteria (LAB) were used. After the fermentation processes have been completed, the products are pasteurised, and probiotic bacteria *(Bacillus coagulans)* were added to increase even more the probiotic potential of the products. After 21 days of fermentation, stable and safe pH and titrable acidity were achieved (3.37-3.54 and 1.35-1.76% lactic acid). For **macroalgae**, macroalgae were previously treated by ultrasounds, mixed with 0.3% added NaCl, and fermented by a consortium of the same LAB and *Debaryomyces hansenii* (a yeast that produces high levels of riboflavin and antimicrobial compounds). Through the fermentation process, pH dropped and titrable acidity increased, reaching food-safety values after 5-7 days (3.72-3.95 and 2.2%-3.6% lactic acid, for *A. esculenta* and *P. palmata*, respectively). In both, **vegetables and macroalgae** fermented products, firmness and consistency decreased. The reduction was more pronounced in the fermented vegetables, with white cabbage and chilli sauerkraut-like product registering the highest decrease in both parameters in comparison with the non-fermented product (77.6 % and 77.3% reduction in firmness and consistency, respectively).

The evaluation of their potential health benefits (antioxidant potential, minerals, content in vitamin B6 and B12, total phenols and flavonoids and mineral bioaccessibility) is being accessed. Due to the action of both LAB and *D. hansenii*, producers of fitases and oxalases that will help the release of iron and calcium, it is expected an increase in the bioaccessibility of these minerals.

Fermentation of vegetables and macroalgae can be considered a way for tailoring the technological and health-related functionality of food products while increasing their sustainability and market value.

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Chemical Characterization and Bioactive Properties of Winemaking Residues Towards their Possible Exploitation

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Grape pomace has been widely studied concerning its chemical composition and bioactivity since there is considerable interest from the food, cosmetic and pharmaceutical industries to turn this by-product rich in bioactive compounds into innovative ingredients [1]. However, other residues from the winemaking process, namely the wine lees and the diatomaceous earth used for wine filtration are less studied and underexploited products. In this study, these were compared with other more studied wine residues. The wine lees and the diatomaceous earth demonstrated to be potential sources of phenolic compounds, with the latter being rich in flavan-3-ols and presenting the highest amount of epicatechin among all extracts. The diatomaceous earth extract also presented one of the highest total phenolic contents in the Folin-Ciocalteu assay and showed the highest amount of non-anthocyanin phenolic compounds, mainly due to its relatively high content in myricetin-*O*-hexoside. The diatomaceous earth extract also revealed a promising antioxidant activity, showing the best results in the reducing power assay. Despite not showing results as good as the other extracts, the wine lees presented EC_{50} values ranging from 0.51-0.93 mg/mL, 0.68-0.78 mg/mL and 0.35-0.61 mg/mL in the DPPH, reducing power and TBARS assays, respectively. The red lees extract showed the highest capacity to inhibit bacterial growth, presenting a bacteriostatic effect against the 8 tested bacteria and lowest minimum inhibitory concentration (MIC) values particularly against methicillin resistant *Staphylococcus aureus* (2.5 and 5 mg/mL).
To estimate the potential of the extracts being relevant for other industries in a circular economy perspective, they were evaluated for their capacity in inhibiting skin enzymes, toxicity in skin cells and antimicrobial activity.
In general, the safety of the extracts for skin cells was demonstrated by the absence of toxicity in HFF-1 fibroblasts at the highest tested concentration (400 µg/mL). Nevertheless, the highest tested concentration of the lees and diatomaceous earth extracts exhibited some toxicity in the HaCaT cell line (human keratinocytes), which was not observed for lower concentrations. Overall, the extracts of grape pomace, seeds, skins and stems, were able to inhibit collagenase and tyrosinase, with best results observed for the red grape pomace (89±2%) and the seeds, (45.3±0.1%), respectively. However, a low or even absent inhibition was observed for the red wine lees, white wine lees and diatomaceous earth.
In addition, because there is evidence that the combination of bioactive compounds with antimicrobial agents can exert a synergistic outcome allowing the use of lower quantities of preservatives [2], possible synergies were evaluated between a grape pomace extract and food preservatives (nisin and citric) by the checkerboarder assay. Results showed the absence of synergy or antagonism, however additive antibacterial effect was observed for some combinations and bacteria, with best results for the extract + nisin being obtained against *S. aureus* and extract + citric acid against *B. cereus*.
Overall, the wine lees and diatomaceous earth showed potential to be used for the extraction of added-values compounds (anthocyanins, epicatechin and myricetin derivatives), while grape pomace, seeds and stems presented results that suggest their potential interest as ingredients in cosmetic formulations.

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PreVineGrape: Development of a biofungicide against grapevine pathogens using plants and food agro-industry bio residues

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Grapevine (Vitis vinifera L.) is one of the most important crops worldwide. Commercial cultivars are greatly affected by many pathogenic microorganisms that cause diseases during pre- and/or post-harvest periods, affecting production, processing, and export, along with fruit quality [1]. Plant/bio residue extracts are a valuable source of a wide variety of biologically active constituents. Several plant/bio residue bioactive compounds have been identified as possible antifungal agents, associated with their antioxidant properties [2]. In this sense, the valorization of plants and bio residues from the agro-food industry through the development of a natural product against fungal pathogens of grapevines is of great environmental and economic importance. For this purpose, the “PreVineGrape” project aims to provide to the wine industry a natural fungicidal agent, active against downy mildew, powdery mildew, and Botrytis cinerea, which cause massive damage to vineyards. A multidisciplinary team comprises this project, with recognized know-how and expertise regarding the development of natural antimicrobials and establishment of in vitro cultures (Instituto Politécnico de Bragança and Deifil); application of natural agents in vineyards (Sogrape Vinhos, S.A. and João Nicolau de Almeida & Filhos, Lda.), technical know-how in sustainable viticulture and management entity of the Vine and Wine Cluster Association for the Development of Douro Viticulture (ADVID). This project covers an extensive range of activities to obtain a biofungicide validated in vitro and in loco, exploring all its valences.

The first part of the project consists of developing a database containing all the detailed information on the phenolic profile of each of the analyzed plants using high performance chromatographic methods. To determine the ideal extraction conditions, these molecules will be recovered using maceration and ultrasound-assisted extraction and applied in the active doses established by the bioactive potential. Additionally, the severity index of grapevine pathogens after applying the treatments with the plants/bio residues product, by comparison with untreated vines will be assessed. The evaluation of the effect of the treatments on the physiological parameters of the vine will also be evaluated. Furthermore, the presence of active compounds in leaves and bunches over time will be chromatographically monitored. In addition, the effects of the treatments applied on the quality of the grapes will be evaluated through the analysis of the evolution of the physicochemical parameters by wet chemistry during the maturation of the grapes, as well as on the quality of the resulting wine. Finally, a new natural fungicide agent based on more sustainable viticultural practices will be developed, by reducing the use of artificial ones in the treatment of diseases that affect the quality and grapes’ production.

In this way, during this project, it is intended to carry out an integrated approach to valuing natural matrices and agro-food by-products with bioactive properties, namely from a wide range of species of plants rich in antimicrobial compounds, maximizing their use through its transformation into value-added products, with profitable application. Moreover, it intends to streamline micropropagation techniques and expand them to a wide range of plants of high commercial interest.

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ORAL PRESENTATIONS

T3

Novel foods

Food industry clean label strategies provided by carbohydrates

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Carbohydrates are food key components able to confer different properties, including sweetness, thickness, or even preservation. Their structural features are not yet always understood. The arise of new methodologies and technologies permit their revisiting, allowing to explain and propose novel food functions and applications. This contributes to the new trends on demand by citizens to have clean labels in a sense that food labels are informative labels, complementing tastier foods with recognizable, balanced and heathy attributes. In this work it is shown that it is possible to have reduction/elimination strategies to substitute phosphate in ham using polysaccharide-derived compounds. The hams have 75% of water content and a hardness of 30 N, comparable with the commercial ones. Another example of the challenge and solutions achieved so far as a clean label strategy is the increment of soluble dietary fibre and oligosaccharides in apple and pear juices. These oligo- and polysaccharides are mainly composed of degraded starch and pectic polysaccharides rich in uronic acids, arabinose and galactose residues. Turbid juices are richer in dietary fibre than the clarified ones, although clarified juices are richer in pectic oligosaccharides. A higher temperature of clarification tended to increase the content of dietary fibre. Also, chitosan films can replace conventional sulfur dioxide treatment of white wines [1,2]. This technology can be extended to vinegar when α-hydroxypolycarboxylic or α-cetopolycarboxylic acids are present, as in cider or wine vinegars [3].

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Development of a novel food ingredient from pine nut skin with prebiotic activity

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Pine nut skin (PNS) is a by-product recovered during pine nut processing, which is usually discarded for heat production. The exploitation of by-products as sources of valuable compounds agrees with the current demand for the reduction of waste, and a transition to a more sustainable production and consumption [1]. Therefore, PNS - an under-explored material - was characterized and its prebiotic potential was assessed.

PNS subcritical water extraction using microwave was optimized and the obtained extracts were separated into low-molecular weight (rich in phenolic compounds and oligosaccharides) and high-molecular weight (rich in carbohydrates). Their prebiotic potential was assessed by evaluation of their gastrointestinal digestibility and fermentability by human gut microbiota. Short-chain fatty acids produced during the faecal fermentation were evaluated. The impact of the extracts on the microbiota composition was analysed by 16S rRNA gene sequencing.

The digestion decreased the phenolic compounds content, but the oligo- and polysaccharides were not affected. Thus, it is proposed that these carbohydrates reach the large gut intact, where they can be fermented by the microbiota.

In the first 12h of fermentation, approximately 75% of the compounds (phenolics, oligosaccharides, polysaccharides) were consumed, which corresponds to the bacterial exponential phase. Nevertheless, during the 48h of fermentation it can be observed a preference regarding the polysaccharides’ degradation. The glycosidic residues from xylans and xyloglucans were the first to be consumed, whereas the rhamnose residues were higher in the end of the fermentation.

The fermentation of both low- and high-molecular weight extracts resulted in an increased production of acetic, propionic, and butyric acids as compared to a commercial inulin-type prebiotic. The analysis of the gut microbial population showed that the low-molecular weight extract did not impact the initial microbiota, while the high-molecular weight extract had an impact comparable to the inulin-type prebiotic. Therefore, both extracts revealed to have prebiotic activity, with the polysaccharide-rich extract having a more pronounced beneficial effect.

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Identification of potentially bioactive peptides from house cricket (*Acheta domesticus*) by *in silico* gastrointestinal digestion

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In recent years, entomophagy has gained relevance as a sustainable alternative source of proteins for human nutrition. Although it is an ancient practice that is still very common in several regions of the world, its implementation in Western countries faces some resistance due to cultural issues. The house cricket, Acheta domesticus, is one of the three insect species that complies with the European Union Regulation on novel foods [1]. It is easy to farm, highly nutritious, has a high protein content (20.5 g/100 g) [2], and originates a flour with a better flavour and taste profile than other edible crickets [3]. Recent studies have related the consumption of edible insects with several health benefits including anti-hypertensive, antidiabetic, antioxidant, and anti-inflammatory properties [4, 5]. Some of these properties are mediated by peptides obtained from the gastrointestinal (GI) digestion of insect's proteins, but so far there are no reports identifying bioactive peptides originated from the GI of A. domesticus. The aim of this study is to apply an in silico approach to identify new bioactive peptides obtained from the GI digestion of A. domesticus with potential anti-hypertensive and/or antidiabetic properties.

Using an in silico approach to simulate GI digestion of six A. domesticus proteins, 43 peptides were obtained and ranked with a probability >50% of being bioactive. These peptides were further submitted to a molecular docking protocol to evaluate their binding interactions with the two catalytic domains (N- and C-) of the somatic Angiotensin-I converting enzyme (sACE) and/or dipeptidyl peptidase 4 (DPP-4). Five peptides (AVQPCF, CAIAW, IIIGW, DATW and QIVW) showed high docking scores for both enzymes, suggesting their potential to inhibit DPP-4 and both catalytic domains of sACE, thus possessing multifunctional antidiabetic and anti-hypertensive properties. Interestingly, other two peptides (PIVCF and DVW) (Fig. 1) showed higher docking scores for the N-domain of sACE, indicating a potential action as sACE selective inhibitors. These peptides have potential anti-cardiac and pulmonary fibrosis bioactivities and their specific interactions with the enzyme's active site can be further explored for the design and development of specific inhibitors targeting the N-domain of sACE, avoiding the side effects related to the non-selective inhibition of both sACE domains.

In summary, this is the first study identifying peptides originated from the simulated GI digestion of A. domesticus with potential activities against hypertension, diabetes, cardiac and pulmonary fibrosis.

Fig.1. Pymol representation of the intermolecular interactions between the residues of the active site of the N-domain of sACE and peptides PIVCF and DVW.

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Utilisation of lentils as valuable raw materials in gluten-free products

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Celiac disorder, a systemic immunological illness, affects about 1% of the world's population [1]. Related diseases, such as non-celiac gluten sensitivity, dermatitis herpetiformis, gluten ataxia or wheat allergy have also been linked to gluten ingestion [1,2]. The only known treatment is to strictly adhere to a gluten-free (GF) diet in order to prevent life-threatening diseases.

On the other hand, due to the absence of gluten proteins, GF products often have a lower protein level, so this difference should be made up for by other sources. GF diets also tend to be high in sugar and saturated fat. There have also been reports of deficiencies in a number of nutrients, including fibre, iron, zinc, magnesium, calcium, vitamin D, and several group B vitamins (B12 and B9) [1,3].

Finding GF substitutes that are both nutrient-dense and palatable to celiacs is a continuous challenge. The production of foods that have higher nutritional content than the conventional rice- or corn-based variants would be a possible choice for enhancing celiac patients' dietary profiles. Pulses can be used in the development of GF foods, balancing the nutrient intake of GF diets. They are also high in fibre and phytochemicals like phenolic compounds.

Lentils are distinguished from other pulses by higher protein, fibre, and iron contents. They also have an exceptionally high vitamin B content, particularly folate (B9). Prebiotics make up a portion of their substantial carbohydrate content, and their resistant starch content may lower the glycaemic index of diets. Depending on the cultivar and the makeup of the seed coats and cotyledons, lentils (Lens culinaris Medik.) can be yellow, orange, red, green, brown, or black. Recent scientific research claim that beans can be used to create GF cookies that are both acceptable and may improve the nutritional value, antioxidant properties, and glycaemic index. However, despite their advantageous qualities, lentils are currently underutilized in GF products. Our objective was to create and characterize various lentil-based cookies as healthier GF snack options for celiacs. Cookies were prepared with varieties of lentils in five different colours (black, brown, green, red, and yellow) and by application of different enrichments (protein, fibre).

Self-developed lentil cookies were assessed for geometry, baking loss, and texture profile. Raw materials (lentil flours) and cookies were characterized in terms of physicochemical characteristics (such as crude and digestible protein content, antioxidant properties, colour, and pH). A sensory acceptance test was also carried out to determine customer preferences for rice-based cookies as opposed to various lentil-based cookies. Results showed that lentil cookies were superior to rice control in terms of higher crude protein (12.1-14.8 vs. 3.8 g/100 g), digestible protein (57.05-53.00 % vs. 52.76 %), phenolics (136.5-342.3 vs. 61.5 mg GAE/100 g) and flavonoids (23.8-75.9 vs. 13.1 mg CE/100 g) content and antioxidant capacity (0.60-1.81 vs. 0.35 mmol TE/100 g), as well as lower hydroxymethyl-furfural content (< 1 vs. 26.2 mg/kg). Lentil cookies were more popular with consumers compared to rice cookies (overall preference: 6.1-7.0 vs. 5.6, there are significant differences between cookies) [4]. Green and red lentil-based cookies were further enriched with different combinations of whey protein, dietary fiber (inulin) and xylitol as sweetener. Different additives significantly affected in different ways many technological, textural and sensory properties of both green or red lentil cookies, particularly regarding the impact of inulin and xylitol on the volume and colour of green lentil cookies. The same trends were seen for textural characteristics of both lentil products: the presence of xylitol and whey protein made the cookies softer, while adding inulin made them harder. [5].

Our findings showed that lentil flours can be promising raw materials for GF bakery products, having nutritional and sensorial quality superior to rice. It is anticipated that lentils will become increasingly important in the creation of new GF items, such as cookies. Yet, when developing a product, both the type of lentil and the enrichment should be chosen appropriately.

Acknowledgments: Authors are grateful to their students and volunteers who participated in the sensory tests.

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ORAL PRESENTATIONS

T4

Food and health, functional foods and ingredients

Neurobiological regulation of food intake: Unbiased identification of food bioactives by virtual screening

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The dopamine D2 and the μ opioid receptor are involved in the neurobiological regulation of food intake. In order to understand if food ingredients may be able to directly modify food intake behaviour, we aimed to identify novel food components, which bind to D2R and μOR, respectively. In order to be able to find entities with previously unknown physiological activity, truly unbiased approaches are required.

In the present studies, computational workflows were therefore established, which allow a comprehensive and unbiased search of food derived D2R and μOR ligands. For this purpose, an *in silico* 3D food compound data base was created compiling >13,000 entries. The food compound data base was then subjected to virtual screening for D2R and μOR ligands. The screening workflow comprised pharmacophore models, subsequent hit clustering and finally docking using a D2R homology model or a μOR crystal structure. Experimental binding and functional assays revealed hordenine as an active functional selective D2R agonist [1]. Quantification of hordenine in foods by LCMSMS sMRM showed that it is prominent in barley malt from which it is almost completely transferred into beer indicating an uptake in the mg range by beer consumption [2]. Human intervention studies were performed to quantify resorption, metabolization and excretion of hordenine after oral uptake with beer [3]. Finally, a set of behavioural paradigms were applied in a mouse model of alcohol addiction indicating that hordenine essentially modulates alcohol-addiction associated behaviour.

Further experimental analysis of the virtual screening hits showed that the structural isomers kukoamine A and B bind as agonists to the μOR with EC_{50} values in the low μM range. Kukoamine A and B were quantified by LCMSMS sMRM in plant foods from the Solanaceae family, showing their prevalence in potatoes, where both bioactives are strongly enriched in the skin. Additional to its central expression, μOR is located in the gut, where it is involved in the regulation of gut motility. Taking their concentrations in potato and their EC_{50} value into account, the intake of a regular portion of potato may be sufficient to modulate gut health via μOR.

Conclusion: The food compound data base was successfully applied for virtual screening to identify food components with can interact with physiological targets involved in food intake behaviour. In the future, this unbiased approach can be extended to a wide range of dietary targets in the human organism.

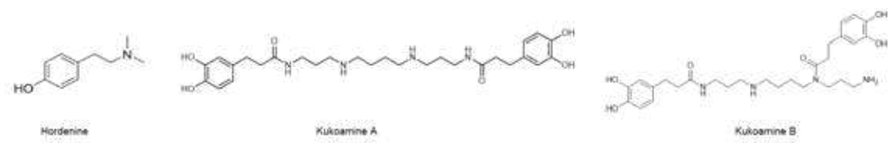

Fig.1. Structures of the malt and beer component hordenine and the potato components kukoamine A and B

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Development of functional ingredients from guelder rose (*Viburnum opulus* L.) fruit pomace and their application for increasing nutritional value of bread

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Viburnum opulus L. (common names - guelder rose, European cranberry bush) is a deciduous shrub yielding the globose bright red drupe 7–10 mm diameter fruits (commonly called berries) with a single seed. Guelder rose fruits are not consumed as a fresh food due to the unpleasant flavour [1]; however, they may be processed into various ingredients containing valuable health beneficial phytochemicals and other nutrients [2]. For instance, pressing berries results in the main product, the juice, and significant fraction of a by-product, which is commonly called pomace or press-cake. Berry pomaces are still used inefficiently or even discarded as a waste causing the loss of valuable nutrients and increasing environmental pollution burden. It was demonstrated that consecutive fractionation of guelder rose berry pomace using green high-pressure extraction methods enables obtaining lipophilic, polyphenolic, and high dietary fibre and protein fractions [3-5], which may be promising functional ingredients for foods and nutraceuticals.

The aim of this study was to evaluate the methods of enzyme (EAE) and ultrasound-assisted (UAE) extraction for the processing of defatted guelder rose fruit pomace into functional ingredients that could be used for increasing the nutritional and biological value of bread. Dried fruit pomace was ground and defatted by supercritical CO_2 extraction ($scCO_2E$), which gave 16.8% of lipophilic extract consisting of unsaturated fatty acid rich oil, tocopherols, phytosterols and carotenoids. Lipophilic fraction was not used in further studies; it is a valuable ingredient by itself and is particularly promising for development of nutraceuticals. The pomace also contained proteins (9.3%), insoluble (62.0%) and soluble (4.8%) dietary fibres and minerals (2.3%). After $scCO_2E$ the proportion of some non-lipid components in the remaining residue increased. The process also slightly reduced the particle size of the defatted pomace. The antioxidant characteristics of the pomace were also determined: total phenolic content TPC) was 39.1 mg of gallic acid equivalents (GAE)/g, the $ABTS^{\cdot+}$ scavenging capacity - 120.2 Trolox equivalents (TE)/g, and the oxygen radical absorption capacity (ORAC) - 52.9 mg TE/g. The antioxidant content of the $scCO_2E$ residue was by 14-17% higher compared with the raw pomace.

The defatted guelder-rose berry pomace was further fractionated by treating with different enzymes and/or ultrasound. The highest yield (35.1%) of soluble compounds was recovered by EAE and UAE with a cellulolytic enzyme complex from *Aspergillus* sp. (Viscozyme L), whereas the highest antioxidant capacity was determined for the extracts obtained by EAE and UAE with proteolytic enzyme from *A. oryzae*: TPC - 108.3 mg GAE/g; $ABTS^{\cdot+}$ scavenging capacity - 195.3; and ORAC - 219.3 mg TE/g of extract dry mass. Screening the composition of the metabolites in the extracts by UPLC-Q-TOF showed the presence of various phenolic compounds, peptides and ascorbic acid, *i.e.* the constituents, which may have the most important influence on their antioxidant properties.

Guelder-rose berry pomace ingredients were further tested in bread formula by replacing part of the wheat flour. The addition of various pomace products improved some general quality characteristics of the baked bread. For instance, increasing the amount of fermented and ultrasound-treated pomace in the dough up to 7.5% significantly increased bread volume and porosity. Pomace ingredients remarkably increased antioxidant potential as well. Thus, antioxidant capacity of bread with 10% pomace was 2.7-3.6 times higher than that of bread without additives. Finally, *in vitro* studies revealed remarkably higher release of antioxidants from bread with pomace ingredients during simulation of gastrointestinal digestion.

Similar experimental studies were performed with other fruit pressing by-products, namely rowanberry, sea-buckthorn, black currant, blueberry, bilberry, blackberry, lingonberry, cranberry and raspberry pomace. The effect of different processed berry pomace ingredients was highly dependent on the fruit species, doses and their treatments. However, it may be concluded that purposively processed berry pomace fractions are promising functional ingredients for foods and nutraceuticals.

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Prebiotic effect of enzymatic treated cocoa bean shells (CBS): a static *in-vitro* digestion

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Cocoa bean shells (CBS) are a by-product of the cocoa production chain. About 700 thousand tonnes every year of CBS are produced, **[1]** specifically during the roasting phase when bean shells are de-hulled from the rest of the bean and discarded**.** CBS are characterized by a significant dietary fibre (DF) content, up to 59% of the dry matter **[2].** DF, and particularly the soluble dietary fibre fractions (SDF), are often prebiotic ingredients able to boost the production of Short Chain Fatty Acids (SCFAs) by the colonic microorganisms at gut level, thus conferring a health benefit to the host **[3].**

The aim of this work was 1) the chemical characterization of CBS and 2) the evaluation of the prebiotic activity of DF isolated from raw CBS and CBS pre-digested with different enzymes mixtures, to increase the fermentable fibre portion.

CBS and enzymes were kindly provided by Italian Companies. Enzymatic hydrolysis was performed on CBS and CBS pre-treated by lipid and polyphenols removal. Two different industrial enzymes (hydrolases) were used: a mixture of cellulase (cellulase, polygalacturonase and xylanase) and a protease. The treatment was performed in suitable conditions adapted to each enzyme; biomasses were then stabilized by freeze-drying.

The different samples were characterized on their Insoluble (IDF) and Soluble Dietary Fiber (SDF) fraction, the radical scavenging activity (DPPH° assay), and Total Phenolic (Folin-Ciocalteu assay) **[4]**. To evaluate the potential prebiotic effect, the various samples were then submitted to a gastrointestinal simulated in vitro digestion and a subsequent *in-batch* fermentation in two different colonic regions (proximal and distal colon) with a stable faecal microbiota stabilized in the SHIME® gut model following protocols reported in **[5] [6].** SCFAs were finally quantified in each simulated vessel (GC-FID) to evaluate the microbial metabolism.

Results showed a significant amount of SCFAs produced, in particularly acetate from fermentation of the lipid- and polyphenols-free CBS treated with the cellulase mixture. In both colonic regions, this sample boosted the production of SCFAs, suggesting the potential usefulness of this enzyme-driven processing, improving the prebiotic effect.

Despite these results, we didn't observe a drastic change in the DF content of the enzymatically treated samples, especially regarding the SDF fraction. This outcome suggested us that the soluble fermentable fraction, as indicated by the literature, did not changed in quantity but we can theorize a potential qualitative change, releasing souble prebiotic oligosaccharides. Total phenolic content was dramatically reduced (80%) after lipid and polyphenols removal, contrarily, a slightly increase was observed in the sample digested with cellulase. The DPPH° assay confirmed the previous results by showing a significant decrease of antioxidant activity (8x fold) in pre-treated samples.

All this data confirmed that the enzymatic hydrolysis drastically induced an augmented SCFAs production in CBS, probably favoured by the previous removal of lipids and polyphenols.

These outcomes open a new scenario in the bio-valorization of CBS, according with the concepts of the bio-based industry and the circular economy.

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Recent results on heat induced carcinogens – formation of furfuryl alcohol during roasting of coffee

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Intensive heating processes result in significant chemical transformations in foods. Especially, roasting of coffee – during which temperatures of up to 270 °C are reached – results in complex reactions. Besides the formation of melanoidins, small molecules can be formed from the degradation of carbohydrates during the course of the Maillard reaction. A series of furan derivatives occurs in the roasted coffee comprising furan, HMF, hydroxymethyl furoic acid, furfural, and furfuryl alcohol. It was shown that the major occurrence of furfuryl alcohol is in roasted coffee [1]. Other heat treated foods contain significantly lower amounts of this compound. Furfuryl alcohol is volatile to a certain extend which reduces the concentration in the roasted product. In addition, it can react to a brown polymer.

It was shown earlier by a Norwegian/German consortium that furfuryl alcohol forms DNA adducts in presence of hu sulfotransferase 1A1 different organs [2]. This is an indication that furfuryl alcohol could be a substance of carcinogenic relevance.

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Promoting Innovation of ferMENTed fOods (PIMENTO) - COST ACTION CA20128

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Present in all European diets, fermented foods (FF) hold a strategic place due to the benefits they offer in terms of nutrition, sustainability, innovation, cultural heritage and consumer interest. The potential of FF for improving human health but also driving food innovation and local production in the next decades has become highly relevant. The PIMENTO project, a COST Action CA20128 (Promoting Innovation of ferMENTed fOods; https://fermentedfoods.eu/), which started in November 2021, is supported by COST (European Cooperation in Science and Technology; www.cost.eu). The challenge of PIMENTO is to federate the scientific community and other key stakeholders working on FF. The long-term goal of PIMENTO is to place Europe at the spearhead of innovation on microbial foods, promoting health, regional diversity, and local production at different scales, contributing to economic and societal development as well as food sovereignty in order to promote multi-modal innovation and respond to the expectations of European communities.

The wide variety of stakeholders engaged will enable CA PIMENTO:
i) to tightly connect and clarify scientific knowledge on health aspects of FF
ii) to tackle technical, societal and legislative bottlenecks behind FF-based innovations
iii) to contribute to the establishment of long-term scientific collaborations on FF
iv) to disseminate widely defined scientific knowledge on FF
v) to outline a strategic roadmap for future joint research.

PIMENTO will contribute to the European Green Deal and the “Farm to Fork” strategy by enhancing research and innovation into fermentation-based solutions for food products and processes, improving nutritional, sensory and functional properties. This collaborative network of researchers that includes food scientists, innovators, entrepreneurs, microbiologists, biochemists, and nutritionists has a very broad geographical coverage with 396 partners from 283 institutions of 50 countries. This regional diversity will play an important role through considering a differentiated panel of FF in diets.

COST Action CA20128
PIMENTO
Promoting Innovation of ferMENTed fOods

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Polysaccharides hypocholesterolemic potential: from structure to function

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Polysaccharides are high molecular weight carbohydrates very predominant in most of foods. They have been related with several bioactivities, namely hypocholesterolemic function.[1]namely cholesterol bioaccessibility and bioavailability. This review will highlight the main mechanisms by which polysaccharides are known to affect cholesterol homeostasis at the intestine, namely the effect (i Yet, relationships between carbohydrate structure and cholesterol lowering function are still scarce. In this work, a large portfolio of polysaccharides, such as arabinogalactans, galactomannans, glucans, fucoidans and chitooligosaccharides, from different food sources and by-products (such as coffee, mushrooms, algae, and shrimp), were extracted and chemically characterized. Their hypocholesterolemic potential was accessed measuring their effect on bile salt binding and cholesterol accessibility using an in vitro intestinal simplified model. Arabinogalactans and galactomannans from coffee were shown to affect hypocholesterolemic properties. [2]the effect of commercial espresso coffee and coffee extracts on cholesterol solubility are studied in an in vitro model composed by glycodeoxycholic bile salt, as a measure of its bioaccessibility. (2 The increase of galactomannans degree of branching was shown to decrease cholesterol accessibility. Hot soluble water glucans rich extract from mushrooms didn’t show any effect on bile salt sequestration or cholesterol accessibility. The negatively charged fucoidans from algae were shown to affect cholesterol accessibility being this effect higher for less charged polysaccharides. Considering positively charged polysaccharides, such as chitooligosaccharides, these were shown to be effective, lowering cholesterol accessibility, resulting from sequestration of bile salts promoted both, by charge and hydrophobic interactions. This work highlights that polysaccharide’s chemical structure diversity and branching degree can be used to optimize their hypocholesterolemic properties and should be considered in the development of innovative hypocholesterolemic food ingredients based on carbohydrates.

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Widening potentials of whey proteins and a look towards unexplored application fields

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Whey proteins (WPs) are recovered by cheese whey or other dairy industry by-products (ricotta or scotta) through sophisticated strategies promoting their conversion into high value-added products to accrue valorisation of the final product [1]. Due to the high nutritional value and specific functional properties, these products are employed both in the food and pharmaceutical industries for different applications/purposes [2,3]. Recently, great interest has been devoted to WP derived peptides for their well-known biological activities. Bioactive peptides are amino acid sequences that are encrypted within the native structure of proteins, which required hydrolysis for their release. Biological activities such antimicrobial, hypocholesterolemic, antioxidant, angiotensin converting enzyme (ACE)- inhibitor, opioid-like have been explored by in vitro and in vivo studies [4]. In this note the biological activities of WP derived peptides obtained by enzymatic hydrolysis, will be presented and discussed along with the exploitation of bioinformatic tools on line available, and a summary of applications in food or clinical sector will be also illustrated. Special attention will be placed on antimicrobial and antibiofilm peptides obtained by lactoferrin hydrolysis followed by characterization and identification by LC-MS/MS of the peptide fractions endowed with biological activities, part of which was assayed in fresh foods (dairy products and ready to eat vegetables) to improve their shelf life under storage conditions [5-9]; other applications of the identified bioactive peptides as a control strategy against skinborne staphylococcal biofilms will be also illustrated paving the way for interesting future applications in cosmetic formulation for skin care [10]. We herein report how the use of complementary experimental and theoretical investigations allowed the identification of novel angiotensin converting enzyme (ACE) inhibitory peptides obtained from a WP hydrolysate and addressed the rational design of even shorter sequences based on molecular pruning [11]. The identified peptides (IAEK, IPAVF, MHI), endowed with high ACE inhibitory activity proved also to be promising for their antiviral activity against the SARS-CoV-2 3C-like protease (3CLpro) and Human Rhinovirus 3C protease (3Cpro) highlighting the high value of WP derived peptides for performing multitarget studies [12]. The herein presented studies highlight the high potential of WP derived peptides as promising molecules to be exploited in the development of target-specific or multi-target agents in pharmaceutical sectors and as unexplored preservatives in food sectors.

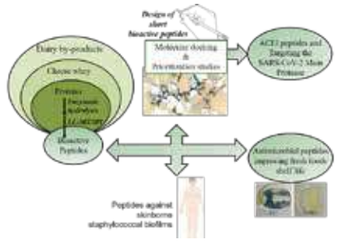

Fig.1. Graphical abstract summarizing fields of applications of WP derived peptides

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Acrylamide bioaccessibility in cereals, potatoes and chips. Effect of the food matrix and colonic fermentation

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Acrylamide is a carcinogenic processing contaminant mainly formed in foods under high temperatures and low moisture conditions through the Maillard reaction between the free amino acid asparagine and the alpha-hydroxycarbonyl group of reducing sugars [1]. Information about acrylamide bioaccessibility is scarce and more specifically, it is unknown what happens during the digestive process when foods are consumed not individually but in combination, within a complete diet. In addition, there is no information concerning the effect of colonic fermentation on the acrylamide trapped in the non-soluble portion after digestion. In this sense, the objective of this research was to evaluate the acrylamide bioaccessibility in several cereal and potato-based foods, including both isolated and combined foods in order to analyse possible interactions in the food matrix. In a second study, the assessment was performed in different snack samples, in which an in vitro fermentation procedure of the undigested fractions was included to analyse the possible influence of the different colonic microbiota in the final acrylamide potentially absorbable. For this purpose, different cereal and potato-based foods were digested individually and in combination with a protein source (i.e. biscuits + milk, breakfast cereals + yogurt, French fries + roasted beef steak, processed potatoes + eggs) applying the standardised in vitro gastrointestinal digestion protocol INFOGEST [2]. Acrylamide evolution in soluble and non-soluble fractions was monitored throughout different stages of the digestive process (oral, gastric and intestinal phases). In parallel, four different snack samples, including potato and veggie chips (beetroot, carrot and sweet potato), were also in vitro digested and the non-bioaccessible fraction was submitted to an in vitro fermentation under colonic conditions using faecal inoculums from different population groups (children, adolescents and adults). Acrylamide was determined by LC-ESI-MS/MS in foods and in the bioaccesible and non-bioaccesible fractions obtained after different stages of the digestion and fermentation process [3]. In the first study, the non-bioaccessible fraction of acrylamide tended to decrease during the digestion process in all the systems. However, the final bioaccessible acrylamide was affected by the food matrix composition (fibre, protein, sugars and lipids). The digestion of breakfast cereals, biscuits with milk, processed patatas and processed potatoes with eggs led to acrylamide bioaccessibility below the initial content of the contaminant in the meals. In absolute values, the combined consumption of biscuits and milk significantly reduced the bioaccessible acrylamide compared with isolated biscuits (from 212 to 122 ng; $p < 0.05$). The presence of protein sources (egg or meat steak) in the potato-based products significantly decreased the acrylamide bioaccessibility, which was more prominent in the French fries-meat steak system (from 2100 to 1698 ng; $p < 0.05$) [3]. In the second assay, acrylamide bioaccessibility was significantly lower in veggie chips (59.7–60.4%) than in potato chips (71.7 %). Potato and sweet potato chips showed the significantly lowest acrylamide content in the non-bioaccessible fraction (22.8 and 24.1%, respectively) as compared with beetroot chips (28.4%). After the fermentation step, acrylamide percentage in the soluble fraction of veggie chips ranged from 43.03 to 71.89%, the highest values being observed in sweet potato chips fermented with microbiota from children. This fact would involve that the acrylamide was released from the non-bioaccessible fractions by the microbiota [4]. These findings establish the importance of considering complete meals and not only isolated foods as well as the study of the non-bioaccessible fractions for a better understanding of acrylamide bioaccessibility, its recovery and interactions during gastrointestinal digestion. Moreover, results point out that the levels of potentially absorbable acrylamide after the complete gastrointestinal process could be modulated not only by the food matrix composition but also by the microbiota. These factors should be further considered for a more precise risk assessment of dietary acrylamide in humans.

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ORAL PRESENTATIONS

T5

Chemical reactions and interactions of food components

Chitosan films enriched with rapeseed cake extract obtained using a deep eutectic solvent

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Nowadays, there is a growing demand on replacing petroleum-based materials (e.g. polyethylene, polyester) with environmental-friendly alternatives. It is particularly crucial in the food and cosmetics industries, where the consumer has direct contact with a product and its packaging. Therefore, biodegradable natural polymers became a focus of a wide range of research to apply them as alternatives to plastics [1]. Among the most popular and well-studied biopolymers used in the food packaging area, chitosan gained significant interest. Pure chitosan (Ch) presents desirable features such as non-toxicity, biodegradability, and film-forming ability. Additionally, it is commonly known that Ch has significant antibacterial properties [2]. For these reasons, a properly modified (e.g. plasticized, cross-linked, blended with other biopolymers, or mixed with active substances) Ch could be successfully used as food packaging material [3].

Several different types of additives (such as plant extracts, essential oils, and metal nanoparticles) that make Ch films functional have been applied so far. Lastly, increased interest in deep eutectic solvents (DESs) has been observed. DESs are mixtures of two or more safe components capable of associating with each other through complexation via hydrogen bonds [4]. Until now, DESs were successfully incorporated in biopolymer films, e.g. chitosan, as plasticizers [4]. Beyond that, DESs have become the object of much research in analytical chemistry, due to their usage as solvents in the extraction of various bioactive compounds was found to be uniquely efficient [5]. Therefore, it is pertinent to investigate the impact of incorporating extracts obtained using DESs on the properties of biopolymer films.

This study aimed to investigate the effect of rapeseed cake extract (RCE) on Ch film properties. RCE was obtained using a mixture of choline chloride (ChCl), lactic acid (LA), and water (H2O), regarded as DES. Subsequently, novel Ch films containing different amounts of RCE (40-70 wt.%) were prepared by casting method. Films with pure DES and neat Ch film were obtained as control samples. Several studies have been conducted to estimate the influence of different amounts of RCE or DES in Ch films on their properties. The Quick, Easy, New, CHEap and Reproducible (QUENCHER) methodologies using 2,2-diphenyl-1-picrylhydrzyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were applied to measure antioxidant capacity (AC) of prepared films. Moreover, mechanical, barrier, and color properties were investigated.

It was found that the AC of films was increased with the higher amount of DES or RCE in the Ch formulation. A similar effect was observed for elongation at break. However, an inverse relationship resulted in Young's modulus (YM) of the samples – the highest YM values were noticed for neat Ch film and samples with 40 wt.% of additives. The gravimetric method was used to determine the water vapor transmission rate (WVTR) of modified Ch films. The highest WVTR was observed for samples with 70 wt.% of RCE or DES. The CIELab method was chosen to evaluate the effect of RCE or DES incorporation of color parameters of Ch films. The obtained results were consistent with visual observations. All of the prepared samples were transparent and uniform in color. In addition, Ch films incorporating RCE resulted in more yellow samples.

Based on the obtained results, it may be concluded that supplementation of Ch films with DES or RCE significantly changed their characteristics. In both cases, samples with additives were plasticized, but materials enhanced with extract were characterized by better antioxidant properties. These findings have significant implications for the development of sustainable and functional food packaging materials, particularly for food prone to oxidation.

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The role of amines in glucosinolate hydrolysis in *Brassica* vegetables

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Glucosinolates are sulfur rich plant secondary metabolites in Brassica vegetables such as cabbage or broccoli. They are precursors of bioactive and health-promoting isothiocyanates (ITCs). Upon enzymatic hydrolysis by the enzyme myrosinase, glucosinolates in Brassica vegetables often form mainly nitriles and epithionitriles and ITCs often are found in lower amounts [1]. Here, we show that amines can be additional main enzymatic hydrolysis products of glucosinolates in cabbage (Brassica oleracea var. capitata). Likely a plant endogenous ITC hydrolase (ITCase) converts ITCs to amines in cabbage samples. A structure dependent conversion from ITCs to amines was observed: while alkenyl ITCs like allyl ITC (and to a lesser extend methylthioalkyl ITCs) were converted to amines, methylsulfinylalkyl ITCs like sulforaphane were not converted [2].

However, amines can be also yielded from ITC by chemical mechanisms. During heat treatment of homogenized cabbage for up to 2 h, methylsulfinylalkylamine levels increased by up to 400%. Here, alkenyl amine levels did not change due to heat treatment, likely as the corresponding ITC was already converted to the amine by enzymatic-like mechanism [2].

Finally the effect of long-term cold storage of cabbage on glucosinolate hydrolysis and amine formation was investigated. While glucosinolates overall were stable during storage, in red cabbage storage led to an increased formation of isothiocyanates and methylthioalkylamines, which could be linked to a decline in epithiospecifier protein 1 abundance. The results show that amines from glucosinolates are part of the human diet.

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Non-Enzymatic Browning Reactions of Phenolic Compounds – Formation of Melanin-like Colorants

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For consumers, sensory impressions are crucial for evaluating the quality of food. Consequently, browning of fresh fruits and vegetables is generally associated with a poor quality, which reduces consumer acceptance. The formation of the corresponding dark pigments, called melanins, is well-known to result from enzymatic polymerization of the phenolic compounds contained in plant-based food [1]. However, browning of other foods such as coffee, cocoa, or cereals, as induced by thermal processing, is accompanied by desired changes of flavor, texture, and shelf life [2]. In contrast to enzymatic browning, these thermally-catalysed reactions are classified under the term non-enzymatic browning. These reactions comprise a vast number of parallel and subsequent reaction pathways leading to the formation of complex, heterogenous copolymers (‘melanoidins’) [3]. In this context, melanoidins are the most prominent group of end-products formed by non-enzymatic browning reactions of reducing sugars with amino compounds. Although, phenolic compounds are also described as constituents of melanoidins, literature clarifying their contribution to the formation of phenol containing melanoidins is limited [4].

To establish a basic understanding of the reactivity of phenolic compounds in non-enzymatic browning reactions, different hydroxycinnamic acid derivatives prevalent in a high number of plant-based food were selected for an in-depth investigation of their reactivity. More specifically, 5-caffeoylquinic acid, caffeic acid, p-coumaric acid, ferulic acid, and hydrocaffeic acid were heated under roasting conditions at 220 °C for up to 10 min. The reactivity of the model systems was characterized by color formation (Vis spectroscopy), changes of pH value, the conversion of the reactants (HPLC-DAD), and the antioxidative properties (TEAC). The composition of the colored reaction products was analyzed by means of high-resolution mass spectrometry (HRMS) and multiple-stage mass spectrometry (HRMSn) experiments. After isolation, selected products were identified by 1D and 2D nuclear resonance spectroscopy (NMR).

Overall, heat treatment of caffeic acid resulted in the most intense color formation. The high reactivity of caffeic acid was also reflected by its fast conversion which was accompanied by an increase of the pH value. This correlation was proposed being a consequence of the decarboxylation of caffeic acid and, additionally, oligomers consisting of up to five units of its decarboxylation product, 4-vinylcatechol, were detected via HRMS. Further, three isomers of a vinylcatechol dimer were identified and a novel reaction mechanism for the oligomerization was postulated.

In conclusion, the present investigation gives novel insights into non-enzymatic browning reactions of prominent hydroxycinnamic acids contained in a huge number of plant-based food. Decarboxylation was identified as the driving force of color formation, which was significantly impacted by the electron density of the aromatic system. The subsequent oligomerization was also found to attribute to increased antioxidant properties of the reaction mixtures. As the oligomers described herein still possess reactive centres, these could be incorporated into food melanoidins and thereby contribute to their characteristic properties, especially color and antioxidant activity.

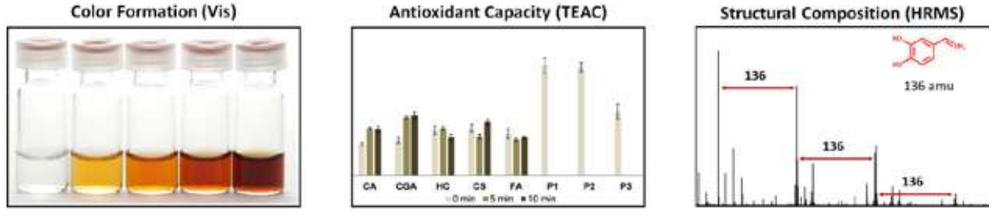

Fig.1. Characterization of the reactivity of phenolic compounds in non-enzymatic browning reactions.

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Saliva – a suitable matrix to study the metabolic transit of food components and metabolites

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Saliva gained significant importance as analytical matrix for the early diagnosis of diseases and for the detection of substance abuse.[1] In our studies, we used saliva to analyse the metabolic transit of food components and metabolites and to draw conclusions concerning dietary habits. This will be demonstrated with three examples.

In the first project we were interested whether dietary glycation compounds are transferred to saliva following ingestion and digestion of heated food. Glycation compounds arise from amino compounds and reducing sugars during storage and heating of food. The analysis of glycation compounds was performed by using liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) as stable isotope dilution assay with the respective isotopologue internal standard. As a result, we were able to demonstrate that the amount of the lysine derivative pyrraline is significantly enhanced following oral uptake, thus making the compound to a hallmark for dietary glycation compounds.

In the second study, we wanted to clarify the question how caffeine intake affects the activity of the caffeine-metabolizing enzyme CYP 1A2. For assessing the CYP1A2 activity, we used the paraxanthine-caffeine-ratio. The concentration of caffeine and its metabolites in saliva were quantitated with HPLC-UV. We could show that a higher caffeine intake results in an increase of the CYP1A activity, expressed as paraxanthine-caffeine-ratio. However, this enzyme system does also depend on external factors, such as medication.

The objective of the third study was to find out if the proportion of salivary proline-rich proteins, which contribute to tannin-derived protein precipitation is enhanced by tannin supplementation with chokeberry juice. The proportion of prolin-rich proteins was determined with a photometric tannin-binding capacity assay. Daily drinking of chokeberry juice did not affect the proportion of proline-rich proteins in saliva, though we could observe a relation between a high tannin-binding capacity and the detection threshold for taste of bitterness and adstringency.

In summary, saliva offers a non-invasive sampling and an easy sample handling which thus allows the detection of a plenty of analytes. Depending on the question, saliva analysis allows to draw conclusions to nutritional habits.

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Formation of volatile methyl ketones during lipid oxidation

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Lipid oxidation is, besides the MAILLARD reaction, one of the most important reactions in food chemistry. In contrast to the MAILLARD reaction, which is predominantly associated with positive properties through the formation of aroma, flavour and colorants [1], lipid oxidation is associated with a loss of the essential function of ω-3- and ω-6-fatty acids and the formation of a variety of secondary volatile degradation products which are widely known as marker for the spoilage of lipid-rich foods [2]. In addition to the typical fatty acid degradation products such as alkanes, aldehydes, and alcohols, methyl ketones (2-alkanones), e.g., 2-hexanone and 2-heptanone, are also frequently described as compounds with an aroma-active character.

While the formation mechanisms of most volatile compounds are largely well understood [3], the origin and precursor compounds of methyl ketones are unknown or not yet comprehensively discussed. One postulated reaction mechanism for methyl ketones starts with the oxidation of saturated fatty acids to 3-oxo-acids. Decarboxylation leads to methyl ketones that are shortened by one C-atom, for example described in milk fat [4]. However, the profile of methyl ketones in milk fat does not correspond to its fatty acid composition. The main methyl ketone, 2-heptanone, should be formed from octanoic acid, but only about 1 % of this precursor is present. This mechanism also does not adequately explain why methyl ketones are found in foods rich in unsaturated fatty acids.

In the present study, the formation of methyl ketones in food-relevant fats and oils as well as in model systems with linoleic acid or secondary degradation products (2,4-alkadienals, 2-alkenals, aldehydes) was investigated. Up to seven different methyl ketones were determined with static headspace GC-MS in milk fat, vegetable oils, and model systems. It was found that methyl ketones are formed in particular from secondary degradation products such as 2,4-decadienal and 2-octenal. When using isotope-labelling experiments, it was shown that the position of the double bond in the precursor compound determines the chain length of the methyl ketone and amino compounds might promote the formation of methyl ketones to varying degrees. As foods naturally contain both, lipids and amino compounds, the proposed pathway might not be neglected for the formation of aroma-active methyl ketones in foods.

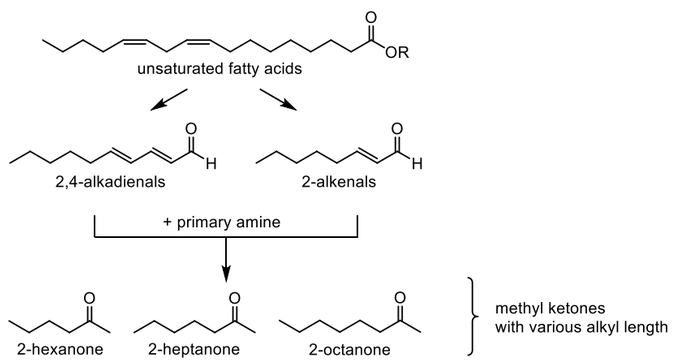

Fig.1. General overview of the formation of methyl ketones during lipid oxidation.

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Colorants of the MAILLARD Reaction: Formation and Structure of Food Melanoidins

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Melanoidins – the brown colorants formed in the MAILLARD reaction of reducing carbohydrates and amino acids – are present in substantial amounts in our daily food. An intake of up to 10 g melanoidin per day is estimated from bread, biscuit, and coffee.[1] Nevertheless, the structures of these compounds have not yet been elucidated and their formation mechanisms are not understood. This is mainly due to the complex nature of the MAILLARD reaction which leads to the formation of hundreds of reactive intermediates that are all involved in the formation of these brown end-products. To gain a general understanding of the main reaction pathways, the isolation of each and every potential colorant is neither reasonably possible nor that useful. Instead, the characterisation of the complex reaction mixtures by means of high-resolution mass spectrometry (HRMS) provides valuable information about reoccurring substructures and helps to identify predominant reactions steps that could lead to the formation of certain structural units.[2] The analysis of the complex data sets is assisted by VAN KREVELEN and KENDRICK mass analysis,[3][4] techniques commonly applied in polymer and petrol chemistry. Nevertheless, in many cases simplified reaction systems based on carbohydrates or their prominent degradation products in presence of amino acids are still necessary to limit the reaction pathways of the MAILLARD reaction.

In the present study, the prominent MAILLARD reaction intermediate methylglyoxal (MGO) was incubated with ten different amino acids at 100 °C and pH 5. The reaction mixtures were analysed on conversion of the reactants by HPLC-DAD or HPTLC, formation of colorants by UV/Vis photometry and HRMS, and antioxidant activity by TEAC and DPPH assays. In addition, the reaction mixtures were dialysed to remove low-molecular-weight MAILLARD reaction products (< 1 kDa) and to isolate high-molecular-weight melanoidins (> 12–14 kDa). The obtained fractions were analysed in regards of their contribution to colour and antioxidant activity of the reaction mixture.

MGO was converted considerably faster than the different amino acids when both were incubated as equimolar aqueous mixtures indicating that the latter mostly accelerated the reaction as catalysts. However, the small proportion of the amino acids that did react mostly underwent STRECKER degradation and the amino nitrogen was integrated in the melanoidin backbone in form of aminoacetone – the corresponding α-aminoketone of MGO. The respective sidechains of the amino acids were identified by VAN KREVELEN analysis in MAILLARD colorants either in form of the complete amino acids or in form of their STRECKER aldehydes. Even though only relatively small proportions of the amino acids were converted (5–30 %, whereas MGO was converted to 75–95 %) the resulting colorants did significantly differ in their water solubility based on the polarity of the respective amino acid sidechain. Overall, the structure of the MAILLARD oligomers formed in the model experiments was analysed by HRMS and additionally, two pyrrole intermediates were isolated from the reaction mixtures by preparative HPLC. The identified predominant reaction steps, aldol and MICHAEL reactions, as well as the resulting substructures help to understand more complex reactions in real food systems rich in carbohydrates like caramel products and baked goods.

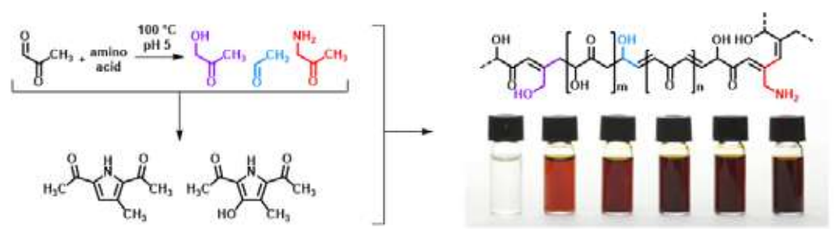

Fig.1. Formation of melanoidins from MGO and amino acids at 100 °C and pH 5 for 0–300 min.

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Development of α-dicarbonyl compounds from oligosaccharides

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α-Dicarbonyl compounds such as (deoxy)glycosuloses and methylglyoxal, are key intermediates of the sugar transformation under caramelization and Maillard conditions. In food, they are involved in the decrease of nutritional value, in sensory and redox changes as well as changes in technological functionality and properties of proteins. In the human body, they can propagate carbonyl and oxidative stress. While the formation of α-dicarbonyl compounds from monosaccharides is quite clearly described, their formation from oligosaccharides is more complex [1,2]. We performed a series of experiments focused on the formation of α-dicarbonyl compounds from oligosaccharides possessing (1→4)-glycosidic bond - maltose, lactose, and their isomers and homologues. Identification and determination of α-dicarbonyl compounds after derivatization to quinoxaline derivatives were performed using HPLC-PDA and LC-MS methods. The levels of α-dicarbonyl compounds and kinetics of their formation/degradation were evaluated in complementary sets of reaction models and foods (syrups, dairy products). The model reactions systems varied in several parameters such as reactants, caramelization vs. Maillard reaction, aqueous vs. low-aw systems, and with α-dicarbonyl compounds derivatized after sampling vs. in statu nascendi. The effect of the addition of phenolic agents on the amount reduction of αdicarbonyl compounds in selected samples was also monitored. The identified oligosaccharide-specific or typical products cover several α-dicarbonyl compounds from (1)3-deoxylactosulose to 3,4-dideoxypentosulose. We focused more on the reactions of β-galactooligosaccharides since the Maillard reaction is favourable in the processing of many foods but usually not in technology of milk and dairy products [4]. In addition to the information about the development of oligosaccharide-related α-dicarbonyl compounds, their specificity and possible mechanisms of their formation, the results revealed the saccharides with a ketose structure at the reducing end as much effective sources of α-dicarbonyl compounds than those composed entirely of aldoses. The results may also contribute to better controlling of the Maillard reaction in dairy products.

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ORAL PRESENTATIONS

T6

Food sustainability, including byproducts valorization

Chemical characterization and bioactive properties of industrial residues from walnut oil production (*Juglans regia* L.).

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Walnut dregs obtained in the production of walnut oil can be considered one of the main by-products of this industry, being currently reused as fertilizer or animal feed [1]. In this sense, with the final objective of valuing this by-product, this work consisted of the chemical characterization and evaluation of the bioactivity of the unprocessed dregs and final residue obtained by supercritical fluid extraction of walnut dregs.
Both by-products were nutritionally analyzed according to official AOAC methods, with energy estimated by the Atwater system. With regards to the chemical parameters, free sugars and tocopherols content were analysed using liquid chromatography coupled to refractive index (HPLC-RI) and fluorescence (HPLC-FL) detectors, respectively. Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID), organic acids by ultrafast liquid chromatography coupled to a diode detector (UPLC-DAD) and phenolic compounds by HPLC with DAD and spectrometry mass detection (HPLC-DAD-ESI-MS/MS). Additionally, the antioxidant and antimicrobial activity of the hydroethanolic extract obtained from the unprocessed (WD) and supercritical processed dregs (FR) were evaluated. Antioxidant activity was determined using tree *in vitro* assays (inhibition of lipid peroxidation by thiobarbituric acid reactive substances (TBARS) in brain homogenates, reducing power, and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. Antimicrobial activity was tested by the microdilution method against a panel of bacteria and fungi. Furthermore, to evaluate the cytotoxic potential four tumoral cell lines were tested, namely AGS (stomach carcinoma), CaCo2 (human colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), and NCI-H460 non-small cell lung carcinoma) and two non-tumor cell lines (porcine liver primary cells, PLP2 and renal epithelial cells extracted from an African green monkey, VERO). Murine macrophage cells (RAW 264.7) were used to evaluate the anti-inflammatory potential of both samples.
WD had a predominance of polyunsaturated fatty acids, with linoleic acid being the major one, with a content greater than 55%, while FR had a profile richer in saturated fatty acids (48.6%) despite having oleic acid as main fatty acid (31.3%). Regarding tocopherols, α, γ and δ isoforms were observed in both samples, with γ-tocopherol being the predominant compound for WD and FR with 89 mg/100 g and 200.4 mg/100g, respectively. Only sucrose was identified in terms of free sugars, which suggests that there was no hydrolysis of oligo or polysaccharides during the process of obtaining the WB and FR. Among the five identified organic acids, quinic acid was the major in walnuts dregs (1.06 g/100 g) and succinic acid in the supercritical extract residue (2.8 g/100g).
The phenolic composition of the extract showed a predominance of catechin derivatives (flavan-3-ols) and hydrolysable tannins were observed in both samples. In general, the hydroalcoholic extract of WD and FR showed interesting antioxidant activity. Lower EC_{50} values were observed for FR in the 3 assays performed, with the lowest inhibition concentration being obtained in TBARS (WD 32.84 μg/mL and FR 9.1 μg/mL). Notwithstanding, both extracts did not show significant activity against the tested bacteria and fungi. Regarding the cytotoxicity potential, FR had positive results (AGS and CaCo2), while WB showed no positive results of inhibition at maximum concentration tested. Furthermore, FR and WB did not show cytotoxic values at non-tumor cell lines. Finally, the walnut by-products presented no activity against Raw 264.7 cells at the maximum concentration tested (400 μg/mL). The results of this work demonstrate the potential of this industrial residue given its high antioxidant activity and composition in nutrients and bioactive compounds. WB residue can be valued for its vegetable oil and the residue obtained further exploited for its bioactive compounds and fiber. Both residues showed a good potential to be explored in the future through its incorporation into food products, adding value to the walnut oil production chain.

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Hydrothermal treatment for hemicellulose extraction: investigation of temperature effect on fibre structure and study of degrading compounds in hazelnut shells

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In a historical period in which consumers' food choices are changing, and moving towards healthier foods, dietary fibres naturally play a fundamental role. Due to the increase in the global population and because of environmental, ethical, and economic reasons, an increasing need to find new sources of dietary fibre has emerged. Since 90% of plant biomass is represented by lignocellulosic material [1], hemicelluloses in particular have acquired a great global interest. Hemicellulose extraction from lignocellulosic biomasses has therefore been investigated and hydrothermal treatment is nowadays one of the most common methods employed for this purpose [2]. However, the effects the extraction parameters (such as time, temperature, and pH) have on the products outcome have not been deeply investigated.

Our work aimed to evaluate the effect of different hydrothermal treatment temperatures, namely 125 °C, 150 °C, 175 °C, and 200°C both on the type and structure of dietary fibre of hazelnut (Corylus avellana L.) shells and on the formation of various products derived from matrix degradation. In fact, it is commonly accepted that different extraction conditions can lead to modifications in the structure of dietary fibres, and also that hydrothermal treatment presents some drawbacks related to the formation of undesired compounds, mainly originating from sugars and lignin degradation [3]. The chemical structure of the dietary fibres extracted was determined by GC-MS, HPSEC-RID, UPLC/ESI-MS, and 1H NMR, whereas the degrading compounds were deeply investigated by an innovative "reactomics" approach based on liquid chromatography coupled with ion mobility separation and high-resolution mass spectrometry (UHPLC-IM-Q-TOF-MS).

Our results indeed showed that different process temperatures led to the presence of diverse polysaccharides in the hydrothermal extract. First, mainly pectin was identified for the first time within hazelnut shells when experimenting at 125 °C. The highest extraction yield of total fibres and xylan was obtained at 150 °C and 175 °C, respectively, then they both decreased at 200 °C.

Moreover, as expected, the temperature of the process also had an impact on the co-extraction or neo-formation of a huge number of compounds, depending on the heat treatment severity. A generally high content of phenols and phenyl compounds derived from lignin was observed, together with oligosaccharides, anhydro-sugars, and furans derived from sugars. However, the abundance of these compounds was variable as well, depending on the temperature and the purification steps eventually performed to further isolate polysaccharides. To the best of our knowledge, this deep investigation of the chemicals that are generated following hydrothermal treatment has never been proposed. Since one of the main aims of reusing hemicellulose from vegetable by-products is its transformation into healthy ingredients for food companies. it is of enormous importance to further investigate the presence and the formation of all these compounds, and especially to evaluate them in terms of potential toxicity.

As a whole, our results suggest the possibility to obtain from the same hazelnut shells biomass different types of fibres. The latter can be potentially extracted with a sequential fractionation approach, as a function of the severity of the extraction parameters, keeping an eye on the co-extracted, potentially hazardous chemicals.

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Sustainable use of pumpkin: characterization of the pulp and valorisation of by-products in obtaining preservative extracts

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In the search for more sustainable industrial processes, the use of by-products from food production is an important strand [1]. Currently, the food industry has increasingly developed practical, ready-to-eat, and long shelf-life food products. However, this demand involves the use of synthetic food additives, which are associated with harmful effects on consumers health. Aiming to promote sustainability allied to the replacement of synthetic additives by natural alternatives, this work proposed the use of by-products from pumpkin processing, as a matrix for obtaining preservative compounds with potential to be applied in a product of pumpkin pulp. For that purpose, five different pumpkins cultivated in Egypt, namely 'Butternut Squash', 'Golden Cushaw', 'Dickinson', 'Halloween', and 'Honey Delite' were assessed. The pulp was evaluated regarding its nutritional value and chemical composition, in terms of free sugars (HPLC-RI), fatty acids (GC-FID), tocopherols (HPLC-FLD) and organic acids (UFLC–PDA). The by-products, more specifically the seeds, fibers, and peel, were evaluated for their antioxidant and antimicrobial capacity, as well as their cytotoxicity.

All the pulp samples presented carbohydrates as the major compounds, followed by protein and fibers, with low content of fat. Regarding free sugars, fructose was predominant in all samples, except for the 'Golden Cushaw', which presented high levels of sucrose. Glucose was also present in high levels in most of the samples, and considerable contents of trehalose and raffinose were also found. Regarding fatty acids profile, the pulp revealed to be rich in saturated and polyunsaturated fatty acids, representing, respectively, about 40-50% of the total fatty acids, composed mainly by palmitic (C16:0), linoleic (C18:2n6c), α-linolenic (C18:3n3), and stearic (C18:0) acids. Regarding organic acids, all pulp samples presented malic and fumaric acids. In the samples that presented quinic acid, this was present in major quantity. Oxalic acid and traces of shikimic and citric acids were also detected. Also, the samples showed α and γ isoforms of tocopherol, being the last one the most abundant. In addition to the rich nutritional composition of the pulps, as expected, the by-product extracts showed excellent preservative capacity. In the TBARS assay, the samples showed great capacity to inhibit lipid peroxidation, mainly the seeds and two of the five fibers. Moreover, all samples presented antibacterial capacity, inhibiting the growth of one to six of the eight tested bacteria. Also, some samples were capable of inhibiting the growth of the two fungal strains tested, with the peels standing out, protecting against at least three bacteria and one fungus. Additionally, none of the samples presented cytotoxic activity against the non-tumor porcine liver cells (up to 400 µg/mL), thus expressing its safety for food application.

With this, it is possible to verify the potential use of pumpkin by-products as a source of natural preservatives, as well as the great nutritional value of the pulp, which can be further explored in the development of new pulp products preserved with their processing by-products. This favours a circular economy through sustainability.

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Brewer's Spent Yeast as a source of Vegan and Clean Label Additives for Mayonnaise Formulations

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Brewer's spent yeast (BSY) components have been reported to possess thickening and emulsifying properties [1]. The stability of the generated emulsions depends on a suitable combination of emulsifying agents with thickeners that confer viscosity, thus inhibiting the coalescence of the droplets. Besides that, the occurrence of insoluble and charged particles is also favorable to promote emulsion stability, by forming Pickering emulsions [2]. In this sense, this work aimed to attest the simultaneous use of BSY cell wall material as soluble components and when combined with the whole BSY particle for their use as a clean label and vegan source ingredients able to replace food additives, and protein from animal sources. To achieve this, structure/function relationships were performed by isolating polysaccharides with distinct structural features from BSY, either by using alkaline extraction (mild treatment) and subcritical water extraction (SWE) using microwave technology (hard treatment) and assessment of their emulsifying properties.

Alkaline extractions solubilized mostly highly branched mannoproteins (*N*-linked type) and glycogen while SWE solubilized mannoproteins with short mannan chains (*O*-linked type) and (1→4)- and (β1→3)-linked glucans.

Extracts with high protein content yielded the most stable emulsions obtained by hand shacked, while the extracts composed of short chain mannans and β-glucans yielded the best emulsions by using ultraturrax stirring. β-Glucans were found to contribute to emulsion stability simultaneously with *O*-linked type mannoproteins by preventing the Ostwald ripening. The residue (BSY insoluble charged particle) resulting from the extraction was also applied as emulsion stabilizer, acting as a Pickering agent. The combination of BSY charged particles used as Pickering agents together with alkali soluble polysaccharides, with thickening and emulsifying properties, contributed for the increase in emulsions stability.

When applied in mayonnaise model emulsions, BSY extracts presented higher stability and yet similar texture properties as the reference emulsifiers. When used in a mayonnaise formulation, the BSY extracts were also able to replace egg yolks and modified starch at 1/3 of their concentration. BSY mannoproteins and β-glucans can replace food additives and animal protein in sauces. Also, BSY particles were successfully used as Pickering agents together with other vegan polysaccharide-based ingredients recovered from agrifood industries, such as aquafaba and pine nut skin. In this sense, the components of the yeast cell wall can be integrated in the development of food grade emulsions, replacing additives, and originating new clean label products integrated in a circular economy context.

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Formation of the Lipation product 2-Amino-6-(3-methylpyridin-1-ium-1-yl)-hexanoic Acid (MP-Lysine) during Roasting of Peanuts

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Peanuts are rich in fat (35.8-54.2%) and protein (21.0-36.4%) and often used as ingredient in food production [1, 2]. Frequently they are consumed in western countries (e.g., Europe or USA) after roasting [3]. In literature, it is known that continuous roasting of peanuts induces browning reactions and protein modification, accompanied by a decrease in protein solubility due to glycation (Maillard reaction) or lipation reactions of nucleophilic amino acids (e.g., lysine) [4-7]. Within lipation reactions of acrolein, which is formed from degradation of methionine or during lipid peroxidation, and the ε-amino group of protein-bound lysine, the reaction product 2-amino-6-(3-methylpyridin-1-ium-1-yl)-hexanoixc acid (MP-Lysine) is formed. As shown in HPLC-ESI-MS/MS measurements of peanuts roasted at 170 °C, the amounts of MP-Lysine increased sharply after prolonged roasting time (40 min) compared to raw peanuts [8]. Therefore, we propose that MP-Lysine represents a suitable marker of the roasting process.

In our studies, a steady increase of MP-Lysine was observed in peanuts roasted at 110-160 °C for 10 min, 20 min and 40 min by means of HPLC-ESI-MS/MS (Fig. 1). MP-Lysine increased from 16 µmol/kg protein in raw peanuts up to 419 µmol/kg protein in peanuts roasted at 160 °C for 40 min (Fig. 1). At each temperature, no changes of MP-Lysine were observed after roasting peanuts for 10 min, respectively (Fig. 1). Furthermore, amounts of MP-Lysine began initially to increase at a temperature of 130 °C (Fig. 1). Same results as for MP-Lysine were observed for total colour differentiation related to the colour of raw peanuts according to CIELAB skala (EN ISO 11664-4) (raw: 0.0, 160 °C/40 min: 34.6), protein recovery (raw: 100%, 160 °C/ 40 min: 6%), and lysine (raw: 212 mmol/kg protein, 160 °C/40 min: 9 mmol/kg protein). However, for free methionine and threonine, as possible precursors of MP-Lysine, no changes in their amounts were found, probably due to thermal degradation of Amadori rearrangement products. Finally, a highly significant correlation between MP-Lysine and total colour differentiation, protein recovery, and lysine could be determined. Overall, it could be shown that MP-Lysine is a marker for the characterisation of roasting peanuts and explains the phenomena (e.g., increased colouration) that occur.

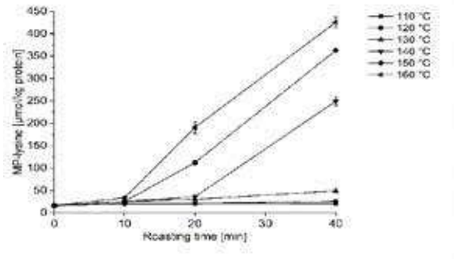

Fig.1. Determined amounts of MP-lysine [µmol/kg protein] in raw and roasted peanuts using HPLC-ESI-MS/MS

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Different Levels and Pattern of Lipid-Derived Gut Microbial Metabolites after Fermentation of Different Lipid-rich Foods

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Next to dietary fiber and proteins, undigested dietary lipids also enter the colon and can be used by human gut microbiota. Although the pathways through which lipids are metabolized by the gut microbiota is partly known, how the nature of the specific lipid-rich matrix modulates lipid metabolism is poorly explored. Here, the differences in the levels and patterns of lipid microbial metabolites produced from different food matrixes, sunflower seed, soybean, and walnut were investigated. Food samples were subjected to a simulated in vitro digestion, after which the undigested material was subjected to an in vitro colonic fermentation using faecal samples from three healthy donors for 48h. Several known linoleic acid (LA) metabolites were quantified by a targeted approach whereas several other FAs metabolites were identified or putatively annotated by the lipidomics untargeted approach using high-resolution liquid-chromatography mass spectrometry. Results showed that digested walnut produced the highest levels of FFAs and conjugated LAs (CLAs) after fermentation. To further explore the matrix effect, the same amount of defatted digested foods, as well as of fibre and polyphenol extracted from the digested materials were prepared and fermented with sunflower oil. The addition of defatted digested walnut to sunflower oil also produced the higher levels of FFAs and detected CLAs. This is ascribed to its fibre and polyphenols which addition produced the higher increase in CLAs than sunflower and soybean. Several LA metabolites, such as di- or tri-hydroxy-C18FAs, were putatively annotated by the untargeted lipidomics approach. Multivariate analysis of the profile of microbial lipid metabolites showed that the lipid profiles produced with sunflower seeds and walnuts were similar but distinct to soybean. In conclusion, foods with different compositions can modulate the microbial production of lipid metabolites and the fatty acid composition after fermentation is more affected by the type of food matrices than oils.

Retention of anthocyanin and antioxidant activity in the presence of pectin in mixed juices for different processing methods

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There is a growing trend of including fruit and vegetable (F&V) juices rather than soft drinks in the human diet, and the global demand for high-quality, fruit juices with natural materials, minimally processed and additive-free are constantly increasing [1]. Mixed vegetable and fruit juices contain several functional substances such as dietary fiber, including pectin, and bioactive compounds, including carotenoids and flavonoids such as anthocyanins [2]. Considering their large production volumes, complementary flavors, and synergistic nutrient profiles, apple and peach are selected as raw materials to combine in mixed juice with anthocyanin-rich F&V. In the process of juice production, the interaction between anthocyanins and pectin is inevitable, which may change the physicochemical properties of the juice, thereby affecting the apparent quality and nutritional properties. The retention of cyanidin-3-O-glucoside (C3G) and antioxidant activity under conventional juice processing (high-speed shear (HSS), high-pressure homogenization (HPH) and heat treatment (HT)) were investigated in the presence of different sources of pectin (apple and peach) and different pectin fractions (Water-soluble fraction (WSF), chelator soluble fraction (CSF) and sodium carbonate soluble fraction (NSF)). Pectin with a low degree of rhamnogalacturonan I branching (RG I) led to a high C3G retention under HT. With HSS applied, more linear structure and high homogalacturonan pectin decreased the antioxidant capacity of C3G. Under HPH treatment, the retained antioxidant capacity was negatively correlated with the pectin degree of esterification. The homogenization process mainly affects the binding on the pectin side chains instead of pectin backbone and peach pectin was found to promote C3G degradation. These results can open opportunities for the use of natural raw materials (peach and apple pectin) to produce more stable and healthy fruit juice containing anthocyanins.

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UHPLC-MS/MS quantification of acrylamide in various foodstuffs: formation and strategies of mitigation

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Since acrylamide has been reported to occur in cooked foods, in 2002, it has become a public health concern, mainly because the International Agency for Research on Cancer classified it as probably carcinogenic to humans (Group 2A) [1]. For this, international food agencies and food industries provided code of practices and guidelines to control and reduce the acrylamide formation at industrial levels. However, the acrylamide formation and its mitigation strategies are still matter of attention from the science community and industrial sector, especially for certain categories of foods known for high acrylamide contents, i.e., potato chips, coffee and baked products. Hence, the present research aimed to optimize an analytical method for acrylamide quantitation by UHPLC-MS/MS system in three different types of cooked foods, i.e., potato chips, roasted coffee and biscuits, with the final goal to study its formation and mitigation. In detail, various mitigation approaches, such as dipping in water, dipping in Aureobasidium pullulans L1 yeast water suspension, dipping in water or in yeast water suspension after pulsed electric fields (PEF), have been employed to reduce the acrylamide formation in potato chips. At the same time, different roasting degree (light, light-medium, medium, medium-dark, dark) in two different coffee bean types (arabica and robusta) and diverse cooking methods (ventilated and static mode for 18, 20, 22, 24, 26 min) for biscuits have been investigated, for evaluating how these processes influenced the acrylamide formation. The yeast water suspension determined a reduction of acrylamide content in potato chips mainly at the longest dipping time (676.4 ± 42.3 µg/kg at 15 min vs. 1384.3 ± 65.0 µg/kg of control) while PEF treatment followed by water dipping was the most promising approach (886.8 ± 9.9 µg/kg at 5 min and 572.0 ± 8.8 µg/kg at 15 min) [2]. On the other hand, at the first roasting degrees for coffee (light and light-medium) higher content of acrylamide was found (730 ± 30 µg/kg for arabica and 1130 ± 10 µg/kg for robusta both at light-medium degree) while it decreased by prolonging the heating process (85% and 88% of reduction from light-medium to dark roasting in arabica and robusta, respectively) [3]. Ventilated mode determined higher contents of acrylamide than static in biscuits cooked for 20 and 22 min, likely because the heat was distributed more evenly compared to the static one [4]. In summary, the present research reports how different strategies and processing methods can influence the acrylamide formation in three categories of cooked foods and provides important knowledge, which the food industry can benefit from.

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ORAL PRESENTATIONS

T7

Food adulteration, authenticity and traceability

Classification, Characterization, and Authentication of Honey by HPLC-UV fingerprinting and Chemometrics. Application to the detection of Honeys adulterated with Syrups.

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Honey is a natural product produced by bees from nectar and other non-floral secretions. They are classified as multifloral or monofloral depending on the pollen content. Honey is considered monofloral if more than 45% of the pollen belongs to the same botanical species. They are also classified as blossom-honeys if they are produced from the nectar of flowers, or as honeydew-honeys if they are produced from plant secretions or sugar-rich materials that plant-sucking insects excrete. Honeydew-honeys tend to be darker and with higher total polyphenol content (leading to higher antioxidant properties) than blossom-honeys. Honey is susceptible to adulteration because of its variable composition depending on the different conditions of production (botanical origin, region of production, etc.), and the similarity with many adulterants (syrup-based products). Hence, the possibility of committing fraud by giving incomplete information about the product or by mixing high-quality honeys with cheaper ones is always present [1]. Thus, the development of feasible and simple analytical methodologies for the characterization, classification, and authentication of honey, as well as for detecting fraudulent practices (for example, based on honey adulterated with syrups), is necessary.

In this work, the characterization and classification of honey according to their botanical varieties using a non-targeted C18 reversed-phase HPLC-UV methodology was evaluated [2]. A total of 137 blossom- and honeydew-honeys of different botanical varieties (blossom, heather, mountain, eucalyptus, forest, holm oak, rosemary, thyme, and multifloral) were analyzed. A simple honey sample treatment based on sample dilution with water followed by a 1:1 dilution with methanol, and separation under a universal gradient elution was used. The obtained HPLC-UV fingerprints were subjected to an exploratory principal component analysis (PCA) and a classificatory partial least squares-discriminant analysis (PLS-DA) to evaluate their viability as sample chemical descriptors for authentication purposes, obtaining good discrimination results between blossom- and honeydew-honey samples.

In addition, the capability of the proposed HPLC-UV fingerprinting methodology to detect honey fraudulent practices based on adulteration with syrups was also evaluated. For that purpose, more than 20 syrup samples of different plant origins (corn, fiber, maple, rice, agave, among others) were also analyzed following the same procedure. A 100% classification ratio of honey samples against syrups was accomplished when building the classification model with 60% of the samples (randomly selected) and employing the other 40% of samples for prediction purposes, showing the good performance of HPLC-UV fingerprints to authenticate honey samples. Besides, several adulteration cases, based on honeys of different botanical varieties adulterated with syrups at different levels, were also evaluated by partial least squares (PLS) regression. For that purpose, calibration models were built with adulterant (syrup) contents of 0, 20, 40, 60, 80, and 100%, while 15, 25, 50, 75, and 85% adulterant (syrup) contents were employed for prediction purposes. PLS results showed good linearity, with calibration and prediction errors, in general, below 12%.

The results obtained in this work demonstrate that HPLC-UV fingerprinting is a simple and feasible strategy to address the characterization, classification, and authentication of honey samples, as well as to detect fraudulent practices of honeys adulterated with syrups.

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Slovak-Austrian cooperation in honey quality assessment

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Honey is a natural bee product with valuable medicinal and immunostimulating health effects. The quality of honey is regulated with the aim to protect consumers from lower quality or adulteration practices. The characterization of botanical and geographical origin of honey is important in terms of supporting the competitiveness of small beekeepers involved in the study. In total of 124 honey samples from Slovakia and Austria of various botanical origins were collected in 2021 and 2022, including acacia, rapeseed, linden, honeydew, forest, and chestnut honeys. They were characterised based on selected physico-chemical parameters, including water content, water activity, electrical conductivity, acidity, enzymatic activity, 5-hydroxymethylfurfural (HMF) and other sugar degradation products content. An important part of the collaboration between the institutions was focused on expertise and training of sensory panels aimed at the characterization of the botanical origin of honeys supported by analysis of specific volatile compounds using GC-MS methods.

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Sesquiterpene chromatographic fingerprinting: lights and shadows for the geographical and varietal authentication of olive oils.

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Virgin olive oil (VOO) is a top-quality product with exceptional sensory and nutritional value. Many VOO producers distinguish their VOO by specifying the olive cultivar and geographical origin. Therefore, it is crucial to verify label-declared variety and geographical origin to prevent consumers from being misled. Currently, verification is only conducted through documentary review, as no official method has been accepted. The presence of sesquiterpene hydrocarbons (SHs) in VOO is highly dependent the olive cultivar and the growing area while it is barely affected by technological factors such as olive post-harvest processing and oil storage conditions. This makes them excellent candidates for authentication models. We present the results of an SH fingerprinting approach to authenticate the varietal and geographical origin of olive oil. Regarding the varietal authentication, the approach successfully discriminates olive oils from the Arbequina cultivar obtained from different geographical origins [1]. Regarding the geographical authentication, the SH fingerprinting approach is also successful at different geographical levels, including the EU, the country [2] and even, the PDO [3]. All models have been developed on large sample sets (>300 samples) including samples from different harvests, cultivars and geographical origins that were analysed by HS-SPME-GC-MS. In all cases, the unfolded matrix built with extracted ion chromatograms of SH specific ions has been used (after chromatographic alignment) to develop classification models based on Partial Least Square-Discriminant Analysis (PLS-DA), supervising it or by the variety or by the geographical origin. All models have been externally validated. The results of the external validation confirm that genetic and environmental factors exert distinct effects on the SH fingerprint and that PLS-DA, depending on the variable selected for supervising the model, finds the features of the SH fingerprint that are characteristic of the variety (beyond the geographical origin) or of the pedoclimatic condition (beyond the cultivar). The tool's success comes from the combination of the SH's biological adequacy, analytical characteristics of HS-SPME-GC-MS, and fingerprinting approach combined with chemometric modelling. However, there are some aspects of chromatographic fingerprinting to be addressed before it can become a universal authentication strategy. Thus, this presentation will also address the current challenges and possibilities of applying the SH fingerprinting approach to other food commodities.

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ORAL PRESENTATIONS

T8

Novel methods for food chemistry

Improving protein extraction from *Chlorella vulgaris* using combined mechanical/physical and enzymatic pre-treatments

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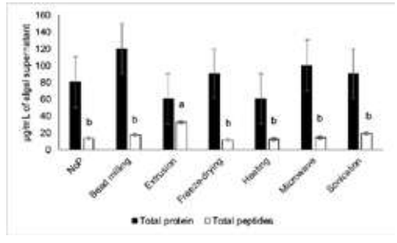
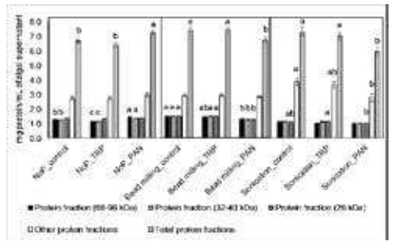
Chlorella vulgaris is a microalga rich in essential nutrients, particularly high-quality proteins (up to 65.5% of dry matter), with food and feed applications [1]. However, the cell wall of *C. vulgaris* represents a major barrier to protein extraction, since it is primarily composed of insoluble carbohydrates, such as cellulose and chitin-like polymers [2]. Proteins from *C. vulgaris* can exhibit a wide range of molecular weights (14 to 116 kDa), according to growth conditions [3], and some of them form complexes with chlorophyll a in thylakoid membranes, which are resistant to anionic and non-ionic detergents [4]. The present study aimed to evaluate different pre-treatments (bead milling, extrusion, freeze-drying after congelation at -80 ºC, heating at 70 ºC, microwave and sonication) combined or not with trypsin (TRP) and pancreatin (PAN) for protein extraction from *C. vulgaris* biomass. The microalga suspension with phosphate buffer (20 mg/mL) was incubated for 16 h with peptidases (20 µg/mL) (n = 5), followed by centrifugation. Total protein and peptides released to the supernatant were quantified using Bradford and *o*-phthaldialdehyde (OPA) methods, respectively [5]. The extracted protein fractions were analysed by 12% SDS-PAGE gel electrophoresis: total protein fractions; protein fraction 1 (66 to 96 kDa) (F1), 2 (32 to 40 kDa) (F2) and 3 (26 kDa) (F3), and other protein fractions. The results showed that pre-treatments had no significant effect ($P > 0.050$) on the amount of total protein compared to control (no-pre-treatment, NoP), except for freeze-drying with pancreatin, which decreased total protein ($P = 0.024$) and non-significantly increased total peptides. Freeze-drying or bead milling combined with trypsin significantly ($P < 0.050$) extracted total peptides and extrusion caused a 3-fold increase ($P < 0.001$) in peptides. Bead milling and microwave were effective ($P < 0.001$) in releasing F2 from algal biomass, whereas sonication could release other proteins. The combination of bead milling with pancreatin hydrolysed F1, F2 and F3 ($P < 0.050$). Both bead milling ($P = 0.018$) and sonication ($P = 0.013$) combined with pancreatin caused a reduction of total protein quantified in the gel. Overall, the pre-treatments promoted protein extraction and increased their susceptibility to enzymatic activity. However, the total protein yield was low (up to 0.75 %) and further research is required to fully exploit their potential for protein extraction from *C. vulgaris* biomass.

Fig.1. Effects of pre-treatments alone **(a)** or combined with enzymes **(b)** on total protein (Bradford), peptides (OPA) and protein fractions (SDS-PAGE) released from *C. vulgaris* biomass (means ± standard error).



(a)



(b)

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Single-tube nested real-time PCR as an efficient tool to quantify allergenic tree nuts in processed foods

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Tree nuts encompass a big group of seeds/nuts, which are widely appreciated for their organoleptic properties. Several health benefits have been attributed to tree nuts and their consumption has been greatly associated with a healthier lifestyle. Currently, different species of tree nuts are widely ingested in their raw state, processed (e.g., roasted) or included as an ingredient in numerous food formulations (cakes, breads, chocolates, etc). However, tree nuts are also a group of well-known allergenic foods, being responsible for eliciting moderate to severe allergic reactions in sensitised individuals. Among the food-related allergy, the prevalence of tree nut allergy is high at global scale, meaning that it is of paramount importance the safeguard of this part of the global population. Currently, tree nuts are required to be labelled in pre-packaged foods, although their unintended presence may still occur due to cross-contamination events during food production/manipulation or due to bad manufacturer practices. In this sense, it is also crucial to have proficient food allergen monitoring programs, ensuring the improvement of analytical methods that can detect traces of allergens in processed foods. In this work, it was intended to exploit the performance of single-tube nested real-time PCR for the quantification of different targets, namely distinct tree nuts in a set of diversified food matrices [1-5].

For this purpose, model mixtures of different matrices (biscuits, wheat material, ice-cream, cakes) containing known amounts of hazelnut, almond, walnut, cashew and pistachio nut (10-0.0001%, w/w) were prepared as calibrators. Different genes encoding for hsp1 protein, Pru du 6, Jug r 3, Ana o 3 and Pis v 2 allergens, were selected as prospective molecular markers for hazelnut, almond, walnut, cashew nut and pistachio nut, respectively. Independent single-tube nested real-time PCR methods using hydrolysis probes were successfully developed, presenting adequate performance parameters (coefficient correlation >0.9933, PCR efficiency 84.9-110.8% and slope in the range of -3.745 to -3.093) for all targets and high sensitivity/specificity for the quantification of different nuts in model foods (Table 1). Each system allowed the unequivocal identification of the desired target, as no cross-reactivity with non-target species was observed for any of the target genes. All systems allowed quantifications between 50 and 5 mg/kg of the target food, presenting the lowest LOD (5 mg/kg) for pistachio nut in ice-cream. This technology provided consistent and highly sensitive/specific tools to target different allergenic tree nuts, with potential to be applied to other allergenic ingredients that might be present in distinct non-processed/processed food matrices [1-5].

Table 1. Performance parameters of different single-tube nested real-time PCR approaches.

Target gene	Tree nut in matrix	PCR efficiency	Slope	Correlation coefficient (R2)	LOD (%)	LOQ (%)
Hazelnut Hsp1						
	Wheat material	107.0	-3.165	0.9956	0.005	0.005
Almond Pru du 6						
	Walnut	95.6	-3.433	0.9933	0.005	0.005
Walnut Jug r 3						
	Dough	109.0	-3.124	0.9962	0.001	0.001
	Sponge cake	108.4	-3.135	0.9962	0.001	0.001
Cashew nut Ana o 3	Wheat material	90.4	-3.574	0.9984	0.005	0.005
	Dough	110.8	-3.093	0.9939	0.001	0.001
	Biscuit	103.2	-3.248	0.9926	0.001	0.001
Pistachio nut Pis v 2						
	Wheat material	84.9	-3.745	0.9940	0.01	0.01
	Ice-cream	104.2	-3.226	0.9970	0.0005	0.0005

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Determination of protein biomarkers by on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry. From biomedicine to food science

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The analysis of protein biomarkers is of great interest in biomedicine and food science. Today, it is widely accepted that high performance separation techniques coupled to mass spectrometry (MS), such as liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS), are the techniques of choice for a reliable analysis of proteins, at the intact level or after enzymatic digestion for bottom-up peptide mapping. However, in many cases the protein biomarkers of interest are found at very low concentration in very complex samples, such as in certain biological fluids or in food contaminated with allergenic proteins. Several strategies have been described to enhance sensitivity in LC-MS and CE-MS, including the on-line coupling of solid-phase extraction for sample clean-up and analyte preconcentration from a large volume of sample. On-line solid-phase extraction capillary electrophoresis-mass spectrometry (SPE-CE-MS) is especially convenient, as it can be easily automated with valve-free microseparation set-ups in commercial CE instruments [1-2]. In this presentation, I will offer an overview of our latest developments in SPE-CE-MS using sorbents based on aptamers [2], which can be selected to recognize protein biomarkers with high affinity and selectivity. I will present different applications for the analysis of protein biomarkers related to neurodegenerative and infectious diseases, and food allergy, at the intact level and from characteristic signatures of peptides fragments [2-4]. The combination of aptamers, electrophoretic separation, and MS detection allows high-selectivity, high-sensitivity, and accurate quantification, with no possibility of false positives due to the unequivocal identification.

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Method development for endocannabinoid analysis in fermented foods

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Endocannabinoids (ECs) and endocannabinoid-like (ECL) compounds are lipid signalling molecules composed of different fatty acid derivatives. They are a large group that includes *N*-acylethanolamines, *N*-acylamino acids, *N*-acylneurotransmitters, monoacylglycerols, and primary fatty acid amides. These molecules play an important role in many physiological processes such as mood, memory, pain control, energy homeostasis, and hunger control. In addition to humans, ECs/ECL compounds are found in plants and certain microorganisms. Recent studies have focused on the formation of microbial metabolites which can affect human mood. It has been reported that some ECs/ECL compounds can be produced by microorganisms, but there is limited research on their occurrence in fermented foods. Therefore, the aim was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) which can provide information about the presence and changes of ECs/ECL compounds during the fermentation of foods.

LC-MS/MS optimization, extraction optimization, and method validation were performed to detect these large numbers of molecules (36 compounds) in a single analysis also by using stable isotope labelled internal standards (7 compounds). Different extraction solvents such as acetonitrile, acetonitrile-isopropanol mixtures, and ethylacetate-hexane mixtures were tested for the selection of the best suitable extraction solvent. Furthermore, single and triple-stage extraction and ultra-turrax homogenization were evaluated for the determination of the appropriate extraction condition.

The developed method was suitable for the separation of isomers and quantitation of ECs/ECL compounds in a wide range of concentrations in fermented foods including fermented Turkish sausage, cheese, kefir, yogurt, bread, olives, cocoa, beer, and wine. The method has low detection (0.01-4.30 ng/mL) and quantification limits (0.02-14.2 ng/mL), good linearity (R^2>0.982), high recovery values (>67%) and good reproducibility (0.1-14.4%) and repeatability (0.3-18.4%). *N*-acylamino acids and *N*-acylneurotransmitters were detected for the first time in fermented foods. Moreover, fermented sausage, cheese, and cocoa powder among fermented foods were found to be rich in ECs/ECL compounds. This LC-MS/MS method can be used to determine the amounts of ECs/ECL compounds in foods and can be used to investigate how these compounds change under various processing conditions, including fermentation.

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HS-GC-IMS: a new rapid strategy to fingerprint volatile compounds in foods: quality and integrity applications

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Each food (or food ingredient) is characterized by a distinct volatilome fingerprint, composed of many volatile organic compounds (VOCs), as result of the natural presence of flavoring compounds, microbial fermentation, industrial processing or external contamination (e.g. migration from packaging). VOCs usually act as indicators of sensorial quality, sometimes useful to assess authenticity, integrity and safety. Gas chromatography, coupled with mass detectors, has been declined in the past in different analytical approaches, including the comprehensive GCxGC-MS technique **[1].** More recently, the bidimensional HS-GC-IMS (Headspace Gas Chromatography coupled with Ion Mobility Spectrometry) **[2]** showed a rapid increase in food applications, as sensitive technique useful to describe the complex volatilome of foods, considering both the targeted and untargeted approaches. The reduction of time-consuming sample preparation, minimizing handling and pre-treatment steps, the rapidity of application as well as the capacity to work at-line, represent the main values of this technique.

This lecture aims to show that a rapid HS-GC-IMS analysis is able to solve food-related authenticity/traceability problems (e.g., integrity of traditional vinegar from Modena, Italy, or the identification of green coffee origin), quality challenges and monitoring of processing impact (e.g. the quality of spreadable hazelnut creams, the flavor of tomato products and ice creams).

Some specific case studies for each specific target will be shown, reporting the advantages of this rapid approach of fingerprinting. HS-GC-IMS is confirmed as high sensitive and reproducible rapid technique, able to reduce time to obtain the outcomes, using a "green" approach, avoiding the use of solvents in sample preparation.

The comparison of HS-GC-IMS with the multidimensional comprehensive GCxGC-MS analysis (green coffee) as well as the implementation of some non-supervised AI-based strategies in the post-analytical processing of data (and the need to improve the quality of the signal) will be also discussed, opening new perspectives in rapid and partially automated food analysis **[3]**. Moreover, the rapid improvement of the analytical sensitivity and detection of volatile compounds exploring the "salting out" method will be discussed with practical examples.

The results confirm and emphasizes the usefulness of the hyphenated analytical platform, particularly as preliminary data set production useful for the data/cluster analysis, but also to identify the presence of in trace VOCs in foods as "molecular markers".

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An innovative nanoplate digital PCR approach for the quantification of allergenic sesame in foods

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Sesame (*Sesamum indicum* L.) has globally emerged as an important allergenic food due to the adoption of healthier dietary habits and the subsequent increase of foods containing sesame seeds or oil. The presence of undeclared traces of sesame due to unintended cross-contamination can be the cause of severe allergic reactions in sensitised individuals [1]. Recently, digital PCR has arisen as a newsworthy alternative to real-time PCR for plant species detection, enabling increased sensitivity and precision, being less prone to PCR inhibitors and providing absolute quantification without the use of a calibration curve. The nanoplate digital PCR (ndPCR) was launched in 2020 as a simpler and faster technology than the current droplet digital (ddPCR). In ndPCR, the partitioning (distribution of the reactional mixture through a multifluidic nanoplate), thermocycling and signal acquisition are all integrated into a single fully automated instrument [2], while in ddPCR the partitioning is based on the formation of individual droplets in a water-oil emulsion, followed by sequential thermocycling and signal acquisition (normally in separate units). This work intended to develop a novel ndPCR capable of detecting traces of sesame in processed foods. Model mixtures containing known quantities (100,000-0.1 mg/kg, w/w) of sesame in wheat dough were prepared simulating the production of biscuits (180°C, 40 min). Sequences of multicopy genes from mitochondrial (CO6b1) and nuclear (ITS) regions were used to design primers and probes to specifically target sesame. Two independent ndPCR approaches were successfully developed for two DNA sequences, achieving limits of detection (LOD) of 5 and 0.1 mg/kg in dough/biscuits, targeting the CO6b1 and ITS regions, respectively (~ 0.5 copies of sesame DNA per µL of reaction). The LOD obtained in the CO6b1 sequence was improved by one order of magnitude comparing with a previously developed real-time PCR method for sesame detection [3]. Additionally, the sensitivity of both methods was not affected by food processing (baking), despite the decreased DNA amount in processed mixtures due to DNA degradation. However, only the CO6b1 system was not affected by food matrix because exhibited the same performance regardless the use of complex matrix (model mixtures starting from 100,000 mg/kg of sesame) extracts or serial diluted DNA. The present work introduces the first ndPCR approach for the detection of food allergens in foodstuffs, reaching a high sensitivity, with optimal performance and without being affected by food processing or food matrix.

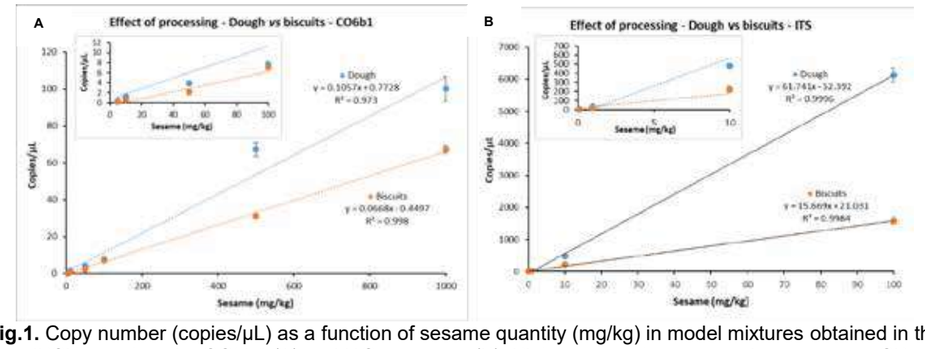

Fig.1. Copy number (copies/µL) as a function of sesame quantity (mg/kg) in model mixtures obtained in the ndPCR targeting the CO6b1 (A) and ITS sequence (B). Plots represents copy number average ± SD.

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Chemical characterisation of biscuits melanoidins using *shotgun* proteomics

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Maillard reaction (MR) is a non-enzymatic browning reaction that occurs between the carbonyl group of reducing sugars and the amine group of amino acids and proteins at high temperatures. Melanoidins are the final products of the reaction and they are responsible for the sensory properties of foods, such as taste, color, and aroma, that occur during the thermal processing of a wide variety of foods.[1,2] It has been estimated that melanoidins have a significant presence in the European diet, especially in bread and coffee. Biscuits are also widely consumed, especially by the younger population, and therefore are among the main sources of melanoidins.[2] Thus, the study of the chemical structure and biological activities of those compounds is mandatory to understand the impact of these foods on consumers' health. Although the chemical structure of melanoidins is not completely established, it is believed that melanoidins from wheat-based products are supposedly generated by colored Maillard reaction products (MRPs) cross-linked with gluten proteins, while other low molecular weight (LMW) MRPs are entangled in the gluten network.[2,3]

In this work, we developed a new method to extract melanoidins from samples of biscuits using successive enzymatic digestions and solvents. We also developed a method to identify the proteins involved in melanoidin formation by *shotgun* proteomics (high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS)). This method was also applied to identify possible protein modifications induced by the Maillard reaction. To validate the efficacy of this method for this kind of assessment, we made Bovine serum albumin (BSA) model systems, simulating the reaction between BSA with several compounds, such as Glucose, Fructose, Glyoxal, Methylglyoxal, Furfural, and Glycolaldehyde.

We were able to identify peptides derived from gluten proteins, such as gliadins and glutenins, as well as peptides from soluble proteins present in wheat flour. We also identified some of the protein modifications previously described in the literature as possibly induced by the compounds used in the BSA model systems, suggesting the efficacy of the method in that regard. It was also possible to identify some protein modifications occurring in gluten proteins, possibly induced by the Maillard reaction, showing that this approach is a promising tool to understand the chemical composition and formation mechanism of biscuit melanoidins.

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Capsule phase microextraction: A green sample preparation strategy for food matrices

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Capsule Phase Microextraction (CPME) device (Fig. 1) has been introduced as a stand-alone sample preparation device that simultaneously filtrates the sample and extracts target analytes. A stirring mechanism is also included in the device, making an external magnetic rod unnecessary.

CPME devices are constructed by two porous polypropylene tubes with i.d. 1.8 mm, o.d. 2.7 mm, and wall thickness 0.45 mm. These tubes are united, including a magnet encapsulated in one of them and the sorbent microparticles encapsulated in the other. Sorbents are synthesized by sol-gel technology, specifically in-situ sol-gel synthesis, while keeping the porous polypropylene tubes submerged in the sol solution. Capsule phase microextraction devices possess equivalent sorbent loading and similar contact surface area compared to FPSE membranes. As the CPME device is immersed in the sample, the aqueous sample quickly permeates through the porous walls (0.2 μm pore size), and the target analytes interact with the sorbent particles trapped inside the porous walls and are rapidly extracted. The porous walls of the tube act like a filter removing any interfering particulate, making the CPME device suitable for a wide variety of complex matrices, including food samples. The magnet in the device facilitates spinning on the magnetic stirrer. At the same time, extracted analytes are back-extracted to a low volume of a suitable solvent rendering the microextraction technique greener than conventional ones and environmentally friendly.

Moreover, the same CPME device can be used several times without sacrificing its extracting power [1].

Till now, CPME technique has been successfully applied to the determination of sulfonamides in milk [2], coumarin in bakery products [3], benzoyl urea insecticides in apple juice samples [4] and are tested for bisphenol A in beverages in unpublished results.

Example protocols with advantages and disadvantages of the technique are briefly described herein.

Fig.1. Capsule Phase Microextraction device.

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Contributions of analytical methods to unveil bioactive compounds in food matrices

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Food matrices pose a significant challenge to analytical chemists due to their complexity and to the different chemical properties of the numerous possible target analytes. Moreover, concentration values may range from gram per litre values for macrocomponents, going down to trace, nanogram per litre levels for some relevant bioactive compounds. In this communication, two recent contributions for assessment of bioactive compounds in food matrices will be considered. First, the interpretation of data from the Oxygen Radical Absorbance Capacity - ORAC method for evaluating antioxidants in food products is addressed [1]. ORAC indexes result from different mathematical approaches often lacking correct elucidation of kinetic features concerning radical scavenging reactions by antioxidant compounds. Two approaches were exploited: the evaluation of the area under the curve (AUC), defined by fluorescein decay after oxidation by peroxyl radicals, and the observation of the time during which fluorescein oxidation is prevented (lag time) as depicted in Fig. 1. Results have shown the importance of choosing suitable calibrator compounds, presenting ORAC kinetics similar to the target food samples (orange juice, red wines) to prevent biased estimation of the antioxidant capacity.

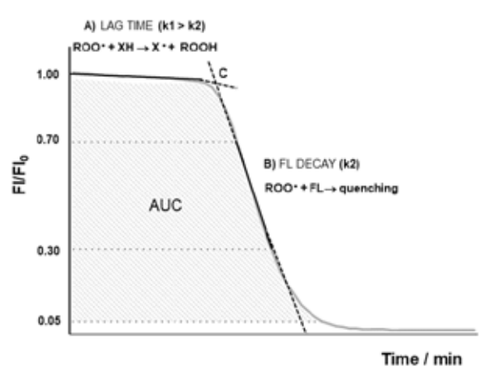

Fig.1. Schematic representation of relative fluorescence vs. time in ORAC assay.

Additionally, considering the current needs of data mining tools for extracting the information provided by comprehensive methods, such as mass spectrometry, a dedicated algorithm was developed [2]. The proposed algorithm was applied to an LC-MS data set generated from the analysis of grapes collected over a developmental period encompassing a 4-month period. The algorithm outcome was a short list of features from metabolites, comprising the retention time, the m/Z value and the signal intensity. The performance of the algorithm was compared with the commercial MZmine software, yielding three-times more features, providing a final set of 99 features (out of 1543 initially identified). In the end, the proposed algorithm demonstrated a higher ability to pin-point features that may be associated with grapes developmental and maturation processes requiring minimal parameters definition.

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Data mining and machine learning methods to predict food contaminants exposure

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Through diet, humans are continuously exposed to a wide variety of food-chain contaminants, namely, heavy metals, pesticide residues, polycyclic aromatic hidrocarbons, heterocyclic aromatic amines, dioxins/dioxin-like PCBs, disinfection by-products, and natural toxins (mycotoxins), among others. The knowledge concerning the global combination and magnitude of contaminants intake associated with diet intake is lacking. This is of major relevance since the combined health risks of those mixtures of contaminants needs to be properly studied in the context of full diet intake. The scientific literature provides a great amount of valuable data, however, information is disperse and summarise it manually is not feasible. To tackle this gap, data mining (DM) - extraction of meaningful knowledge hidden within large datasets and machine learning (ML) by using algorithms that deal with vast data in the most intelligent way and derive relevant insights is needed. The goal of this work was to implement a systematic organization of existing knowledge concerning food contaminants in highly produced/consumed food items, using DM and ML methods, to identify the patterns of contaminants distribution in most consumed foods and establish associations between diet and contaminants intake. FoodMine's code [1] with some modifications was applied and FAOstat database was used for selection of the most consumed foods worldwide. PubMed Advanced Search Builder was used to determine the best terms and search strategies. A total of 96 food contaminants were searched in 80 food items. FoodMine code provided 26,697 articles, which were reduced to 1,887 articles by ML. From those, 438 articles were effectively used to extract data and build a database of contaminants content in most consumed foods (covering the period 2000-2022), while the remaining 1,449 articles were excluded, because they did not relate to quantification of contaminants in foods, or not specify the N or the contaminant prevalence (%). To predict the patterns of contaminants distribution, the 80 food items were classified in 12 groups (most of them according to hieralchical level 1 of FoodEx2). Multivariate statistical analysis of data revealed that heavy metals (arsenic, cadmium, lead and mercury) were the most prevalent food contaminant. The second most prevalent were mycotoxins, followed by polycyclic aromatic hidrocarbons, pesticide residues, and heterocyclic aromatic amines. Pesticides and mycotoxins achieved the highest contents.

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ORAL PRESENTATIONS

T9

Food contaminants

Tropomyosin quantification in seafood samples-right choice of standard makes a difference

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In the last 50 years, the annual per capita consumption of seafood products worldwide has more than doubled, from almost 10 kg in 1960 to over 20 kg in 2014. Seafood protein is an essential part of the diet in many countries, particularly where total protein intake is low [1]. However, as defined by the European Community, fish, and shellfish tropomyosins (TPM) are major allergens and major causes of anaphylaxis [2]. The increasing prevalence of food allergies is consistent with the increasing pollution of soil and water with plastic particles. To investigate the potential link between increasing plastic pollution and increasing food allergy prevalence, we aim to develop methods for precise and accurate monitoring of allergens and plastic in real seafood samples.

TPM was isolated from shrimp (*Litopenaeus vannamei*), clams (*Venerupis philippinarum*), and mussels (*Mytilus galloprovincialis*). The obtained in-house TPM proteins from three different sources were resolved using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The concentration of TPM in seashell samples from different geographical origin was determined using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with prior optimization of adequate TPM standard curve using commercial and non-commercial in-house prepared TPM standards.

TPM standards resolved *via* 2D-PAGE revealed the presence of two isoforms of shrimp and mussels TPM standard, one dominant and one less abundant isoform. Two isoforms from both seafood sources, shrimp and mussels, are slightly different in molecular weight and pI value. As for the TPM standard obtained from clams, the 2D electrophoregram showed possibly eight isoforms with small differences in mass and pI values. Furthermore, the presence of three dominant isoforms can be observed that differ slightly in molecular mass, while other isoforms also differ in pI value. The ELISA results, regarding TPM standard curve optimization, showed that in both the commercial shrimp TPM and in-house shrimp TPM standards, sigmoidal concentration dependence is present in a range of 50 to 0.05 ng/ml, using serial double dilutions. On the other hand, TPM standards isolated from mussels and clams show sigmoidal concentration dependence in the range of 45 to 0.044 µg/ml with using the identical combination of capture and detection antibodies and serial double dilutions. TPM concentrations in clams and mussel samples extrapolated from standard curves of commercial shrimp TPM standard and corresponding in-house TPM standards are presented in Table 1.

Table 1. TPM concentration in real samples using different standard curves

Sample	Commercial TPM standard C (ng/ml)	In-house TPM standard (µg/ml)
Clams Korea Yellow Sea	119.7±14.1	69.4±11.5
Clams Korea Taean	100.6±29.8	49.4±7.6
Clams Croatia	110.6±47.5	57.7±23.0
Mussels Croatia	12.4±2.5	3.8±1.5
Mussels Belgium	8.1±2.4	0.31±0.05

Differences in TPM concentration of the same sample using different TPM standards differ from 40 to 600 times, which strongly indicates that the right choice of TPM standard is a critical step for accurate and precise determination of TPM concentration in seafood samples.

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Detection of food contact chemicals from inks and adhesives in food

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Food contact materials (FCM), all materials designed to into contact with food, contain a wide number of compounds or food contact chemicals (FCC), with estimates of several thousands compounds being present. FCC can be both intentionally added (IAS) or non-intentionally added substances (NIAS). Although legislation in the EU exists on some food contact materials, a large number of these FCC remain unstudied, their presence unknown and their toxicology not evaluated. Inks and adhesives are extensively used on FCM. Inks are used for both marketing and information purposes. Adhesives are used to bind together multiple layers of packaging, for closing them or for adding a label. Previously, our team has performed a risk-based prioritization of almost 7,000 IAS used in the manufacture of inks and adhesives for food packaging [1]. 696 very high priority substances were identified, being the compounds with the highest hazard for human consumption. In this work, we developed analytical methods for a number of these compounds and tested food samples for their presence.

Compounds to be included in the method were chosen from the list of 696 prioritized substances, based on availability of a standard, presence in food and the environment. Other compounds were evaluated analytically and divided between an LC-MS/MS and an APGC-MS/MS method. In total 110 compounds were included in the methods.

Instrumental methods for both LC-MS/MS and APGC-MS/MS were developed and the usual parameters were optimized (mobile and stationary phase, MS parameters, etc.) QuEChERS extraction, combined with dispersive SPE, was used for the extraction of food samples and this was optimized for five food matrices: frozen brussels sprouts (water-rich), apple (water-rich and acidic), olive oil (fat-rich), biscuits (fat- and starch-rich) and cheese (fat- and protein-rich), in order to cover a wide variety of food. The extraction solvent and SPE sorbent were optimized. The extract could be used for both APGC-MS/MS and LC-MS/MS analysis. The developed methods were fully validated in all five tested matrices according to guidelines for FCM testing [2]. Overall, good validation results were obtained, but some classes of compounds are difficult to analyse in multi-analyte methods covering a wide range of chemicals. For example, primary aromatic amines are known toxic compounds being formed from most used inks, but as they are small, hydrophilic compounds, they are not well suited to a standard RPLC analysis and cannot be analysed by GC.

52 samples were bought from Belgian supermarkets, covering a wide range of products: 14 were baby food, 15 were frozen products (vegetables, potato products, etc) and 22 food matrices containing more fat (biscuits, pastry, cheese, meat, etc.). In most of the baby food samples no compounds were detected. This is expected, as they are often packaged in expensive, multi-layered material containing a barrier layer. In one sample, caprolactam was detected at low concentration (30 ppb) and another sample contained phenylacetaldehyde, a natural occurring compound but also an additive to polymerization reactions, at 88 ppb. Frozen food samples often contained low amounts of caprolactam (11 to 100 ppb, found in 10 out of 15 samples). All 15 samples contained one or two of the targeted analytes, but frozen foods are mostly hydrophilic, slowing extraction of FCC and they are stored at low temperatures, again slowing extraction of FCC.

For foods containing more fat, there are more and more problematic compounds being detected. 19 out of these 22 samples contained 2,2-dimethoxy-2-phenylacetophenone, a photo-initiator at concentrations between 21 and 106 ppb, showing a wide variety of sources of this toxic chemical. Another toxic compound that was detected was cyclohexanone oxime, with a NOAEL of 2.5 mg/kg BW per day and detected concentrations between 18 and 38 ppb, for example in packaged cheeses. The highest concentration was found for caprolactam, with one sample as high as 28 ppm, where the SML is 15 ppm. It was detected in 11 of these samples, with five samples at a concentration over 1 ppm. These high concentrations were detected in pre-packaged cheeses and meat.

These positive findings for a very restricted list of compounds show the need for more work on FCC, both in food analysis and toxicological evaluation. Analytical development and legislation need to urgently tackle the issues with FCC. The potential problem is huge, with several thousands being used (IAS), but many more being formed (NIAS) and the risk for public health is a real concern.

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Asparaginase treatment of fruit additives enriching biscuits

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Bakery products as staple foods are a frequently used matrix for enrichment with nutritionally valuable compounds, especially of natural plant origin. Sea buckthorn fruits are the exceptional material used for the production of juices and marmalades, however the pomace remaining from the juice production still has a high content of bioactive substances (carotenoids, tocopherols, flavonoids, tannins, phenolic acids, ascorbic acid etc.) [1]. The partial substitution of wholegrain flour (wheat, triticale, rye) with dried sea buckthorn pomace in the recipe of biscuits significantly increased their nutritional value. On the other hand, due to the high content of the free amino acid asparagine in sea buckthorn berries, this ingredient promoted the undesirable acrylamide formation. This disadvantage was successfully eliminated by introducing asparaginase treatment into the procedure of sea buckthorn pomace processing. The application of the enzyme had to be optimized for the specific acidic conditions of sea buckthorn mash. This innovative procedure is protected as the utility model No 9572 [2]. The final biscuits enriched with enzymatically treated sea buckthorn pomace powder had a reduced acrylamide content by up to 65%, thus complying with the requirements of the EU Regulation No 2017/2158 [3] to the benchmark level of acrylamide content for biscuits below 350 µg/kg.

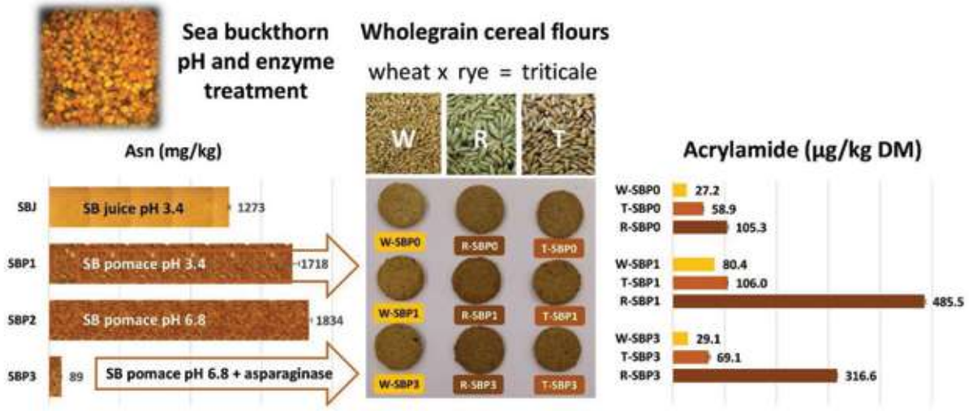

Fig.1. Impact of enzymatically treated sea buckthorn pomace on acrylamide reduction in nutritionally enhanced wholegrain cereal biscuits

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Acrylamide formation in protein fortified flour and bread

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Strategies to provide significant nutritional benefits across society include fortification of bakery products with high protein non-wheat flours such as soya [1], chickpea [2], or fava bean (*Vicia faba* L.) [3]. These leguminous flours also tend to be high in fibre and micronutrients. In particular, the high yields and high nitrogen fixation rates of the fava bean, and the ability to grow it globally, means that it has excellent potential for providing both health and environmental benefits.

However, changing the profile of the proteins, amino acids and sugars present in bakery ingredients can have a significant impact on the generation of acrylamide (a probable carcinogen [4]) during baking. Currently, acrylamide is regulated in the EU by Commission Regulation (EU) 2017-2158, whereby unenforced benchmarks are provided for categories of foodstuffs, but new regulations are being proposed which will enforce legal limits. It is important from both a health and a regulatory perspective that acrylamide levels, and its precursors, are monitored in fortified products, and that we generate an understanding of how acrylamide formation is affected by addition of high protein flours.

The aim of this project is to assess the acrylamide potential of both baked flour, and bread formulations with incorporation of fava flour. In the first experiment, strong white flour samples were fortified with 0, 25, 50, 75 and 100% fava bean flour, heated dry for 0 – 11 min at 180 °C, and their acrylamide, 5-(hydroxymethyl)furfural (HMF), sugar and amino acid concentrations were measured by liquid chromatography-mass spectrometry, liquid chromatography-UV spectrometry, ion chromatography and liquid chromatography-mass spectrometry, respectively. Addition of 25% and 50% fava bean flour significantly increased acrylamide concentrations when the flour was baked at 180 °C but further fortification decreased concentrations to similar levels of wheat flour alone, showing the synergistic behaviour of these two flours in acrylamide formation. We discuss these results in light of the precursor profile in the raw flours, the underlying chemical pathways and also examine the changes in HMF (another processing contaminant) and key aroma compounds.

In a second experiment, the British standard sliced loaf was prepared using the standard Chorleywood process, with 0, 5%, 15% or 25% of the strong white bread flour replaced with either pre-roasted or unroasted fava flour. Samples of raw flour, dough, proved dough and bread were analysed for acrylamide, precursors and a range of standard physical properties. With incorporation of roasted fava flour, the higher protein content tended to increase acrylamide and pyrazine formation as a result of increased Maillard activity, but there was no such increase when the unroasted flour was used. We discuss this in terms of the fermentation process and yeast activity which has been shown to mitigate acrylamide formation [5], and also in terms of the generation of Maillard intermediates during the pre-roasting process. There were also significant changes in the physical properties of the bread loaf: addition of fava flour, particularly at 50% inclusion, reduced the volume and increased the density of the loaf.

We show that the combination of ingredients, pre-processing and processing factors can all have an impact on the generation of acrylamide during baking and recommend that the food industry takes all these factors into account when developing protein fortified products.

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From Data mining to Meta-analysis: Presence of mycotoxins in food.

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Mycotoxins occurrence in food is an important topic regarding the actual human health risk and considering the climate changes that will likely contribute to an increase of these toxicants' outbreaks in food. In addition, the human co-exposure to multiple mycotoxins is a real problem, increasing the concern about their combined impact on health, as reported by Rodrigues and Naehrer [1]. Mycotoxins can exert several deleterious effects, with carcinogenicity being one of the most impacting which lead to the classification of some molecules by the International Agency for Research on Cancer (IARC) [2].

The goal of this work was to perform a systematic review (from 2000 to 2022) using FoodMine's code [3], with some modifications to improve minining and collect data concerning the prevalence of Aflatoxin B1(AFB1, group1), Fumonisin B1 (FB1, group 2B), Deoxynivalenol (DON, group 3), Beauvericin (BEA, no classification) in 10 foods from different origins, and then perform a meta-analysis to assess whether the mycotoxins prevalence and mean values represent a risk in these foods.

Results for food samples varied among mycotoxins, as observed in Fig.1. For AFB1, 3% ($CI_{95\%}$ - 0-12%, I^2=79%) were contaminated, with a mean value of 27.11 µg/kg ($CI_{95\%}$ - 10.42-70.53 µg/kg, I^2=94%). With B1, 44% ($CI_{95\%}$ - 6-89%, I^2=79%) were contaminated, having a mean value of 94.60 µg/kg ($CI_{95\%}$ - 19.69-454.59 µg/kg, I^2=100%). As for DON, 68% ($CI_{95\%}$ - 67-68%, I^2=91%) appeared contaminated, presenting a mean value of 207.74 µg/kg ($CI_{95\%}$ - 145.71-296.19 µg/kg, I^2=100%). Finally, 80% ($CI_{95\%}$ - 28-98%, I^2=14%) were contaminated with BEA, with a mean value of 16.28 µg/kg ($CI_{95\%}$ - 1.25-212.73 µg/kg, I^2=99%).

These results may be concerning when compared with the maximum levels permitted in food by the European Union (2-12 µg/kg for AFB1, 200-4000 µg/kg for Fumonisins, and 200-1752 µg/kg for DON) [4]. Although no limits are established for BEA, its high prevalence should not be overlooked. This study highlights the importance of monitoring mycotoxins to ensure the safety and quality of food products and protect consumers from contamination.

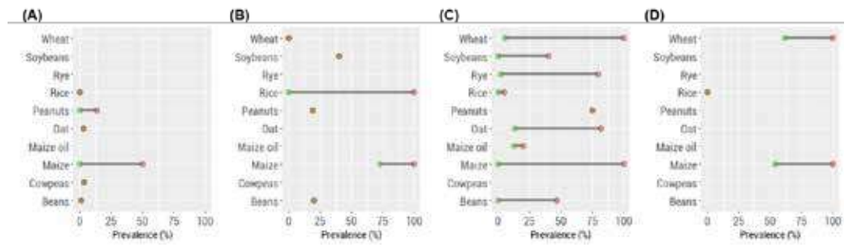

Fig.1. Mycotoxins prevalence among studies for the different foods. (A) AFB1, (B) FB1, (C) DON, and (D) BEA.

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Binding and corona formation of ovalbumin to polystyrene and polyethylene terephthalate microplastics under neutral and acidic conditions

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Microplastic represents one of the major types of pollutants in modern era. Over several years of research in the field of microplastic, there are still many unknown gaps, including the effects and mechanisms of action of these particles on human health. Studies in this field conducted experiments on cells and human tissues or animals like rats and mice. While these studies suggest the toxic effects of microplastic, it is not clear if concentrations used for exposure are relevant for humans. Also, most of the studies used spherical polystyrene, which does not reflect well the diversity of microplastic particles found in nature. Another gap is lack of studies describing direct interactions of microplastics and proteins. While it is generally known that proteins form corona around microplastic particles, affinity studies and consequences on protein structure are usually missing.

The aim of this work was to analyze interaction of a major egg white protein and allergen, ovalbumin to several to microplastic particles, including polystyrene (PS) of 120 and 500 µm in size and polyethylene terephthalate (PET) of 120 µm in size. Binding affinity was determined at both acidic, pH 3 and neutral, pH 7 conditions, at the room temperature, by measuring bulk ovalbumin concentration in supernatants at the equilibrium time. Several binding models, including Langmuir, Freundlich, Redlich–Peterson and Guggenheim-Anderson-de Boer (GAB), were used to determine binding parameters. The formation of soft and hard corona was analyzed according to the published protocol [1]. Structural analysis was performed using near and far-UV CD spectrometry.

Obtained results showed that ovalbumin binds to both PS and PET. All binding models indicated that ovalbumin binds with higher affinity to tested microplastics on pH 3, compared to pH 7, with the highest affinity being calculated for PS 120 µm. Further analysis showed that ovalbumin forms both soft and hard corona onto the surface of all three microplastics. Structural alterations of ovalbumin as a consequence of its interaction with microplastic was shown to be both pH and microplastic type dependent. Also, more pronounced effect on its tertiary structure was observed, compared to secondary. At pH3, tertiary structure of bulk ovalbumin becomes destabilized, especially in the presence of PET 120 µm and PS 500 µm, while at pH 7, structural stabilization is observed, especially in the presence of PS 120 µm.

Considering that the microplastic was discovered in eggs [2], obtained results suggest that direct interactions of native ovalbumin with microplastic particles could have influence on its structure and thus affect its techno-functional properties.

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Enzymatic detoxification of ochratoxin A: *Aspergillus niger* vs. *Alcaligenes faecalis* ochratoxinases

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Ochratoxin A (OTA) is one of the most important mycotoxins attending to its high prevalence and toxicity [1]. It is the cause of a variety of toxicological effects both in humans and animals being the kidney its main target [2]. To ensure food safety, it is necessary to reduce OTA presence to values as low as technologically possible. Once OTA is already contaminating food and feed there are different strategies to eliminate it, mainly: physical, chemical, and biological methods. In this context, biological methods arise as the most promising ones since they can be applied in mild conditions, are environmentally friendly, and more specific. To date, there are numerous examples in the literature of microorganisms capable of degrading OTA. In most cases, OTA detoxification is achieved through the hydrolysis of its amide bond rendering non-toxic ochratoxin α (OTα) and L-β-phenylalanine [4]. Moreover, some enzymes from microbes have been studied for their OTA detoxification activity but few of them have been isolated and further characterized. Two of them are included in this study, the ochratoxinase from *Aspergillus niger* (which is the first reported microbial enzyme able to hydrolyse OTA) [5] and an N-acyl-L-amino acid amidohydrolase from *Alcaligenes faecalis* [6]. Since these enzymes could be good candidates for OTA-detoxification of food matrices, the aim of this study was to elucidate if their biochemical characteristics are compatible with their use in food technological processes. For this purpose, both proteins were recombinantly hyperproduced. Both enzymes possessed similar optimum pH, as both showed maximal activity around neutral pH values. Regarding its optimal temperature, *A. niger* ochratoxinase shows better activity at 65 **ºC while amidohydrolase from** *A. faecalis* is more active between 37 and 55 **ºC.** *A. niger* ochratoxinase maintains its activity after 6 hours of heat treatment at all the temperatures studied while amidohydrolase from *A. faecalis* shows a significant loss of activity. Enzyme hydrolytic activity was assayed against a set of 21 synthetic commercial carboxypeptidase substrates possessing different aminoacyl substituent residues at their C-terminal end. Ochratoxinase from *A. niger* was found to have a narrower substrate specificity, displaying a higher preference for substrates with phenylalanine in their C-terminal end (such as the mycotoxin OTA). Among the properties that an enzyme should have for its use in food matrices, an important one is to display a narrow substrate specificity to avoid altering the nutritional quality and organoleptic characteristics of the food substrates. Considering the results obtained in this study, ochratoxinase from *A. niger* possesses characteristics that might be perceived as more promising for its application in food matrices.

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Combined synergic exposure to food contaminants: A matter of concern?

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Humans are exposed to combinations of different toxicants by several routes, with food ingestion being particularly significant due to the unavoidable presence of chemical residues from soil, air, processing, agrochemicals, and fungi. Many of these molecules are regulated with maximum limit levels established, generally considered safe. Notwithstanding, the current chemical risk assessment within the EU/EFSA mainly relies on the evaluation of individual substances neglecting the potential synergistic effects of combined exposure, particularly in the long-term and at very low levels. Recognizing the need to address exposure to multiple chemicals, EFSA aims to implement comprehensive human health risk assessments for multiple chemicals by 2030 [1]. To achieve this goal, it is crucial to expand the data available on the toxicological interactions of contaminants with high probability of co-exposure. In the last years, our group has gathered *in vitro* data [2] on the toxicological interactions of food contaminants from different groups (mycotoxins, polycyclic aromatic hydrocarbons, pesticides, heterocyclic amines, and metals) concerning their cytotoxicity to different organs, tested in human cell models (Figure 1). The present work aims to compile and explore this data in a context of its importance for combined synergic exposure. Molecules were selected based on their toxicity or greater prevalence in foods: aflatoxin B1 (AFB1), citrinin (CIT), cyclopiazonic acid (CPZ), enniatin B (ENB), ochratoxin A (OTA), sterigmatocystin (STG), deoxynivalenol (DON), fumonisin B1 (FB1), benzo[a]pyrene (BaP), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), cadmium (Cd), and λ-cyhalothrin (CYH). The theoretical biology-based Combination index-isobologram method [3] was used to evaluate the individual and binary effect of these toxicants and determine the type of the interaction. Cytotoxicity was assessed using the MTT test after 72 h exposure across a wide range of concentrations, nM to mM order, depending on the compound. A summary of results concerning the dose reduction index (DRI) – dose reduction rate of each compound in the combination that achieves the same toxicity compared with the doses of each compound alone – for the most synergic combinations in each of the models are presented in Table 1. Synergisms found are particularly relevant at low doses, which are most likely to occur from food exposition, making this data valuable to improve human health risk assessment. Insights into novel approaches to estimate toxicants bioaccessibility, bioavailability, physiological relevant doses and chronic toxicity *in vitro* are explored.

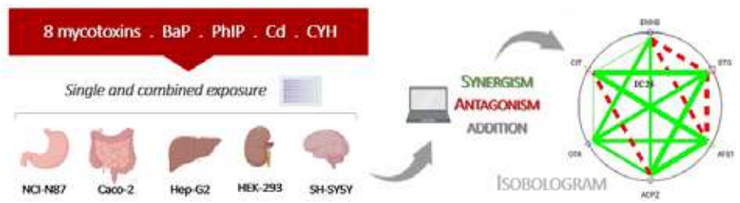

Fig.1. Contaminants, target organs and models used and an isobologram representative of toxicological interactions of mycotoxins in neuronal cells (green solid lines: synergism; dashed red lines: antagonism).

Table 1. DRI and combination indexes (CI) for the most synergic combinations at IC_{25} of food contaminants, for each of the models. Synergism: (+++), strong (++++), moderate (++), slight (+). Antagonism: (---)

	Stomach	Intestine		Liver	Kidney	Brain
Combination	OTA-ACPZ	OTA-DON	PhIP-BaP	AFB1-DON	ENB-STG	STG-CIT
DRI	0.9-2.3	3.5-3.9	2.8-2.9	14.3-10.0	4.9-3.0	7.3-21.8
CI	1.61 ---	0.54 +++	0.70 ++	0.17 ++++	0.54 +++	0.18 ++++

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FLASH PRESENTATIONS

Profiling of carotenoids and tocopherols of a new food ingredient based on sea fennel industrial by-product

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C. maritimum, or sea fennel, is a halophyte wild herb which grows along coastlines, well adapted to water shortage, low soil fertility, and high salinity conditions of the Mediterranean basin. Innovative production systems of sustainable organic crops are being investigated to cultivate sea fennel at an industrial level. Sea fennel is a rich source of bioactive compounds such as essential oils, phenols, ascorbic acid, essential fatty acids, and carotenoids. Recently, sea fennel is gaining scientific relevance as a food ingredient, but only few food applications are available [1].

Essential oils and phenolic compounds of extracts, micro-emulsions, liposomes, and isolates obtained from sea fennel possess multiple pharmacological properties such as antioxidant, antimicrobial, antifungal, anti-inflammatory, and anti-atherogenic activities [2]. Carotenoids and tocopherols participate to the functional role of sea fennel but their in-depth chemical characterization is still missing. Carotenoids were determined in sea fennel only by spectrophotometric methods [3,4], while no data are available for tocopherols. They are powerful antioxidants in halophytes plants with vitamin E and vitamin A activity involved in the salt tolerance system [5]. Carotenoids and tocopherols may be present in both free and esterified forms. It was reported that esters of lutein and zeaxanthin have a different distribution in liposomes for food and nutraceutical applications than free compounds because of lipid solubility and different degradation, oxidation, and *cis* to *trans* isomerization [6].

Thus, this study chemically characterized for the first time carotenoids and tocopherols profiles in sea fennel industrial by-products to develop new functional ingredients for food and nutraceuticals using a zero-waste strategy.

For this purpose, the non-saponified and saponified extracts of by-products were characterized by ultra-high-performance liquid chromatography coupled to photodiode array, fluorescence, and mass spectrometer detectors. The by-products were composed of a mixture of flowers, woody stems, and leaves resulting from the harvesting of cultivated sea fennel in central Italy.

The preliminary results on industrial sea fennel by-products showed free lutein as the most abundant carotenoid in the non-saponified extract, followed by zeaxanthin, while carotenes were not detected. Differently, in the saponified extract neoxanthin and violaxanthin were quantified, meaning esters xanthophylls are present in sea fennel plants. Moreover, alfa- and gamma tocopherols were identified for the first time in sea fennel by-products.

The characterization of free and esterified carotenoids and tocopherols could improve the quality of sea fennel as an ingredient for food and nutraceuticals under the formulation of powders, isolates, micro-emulsions, and liposomes.

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Impact of wood sticks on phenolic compounds and sensorial quality of Touriga National Portuguese red wine

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In recent years, wineries have introduced many winemaking innovations more sustainable, obtaining different red wine styles trying to achieve sensory preferences of consumers [1]. Red wines have been traditionally aged in oak barrels that lead to the modification of their physical-chemical and sensory characteristics [2]. However, oak barrels are generally considered as rather costly. This have driven oenological research to seek alternatives in order to obtain a quality wines with lower costs. The use of chips in substitution of oak barrels is one possible strategy that allows to get good wine’s quality. Wood aging influence the organoleptic quality and complexity, such as aroma, structure, astringency, and persistence, and contributing to color stabilization of red wines [3]. Touriga Nacional is the most important red grape variety of Portugal. Due the high content of polyphenols and especially tannins, the Touriga Nacional red grape variety usually requires aging in wood. Their wines are balanced, full body with good alcoholic graduations. The aroma is soft, round and warm, reminiscent of dark red, very ripe wild berries, with some floral notes.

The red wines used in this work were obtained from Silgueiros winery Dão Appellation, produced from grapes of *Vitis vinifera* L. variety Touriga Nacional. American (Quercus alba) and French (Quercus petraea) wood sticks, each one with light or medium toast were added to the wine, and allowed to stay during 3 months. Chemical characterization and phenolic composition was performed spectrophotometrically, monomeric anthocyanins were determined by HPLC, while sensory analysis was evaluated by a trained panel.

The control wine was composed of 1903 mg GAE/L of total phenolic compounds, 7.6 mM of TE of TEAC, 600 mg/L of total anthocyanins and 1700 mg/L of total tannins.

The addition of oak sticks leaded to wines with higher amount of phenolic compounds and antioxidant activity. The decrease observed on the amount of total anthocyanins, after 3 months, was lower in wines aged with sticks when compared to the control wine, allowing to infer a stabilization of these compounds by the presence of oak sticks. All oaked wines contained higher amounts of tannins (2.0-2.4 g/L) when compared to control wine (1.7 g/L). All samples showed a similar relative composition of monomeric anthocyannins. The sensory analysis showed that wines aged with wood were preferred by panelists. French oak sticks exhibited better results.

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Microwave treatment as a promising strategy to develop functional milk alternatives obtained from by-products of the oil industry

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Non-dairy milk alternatives are surging in popularity due to pathological reasons, changing lifestyles, food practices, environmental care, and animal welfare [1]. Plant-based milk alternatives (PBMAs) are mainly made from soy, almond, oat, or rice. The nutritional properties of PBMAs depend on the type and quality of used plant material and technological processes [2]. The pre-treatment of raw plant materials could enhance the functional properties of PBMAs [3]. Selection of suitable heat treatment of plant materials could increase bioactive compounds content, antioxidant activity (AA), and bioaccessibility after gastric digestion of final PBMAs [4]. Consequently, it is necessary to develop new milk alternatives with similar or better nutrition value than cow's milk, low production cost, and high economic effect. Therefore, by-products of the oil industry (oilseed press cakes) as rich sources of proteins, fats, dietary fibers, minerals and antioxidants could be an attractive option in the development of new PBMAs [5].

The aim of this study was to optimize the production of new PBMAs based on microwave-treated oilseed press cakes to enhance their pro-health properties. Three-level Box-Behnken design (BBD) and response surface methodology (RSM) were used for evaluation of the effects of three independent variables (MP – microwave power; t – time and BCC:RC – black cumin cake (BCC) to rapeseed cake (RC) ratio) and their interactions on the response variables: AA determined by the modified analytical methods 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and mineral composition (calcium, magnesium, potassium, and sodium) of new cow's milk alternatives.

Higher RC content in PBMAs caused a higher AA and TPC in comparison with samples prepared with BCC. The AA of BCC samples increased with increasing the MP and time prolonging (t) of oilseed cakes pre-treatment. The results showed that oilseed treatment with higher MP had a beneficial effect on calcium concentration in investigated samples. During PBMA preparation, treatment by identical MP and t values resulted in a higher content of calcium and magnesium in beverages based on BBC than in drinks containing RC. To evaluate the sufficiency of the proposed mathematical models, verification experiments were carried out at the predicted conditions (MP = 800W; t = 3 min; BCC:RC ratio = 0.11). The predicted and experimental values are listed in table 1. The microwave treatment of RC and BCC was sufficient to increase the content of antioxidants and minerals of PBMAs.

Table 1. Predicted and experimental values of the studied responses under the optimal conditions (MP = 800W; t = 3 min; BCC:RC ratio = 0.11).

	ABTS [mmol TE/100 mL]	**DPPH** [mmol TE/100 mL]	**FRAP** [mmol TE/100 mL]	**TPC** [μmol GA/100 mL]	**ΣMinerals** [mg/100 mL]
Pred.	7.34	1.45	1.06	91.23	188.4
Exp. ± SD	7.06 **±** 0.16	1.14 **±** 0.07	1.01 **±** 0.02	89.06 **±** 2.37	172.6 **±** 1.3

Pred. – predicted data; Exp. – experimental data; SD – standard deviation; TE – Trolox equivalent; GA – gallic acid equivalent; ABTS - 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid; DPPH - 2,2-diphenyl-1-picrylhydrazyl; FRAP – ferric reducing antioxidant power; TPC – total phenolic content; ΣMinerals – sum of minerals (Ca, Mg, Na, K).

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Fig (*Ficus carica* L.) leaves as a source of bioactive compounds: A sustainable approach to valorization of fig bioresidues

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The fig tree is a species with a rich history, whose fruit production in 2019 reached an incredible value of 1.153Tn [1]. Unfortunately, the loss of leaves during the production process is a serious issue that fits in the sustainable development goals for 2030. In response to this concern and the lack of research on the value of fig tree leaves, a sustainable integrated project to recover bioactive molecules from fig leaves was conducted with several scientific and industrial partners, including a Portuguese private agricultural company responsible for the production of the 5 fig varieties used in this study - Pasteliere (PA), Longue d'Aout (LA), Dauphinie (DA), Boujassote Noire (BN), and Marseille (MA) (figure 1). These raw materials were chemically characterized by chromatographic methodologies (Sugars, Tocopherols, Phenolic compounds and organic acids), and evaluated for their bioactive potential, after preparation of hydroethanolic extracts by maceration.

From the obtained results, the leaves from the BN variety showed the highest levels of organic acids (146.5±1.1 g/100g dw), indicating its potential for application in food formulations. Furthermore, the LA variety showed a high concentration of phenolic compounds (42.4±0.2 mg/g dw), while DA was the richest variety in free sugars (17.36±0.08 mg/g dw). Additionally, PA stood out for its high levels of tocopherols (4.14±0.04 mg/100 g dw), while LA and BN varieties had the highest content in fatty acids (70.6±0.1% and 70.5±0.5% of total fatty acids, respectively). Concerning the results of the bioactivity evaluation, hydroethanolic extracts showed that the BN variety presented the highest antioxidant activity (EC_{50}=0.23 ± 0.01 a mg/mL, TBARS assays), while DA showed the most promising cytotoxicity (GI_{50}= 158 ± 13 **μg**/mL, sulforhodamine assay in AGS cells), anti-inflammatory activity (IC_{50}= 82±8 **μg**/mL in in RAW 264.7 cells) and antimicrobial activity (MICs ranging from 1.25 to 5 mg/mL against *Yersinia enterocolitica*, *Eschericia coli*, *Staphylococcus aureus*, and *Salmonella enterica*). In conclusion, fig leaves from the analysed varieties can be considered valuable sources of bioactive compounds are a real opportunity for sustainable development and valorisation of the circular economy in the food and pharmaceutical sector. It is important to highlight that varieties BN and DA showed significant results and can be considered the most promising samples for future studies and applications, in different industries, particularly in the food sector, acting as a potential natural and sustainable preservatives source.

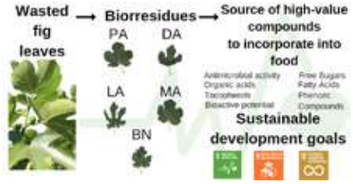

Fig.1. Schematic representation of the sustainable approaches for the valorization of fig leaves.

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Marine polysaccharides valorisation as functional ingredients in food products

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Nowadays, seaweed and microalgae have been incorporated into several food preparations, such as pasta, snacks, candy bars, gums, and beverages. They have been used to enhance the nutritional content of food and further to promote the health of humans and animals. This is due to their capacity to produce biologically active compounds, such as proteins, lipids, polysaccharides, and pigments. Polysaccharides are of increasing interest due to their chemical and biological diversity, namely the negatively charged polysaccharides. Important biological activities, such as anticoagulant, antiviral, immunomodulatory and anti-inflammatory, have been assigned to these polysaccharides [1]. Nevertheless, the structural characterization of these polysaccharides and the relation with the biological properties is still very limited. This knowledge is regarded as a major boost to assess their valorisation.

The approach of this work was the structural analysis of the polysaccharides from different marine resources, namely the water-soluble ones obtained from seaweed and microalgae. Furthermore, the easily assessed polysaccharides present in saltpans water were also studied. The immunostimulatory and hypocholesterolemic activities of the purified polysaccharides was assessed to identify their potential application.

The polysaccharides from brown seaweed (*Saccharina latissima*) and different microalgae species (*Nannochloropsis oculata*, *Chlorella vulgaris*, and *Porphyridium purpureum*) were extracted with hot water and fractionated by ethanol precipitation and/or by anion-exchange chromatography. The fucose-containing sulphated polysaccharides (FCSP) from brown seaweed were rich in uronic acids, fucose, and galactose. *N. oculata* water-soluble polysaccharides are constituted by mixed-linked (β1→3, β1→4)-glucans, (α1→3)-, (α1→4)-mannans, and anionic sulphated heterorhamnans[1]. Besides starch, the most abundant polysaccharide, *C. vulgaris* produces an exopolysaccharide, a sulphated galactan[2]. *P. purpureum* is rich in floridean starch and has the ability to excrete high amounts of sulfated polysaccharides, namely glucuronoglucogalactoxylan[3]. Besides, the saltpan water revealed the presence of highly sulfated polysaccharides (42 mg/L) composed mainly by galacturonic acid, glucuronic acid, and galactose with minor content of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose.

The sulfated polysaccharides from salt pan water, the FCSP from brown seaweed, the soluble polysaccharides of *N. oculata* and the exopolysaccharides from *C. vulgaris* and *P. purpureum* revealed to stimulate murine B-lymphocytes *in vitro*, being the sulfate esters relevant for this activity. In addition, FCSP from brown seaweed showed the capacity to sequestrate cholesterol. This opens a great potential for using the marine environment as a source of sulfated polysaccharides that can be used as functional ingredients in food applications.

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Innovative olive pomace extract as a source of phenolic compounds with antitumoral activity for functional foods

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Olive oil production is a main sector of the Mediterranean food industry but it has a negative environmental impact due to the high generation of by-products. Olive pomace is the largest example, because it is a rich source of bioactives, which are phytotoxic, creating several environmental constraints. However, when extracted, these compounds could have several applications (e.g., foodstuffs fortification). Moreover, cancer is a worldwide epidemic and a leading cause of death. As its incidence continues to escalate, there is a need to find natural solutions to prevent its development. The diet is an important factor that modulates human health. Particularly, the Mediterranean diet has been associated to cancer prevention due to the health promoting properties of olive oil [1,2].

Since 98% of phenolics remain in olive pomace after olive oil production [1], this work aimed to obtain an olive pomace extract (OPE), using a green solvent-free method. Its chemical composition was ascertained and the antitumoral potential was studied in different cancer cell lines (breast: MCF-7, pancreatic: AsPC-1, and colorectal: HT-29, Caco-2).

To prepare the extract, the olive pomace was submitted to the process described in the patent international application PCT/IB2018/060111 (2019). Briefly, it was pressed at specific time, temperature, and pressure parameters, and centrifuged to obtain the liquid extract which was further lyophilized. The chemical analysis included: total fat, protein, ash, carbohydrates; brix of sugar; pH value; vitamin E and fatty acids profiles; total phenolics, flavonoids and antioxidant activity; hydroxytyrosol content [2,3]; and phenolic profile by UHPLC-ESI-QTOF-MS [4]. The cell assays included the assessment of cell viability, proliferation, growth [5], and angiogenesis of a 24 h treatment with 1 mg/mL of OPE.

The freeze-dried extract was composed of sugars (28%), minerals (11%), lipids (8%), mostly oleic acid (72%), protein (1%), phenolics (3 g gallic acid equivalents/100 g), hydroxytyrosol (215 mg/100 g), flavonoids (2 g catechin equivalents/100 g), and α-tocopherol (2 mg/100 g). Moreover, 68 phenolics were tentatively identified by UHPLC-ESI-QTOF-MS. Also, it revealed interesting results in the antioxidant activity assays especially in ferric reducing antioxidant power (2 g ferrous sulphate equivalents/100 g). The OPE significantly reduced cell proliferation in all the studied cancer cell lines. Furthermore, OPE reduced angiogenesis in AsPC-1 (pancreatic). It also presented a cytotoxic effect and increased malondialdehyde levels in HT-29 (colorectal) cells.

These results suggest that OPE can play an important role against cancer. It can be a great source of phenolics with antitumoral activity to formulate functional foods and dietary supplements, aiming their incorporation in diets to promote health and prevent cancer development. Nevertheless, further research is needed to assess the bioaccessibility and bioavailability of compounds after digestion and their mechanistic antitumoral action.

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MERS-SARS-COVID-NEXT- can food help?

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The Covid-19 pandemic has shown us that top science using modern methods can react very quickly and identify possible solutions to inhibit the virus not only at the level of medical therapy, but also in the field of prevention based on the inhibitory effect of substances found in food. The knowledge gained during the local epidemics of SARS and MERS proved to be interesting, which confirmed the connection between the structure of natural substances found in food and their preventive effect against viruses. The results of the investigation of antiviral properties, as well as anti-inflammatory, antimicrobial and antioxidant properties of three groups of potential sources of these substances - preparations based on bee products, complex extracts from plant matrices and biopolymers based on carbohydrates will be presented. Bee pollen was selected based on the high content and activity of the complex of contained phenolic substances; for plant matrices, we focused on plants containing stilbenes and also phenolic substances. The investigated active biopolymers were obtained from herbs and mushrooms.The presentation will also include model examples of the application of the obtained active complexes in food and nutritional supplements, which can play an important role in preventing not only viral epidemics

Picture 1. Candies with 1% of scattered pollen

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Plant extracts as natural agents against *Botrytis cinerea* infection

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mBotrytis cinerea causes important economic losses every year in grape production [1]. In this sense, the development of new natural ingredients against these grape pathogens is of paramount relevance. Plant extracts are a valuable source of a wide variety of biologically active constituents. Several plant bioactive compounds have been identified as possible antifungal agents, associated with their antioxidant properties [2]. In this work, five natural extracts, namely chestnut (*Castanea sativa* Mill) male flower, cistus (*Cistus ladanifer* L.) leaves, eucalyptus (*Eucalyptus globulus* L.) leaves, fennel (*Foeniculum vulgare* Mill) leaves, and orange (*Citrus sinensis* L.) peel, were evaluated regarding the antifungal activity against *B. cinerea.* The matrices efficacy was first evaluated *in vitro* through the microdilution method and further on grape-based culture medium supplemented with the plant extracts in concentrations of 10 and 20 mg/mL established by the microdilution method as effective inhibitory concentrations. In addition, grape berries of the red cultivar Touriga Franca were inoculated with *B. cinerea* and immersed for 3 minutes in the selected extracts: chestnut male flower, cistus leaves, eucalyptus leaves, and orange peel extracts (10 mg/mL). The relative levels of rot severity of the berries treated with the different plant extracts, were determined by the McKinney index, and expressed as percentages in comparison to the non-treated control. The results obtained through the *in vitro* experiments showed that after 5-10 days of incubation, both concentrations of chestnut male flower, cistus leaves, eucalyptus leaves, and orange peel extracts were able to inhibit the growth of *B. cinerea*. All the selected plant extracts revealed the capacity to inhibit the growth of *B. cinerea* at a concentration of 10 mg/mL, which could be related with their high composition of bioactive compounds, mainly phenolics. The plant extracts were also evaluated regarding their ability to modulate *B. cinerea* symptoms in grape berries at 10 mg/mL. The McKinney index of the berries treated with plant extracts were 83% ± 3%, 73% ± 3%, 43% ± 19%, and 8% ± 5% for chestnut male flower, eucalyptus leaves, orange peel, and cistus leaves, respectively; demonstrating that cistus leaves extract presents an interesting potential to reduce the levels of rot severity of the berries caused by *B. cinerea*. The positive antifungal activity of the plant extracts assessed, especially cistus leaves and orange peel, could be attributed to their varied bioactive composition, such as several classes of phenolic compounds (i.e., flavonoids, phenolic acids, and tannins), and organic acids (i.e., citric, oxalic, ascorbic, and malic). In fact, these plant extracts have been described as source of compounds with biological activity against grape pathogens [3,4]. Considering the potential antifungal properties of these plant extracts, these natural ingredients could be applied as a biofungicide in wine industry, acting against grape pathogens, such as *B. cinerea*, thus reducing or avoiding the use of chemical fungicides.

Keywords: *Botrytis cinerea;* Grape pathogens; Plant extracts; Biofungicide

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Design of Functional Food Ingredients through Bioprocessing to Address Food and Mood

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Food provides us with nutrients, but what we eat also affects our mood. Certain molecules in foods have neuroactive properties and their amount can be manipulated using targeted bioprocessing methods. These molecules are derived from amino acids and lipids, and may synthesized by microorganisms. Because fermented foods have many proven health benefits, they are gaining more and more acceptance and are also considered functional foods. A wide variety of fermented foods and beverages are becoming increasingly common, including fermented dairy and cereal products. This presentation will focus on the bioprocessing strategies that we have used in the design of functional foods in the last decade. One such example is the conversion of free amino acids from the sprouting of grains into neuroactive compounds by fermentation. The increased amount of tyrosine due to the increased proteolytic activity during sprouting are later utilized by sourdough microorganisms to form dopamine and L-DOPA. Therefore, sprouting followed by fermentation are synergetic on tyrosine biotransformation into its bioactive derivatives. Overall, this presentation will address how to tailor bioprocessing methods to enhance the concentration of neuroactive compounds in foods, considering their precursors and pathways of formation.

Salt pan waters can be exploited as a source of functional food and feed ingredients

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Marine environments are the warehouse of a variety of novel bioactive compounds prone to be explored. Polysaccharides, that are excreted by marine organisms, are one of these compounds, particularly the sulfated ones. Sulfated polysaccharides have been proposed as bioactive agents, regarding their potential antiviral, anticoagulant, antioxidant, and immunomodulatory activities [1]. Along with these functional properties, that can be used to produce health promoting food and feed ingredients [2,3], some sulfated polysaccharides have already been explored as food additives due to gelling, emulsification, and thickening behaviours [4]. The growing interest in sulfated polysaccharides has led the search for new sources. Seawater is an available source of carbohydrates, which can be naturally concentrated in salt pan waters due to its evaporation by wind and sunlight [5]. Therefore, in this study polymeric material and polysaccharides were analysed along salt production in the evaporation ponds and in the crystallizer water.

Along salt production, polymeric material of seawater (13 mg/L) was accumulated in the evaporation ponds (9-73 mg/L) and in the crystallizer (133-144 mg/L). This polymeric material was composed by 29% of sulfated polysaccharides, with 45 mol% of sulfate esters, 23 mol% of uronic acids, 12 mol% of galactose, and 1 to 6 mol% of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose. The uronic acid pattern was analysed by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) [6]. Galacturonic (57%) and glucuronic acids (43%) were identified, whereas guluronic, mannuronic, and iduronic acids, common in some marine polysaccharides, were not detected.

These results highlight salt pan waters as a source of highly sulphated polysaccharide worth exploring as functional ingredients for food and feed applications.

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Understanding polyphenol adsorption to oral models as a secondary mechanism for astringency

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Astringency is described as a tactile sensation of puckering, tightening and dryness in the oral cavity, commonly induced for by phenolic compounds1. The major mechanism attributed to this phenomenon is the interaction between salivary proteins and polyphenols and respective formation of insoluble complexes that precipitate in the oral cavity. However, more recently, this research line is growing curious about the importance of secondary mechanisms (salivary film disruption, polyphenol-membrane interactions, and mechanoreceptors) in the perception of different subqualities of astringency. However, there is still little to no proof that can substantiate these theories. A recent study from our team as already shown some first evidence that, depending on their structure, polyphenols may bind in different ways to the oral constituents and therefore elicit different mouthfeels2. In this study, the total adsorption of compounds to salivary cellular models was evaluated (Figure 1). Overall, Alum, a strong astringent standard, has shown a higher adsorption potential to the oral models compared to the other mixtures and higher affinity to oral epithelial models. Since alum can't precipitate salivary proteins, these results hint that compound adsorption may be an important mechanism to elicit astringency for certain compounds. Analysing the interaction of Grape Seed Extract (GSE) and Tannic Acid (TA) with oral epithelial cell models, the resultant adsorbed compounds of GSE had up to an eight-fold decreased adsorption when compared to Alum (0.016 mg/cm2), at similar initial concentrations. For Green Tea Infusion (GTI), a higher adsorption was achieved in models where saliva was not present, up to 2-fold. To further substantiate these results a correlation with a certified sensorial analysis is currently being performed.

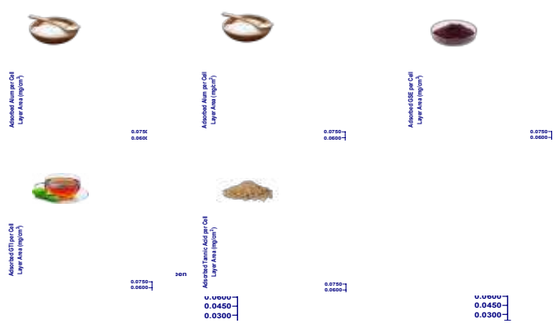

Fig.1. Total adsorption of compounds to salivary cellular models per monolayer area. Oral models constituted by epithelial cells (HSC-3 or Caco-2), whole saliva and mucin, were applied to interact with three concentrations of a sensorial standard and different families of polyphenols: Alum (at 1 $g.L^{-1}$, 1.5 $g.L^{-1}$ or 2.75 $g.L^{-1}$), Grape seed extract (at 0.2 $g.L^{-1}$, 0.6 $g.L^{-1}$ or 1 $g.L^{-1}$), Tannic acid (at 0.1 $g.L^{-1}$, 0.3 $g.L^{-1}$ or 0.5 $g.L^{-1}$) and Green Tea Infusion (at 0.43 $g.L^{-1}$, 0.87 $g.L^{-1}$ or 1.69 $g.L^{-1}$).

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Investigation of structural changes in ovalbumin induced by two types of MPs and its impact on protein digestibility

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Ovalbumin (OVA) is the most abundant protein in chicken egg white. It is one of the major allergens in eggs. Micro- and nanoplatic particles (MNPs) are a widespread contaminant and have been found in food and water. It is still unclear how MNPs might affect human health. However, due to their large surface area they have been found to bind various biopolymers, including proteins. These biopolymers can be bound more strongly or loosely, and are referred to as hard and soft corona, respectfully [1]. MPs have been found in eggs, in the size range of 50-100 μm [2]. It is shown that these particles can interact with proteins and induce structural changes, but there is still not enough information on this topic [3]. These structural changes could lead to a decreased digestibility in the gastrointestinal tract, which could increase the immune response to known allergens.

The aim of this study was to determine whether there are structural changes present in the OVA after incubation with two types of MPs – 120 μm polyethylene terephthalate (PET) and 120 μm polystyrene (PS) and whether they could influence digestion of OVA with gastrointestinal enzymes. 20 mg of MPs were incubated with 1.3 mg/mL ovalbumin for 4 h at room temperature in a 20 mM phosphate buffer at pH 7. Bulk ovalbumin was separated from the MPs by centrifugation and by filtration through a 0.22 μm PVDF filter. Soft corona was obtained by washing the MPs with water, and the MPs were later removed as described with bulk ovalbumin. Formation of amyloids was monitored with a Thioflavin T (ThT) assay at room temperature and after thermal treatment, and additional structural analysis was performed by circular dichroism (CD) spectrometry in the far-UV region. Thermal stability was also determined by spectrofluorimetry. Digestion with two proteases (pepsin and trypsin) was performed to determine whether there is a change in the gastrointestinal digestibility of OVA.

Results from the ThT assay show that at room temperature there is no significant difference between the fluorescence emission obtained for all samples, with bulk OVA from both MPs showing a slight decrease. However, there is an increase of fluorescence after thermal treatment in all OVA samples, where OVA from the soft corona emits significantly less fluorescence than control and bulk samples for both types of MPs. Additionally, soft coronas have been shown to have more β-sheet content than other samples, which is more pronounced for OVA incubated with PET. For the heated samples there is a sharp change from α-helix to β-sheets in all the samples, but it is the most dramatic in the soft coronas. This could impose rigidity to the tertiary structure, which would explain why the ThT molecule does not bind as strongly. Despite differences in both the secondary and tertiary structure, the thermal stability is almost the same in all samples. Digestion of the samples shows that the soft corona incubated with PS tends to be more resistant to trypsin than other samples after 2 min, but it is not significant. For digestion with pepsin there is no difference between the samples. In conjunction with the previous results, which indicates a structural stabilisation of the soft corona at pH 7, it is not surprising that there is an increased resistance to trypsin, compared to pepsin which is a gastric enzyme and for which digestion is performed at an acidic pH.

In conclusion, there is a structural change present in samples upon contact with MPs, particularly in the soft corona, of which the most pronounced is a decrease of α-helix content and increase in β-sheet content as determined by far-UV CD. This leads to a structural stabilization which could further impact the digestibility of the OVA protein and impact its allergenicity. However, this must be confirmed with further experiments.

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Optimization of sample preparation conditions for determination of free amino acids in fermented food

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Fermented foods are those which owe their final appearance, texture, taste and odor as well as chemical composition and nutritional value to the action of microorganisms on certain raw materials used for the production of particular foods. Nowadays the fermentation of fresh raw food is applied in commercial scale and in households not only to prevent food spoilage but also to produce unique and consumer-desired sensory characteristics [1]. Besides the above-mentioned aspects, fermentation of food also improves, sometimes dramatically, the digestibility and nutritional value of food. Of the various food constituents, amino acids are among the most important since they are the building blocks for the biosynthesis of proteins and since they have to be taken up in either the free or peptide bonded form in food. Moreover, free amino acids (FAAs) participate in the synthesis of quality characteristic ingredients and can interact with many receptors and produce abundant tastes of fermented food. According to Wang et al [2], FAAs, as one of the important quality indexes, play a critical role in defining sensory and consumer acceptance of fermented food. On the other hand, amino acids are precursors of many compounds formed as a result of the activity of microorganisms, such as biogenic amines.

Many conventional, modern, and innovative methods for FAAs determination in food have been proposed. However, the chromatographic methods and among them HPLC technique is the most commonly used for this purpose. Regardless of the determination method used, the extraction stage of free AAs from food matrices is a required, tedious and time consuming step. Therefore, this work was focused on the development of fermented food sample preparation method based on ultrasound-assisted extraction and microwave-assisted extraction (MAE). The selected condition of extraction, such as reagents, time, and temperature were tested and compared. Free amino acids were determined by RP-HPLC with UV-Vis and Flu detection after precolumn derivatization. The developed procedures were applied to assess the content of amino acids in various fermented vegetables and fruits available in health food stores.

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Sampling method and quantification of quaternary ammoniums biocides on agri-food surfaces

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To be placed on the market, foodstuffs must meet strict sanitary requirements imposed by the European food law regulation (Regulation (EC) No 178/2002). In order to ensure the microbiological safety of foodstuffs, cleaning and disinfection protocols of surfaces are implemented in agri-food industries. The cleaning step aims to remove remaining food soils from surfaces, mainly using acidic or basic detergents. This step is followed by disinfection with biocides products, in order to reduce bacteria on the surfaces. Only disinfectant biocides classified as Product Type 4 (PT4) of the European Regulation EU 528/2012 [1] are authorized for the disinfection of food contact surfaces. Among them, quaternary ammoniums compounds (QACs) and N-(3-Aminopropyl)-N-dodecylpropane-1,3-diamine (AMPD) are widely used. However, these disinfectant biocides may leave residues on food contact surfaces, especially when procedures (e.g. contact time, rinsing) are not properly applied. They may be also persist in critical areas that are difficult to rinse. As a result, residues could be transferred into food by contact with the chemically impregnated surfaces [2-3].

Our study focuses on the sampling and quantification of disinfectants biocides residues on INOX316L surfaces, a material representative of agri-food industry facilities. The protocol is based on a sampling for QACs and AMPD residues from surfaces using a damp cloth followed by their extraction from this vector with a suitable organic solvent. Considering the physicochemical properties of the compounds, two independent LC-MS/MS methods have been developed for the quantification of QACs and AMPD.

Recovery ranging from 33 to 87% could be estimated depending on the biocide compounds and the presence or absence of food soiling (i.e. milk, orange juice, beef stock and olive oil) on the stainless steel surfaces. The highest recoveries were obtained in presence of food soiling, which represents an incomplete rinsing before cleaning and disinfection procedures.

The developed method allows the quantification of QACs and AMPD residues on the surfaces as low as 4 ng/cm² for most compounds.

The results of this study contribute to a better understanding of the risks associated with biocide use in the agri-food industry and allow the identification of critical materials and critical areas in the food production chain. In this sense, the method has been implemented in a collaborative project involving agri-food industries by searching for critical areas of biocides retention after cleaning and disinfection procedures, which has made its applicability possible and has led to interesting results.

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Effects of Sprouting and Fermentation on the formation of Acrylamide and 5-Hydroxymethyl formation in relation to Free Asparagine and Reducing Sugar Concentrations in Wholemeals

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Sprouted grains have become popular in recent years, especially among the consumers who look for healthier options. Sprouting results in various physicochemical and biochemical changes in cereals. The nutritional value of the grains increase with the increase in the mineral bioavailability, bioactive compounds, and protein digestibility. On the other hand, starch and proteins are hydrolysed into reducing sugars and amino acids, which are Maillard reaction precursors. This should be considered as a new risk when sprouted grains are used in the production of bakery products. This study investigates the risks of acrylamide and HMF formation in heated wholemeal from sprouted grains.

The changes of free asparagine, total free amino acids and reducing sugar concentrations were comprehensively assessed during controlled sprouting and yeast and sourdough fermentations of common wheat (also known as bread wheat), einkorn wheat, rye, oat, barley, and buckwheat. Acrylamide and 5-hydroxymethylfurfural (HMF) were analysed in heated samples. The concentration of asparagine decreased up to 40% after 24-36 h of sprouting (except buckwheat) and then increased to its initial concentration after 48 h. Further sprouting increased its concentration exponentially. The increased amount of reducing sugars after sprouting caused higher acrylamide and HMF formation even if the asparagine concentration was lower. On the other hand, when sprouted grains were fermented, the formation of acrylamide and HMF was decreased due to the consumption of sugars and asparagine by the yeast. In sourdough fermentation, acrylamide formation was decreased, while HMF formation was increased following the pH drop of 3 units via accumulation of lactic acid.

The results have shown that it is possible to produce bakery products with sprouted cereals under controlled conditions. To decrease the risk of the formation of acrylamide and HMF, one should be aware of the importance of the assistance of fermentation procedure. Furthermore, care should be taken not to keep the sprouting period long (over 2 days at 20 °C) for the sprouted grains to be used in heat treated products, as it will cause excessive asparagine and in turn acrylamide accumulation.

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POSTER PRESENTATIONS

T1

Food composition, quality and safety

A comparative study of Ca and Fe concentrations in Hungarian proso millet grains (*Panicum miliaceum L.*) varieties

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Proso millet is a small-seeded grass called millet that is widely grown worldwide as a cereal crop. There are many studies on millet's dietary, practical, and technical aspects as a gluten-free grain. Millet is supposed to be cheaper and healthier than other grains in terms of nutritional value. Due to their nutritional properties, grains are valuable sources of numerous nutrients and minerals that support the optimal health. Consumer awareness of gluten-free food sources has significantly changed the development and refinement of techniques scientists use to improve the characteristics of new varieties through breeding. Mineral deficiency is a massive problem of diet trends concerning nutrition; this is a significant issue in the Western world, especially among vulnerable groups such as children, women, the elderly, and sick people. Up to 30% of women in their fertile age have exhausted their iron reserves, while 40% of young girls may suffer from iron deficiency. The research studies were conducted at the Research Institute of Nyiregyhaza (RINY) Institutes for Agricultural Research and the University of Debrecen's Education Farm. Using ICP-OES (Inductively Coupled Plasma Optical Spectrometer) technology, the mineral concentrations (Ca, Mg, Fe, and Zn) of three red and white *P. miliaceum* varieties were investigated. Calculations of crude protein concentration are based on Kjeldahl's study of nitrogen content were measured. The results show that the highest average of N (21.58 g/kg) and Fe (63.42 mg/kg) were indicated in the case of the Gyongyszem variety.

The statistical test Anova reveals a significant difference ($P < 0.05$) between the varieties. In contrast, the average calcium did not significantly differ between Biserka and Gyongyszem (539 mg/kg) (533 mg/kg), respectively. Furthermore, the size effect test shows a high effect of varieties on mineral concentrations, more significant than 0.14 for all investigated varieties and targeted minerals. The findings show that mineral concentrations indicate variation based on the varieties, allowing for enhancing *P. miliaceum* varieties to develop commercial products rich in mineral concentrations based on whole grain products in Eastern Europe, particularly Hungary.

***Keywords**: Proso millet, grain, mineral concentration*

Chemical composition, antioxidant properties, and *in vitro* digestibility of flour and ground hulls of differently coloured oat varieties

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Whole grain cereals are regarded as key components of nutrition due to their health-promoting properties. Oats (*Avena sativa* L.) are naturally gluten-free cereals suited for the gluten-intolerant and individuals with celiac disease. Oats are mostly utilized as flour or rolled oats in the food sector. Oat flour is produced by milling oat groats or flakes, and it is frequently used as whole-grain flour. The inedible outer hull of the oat grain is typically removed during harvest and processing. The hull typically makes up 25–30% of the kernel weight in hulled oats, with minor fluctuation across genotypes. Oats are also high in dietary fibre, notably β-glucans, which have been shown to provide medical benefits such as lowering cholesterol, slowing glucose uptake, and lowering plasma insulin levels [1,2]. Furthermore, phenolic acids, flavonoids, carotenoids, vitamin E, and phytosterols are plentiful in oats [3,4]. Oat hull is high in fibre and low in protein, which lowers the amount of energy that can be extracted from the kernel. Thus, the use of hulled oats in diets for pigs and poultry is severely constrained, however, ruminant animals can profit from oats' high levels of fat and fibre compared to other cereal grains. Oats are currently sent to mills as blends of many oat cultivars with varying quality characteristics, which presents a challenge for the food industry because it makes it more difficult to forecast the quality of the finished product.

This study aimed to examine the nutritional potentials of the whole grain oat flour and hulls of three oat genotypes with different hull colours: yellow, brown, and black. Ground oat grains and oat hulls were investigated for their levels of total phenolic compounds, phenolic acids, β-glucans, antioxidant capacity, and *in vitro* digestibility. An *in vitro* multi-step digestion method was used to determine the potential digestibility of the oat samples for human consumption as a function of processing variables. The oral, gastric, duodenal, and colon phases of the method proposed by Papillo et al. [5] and modified by Hamzalıoğlu and Gökmen [6] were carried out without attempting to closely resemble gastrointestinal digestion. Significant variations were found amongst the analysed samples, particularly when comparing parameter values found in the hulls to those found in the whole-grain flour. In comparison to flour (841.89-982.08 μg GAE/g d.m.), oat hulls had more total phenolic compounds (11320.11-24352.48 μg GAE/g d.m.), as well as the phenolic acids: *p*-coumaric, ferulic, isoferulic, vanillic, and syringic acid. Ferulic acid was predominant in both the whole grain flour (395.88-589.14 μg/g d.m.) and the hulls (4987.02-13794.82 μg/g d.m.). The antioxidant capacity was higher in oat hulls, ranging from 42.31 mmol Trolox/kg d.m., in yellow hulls to 53.16 mmol Trolox/kg d.m. in brown hulls, and from 22.61 mmol Trolox/kg d.m. in black grain, to 25.06 mmol Trolox/kg d.m. in brown whole-grain flour. On the other hand, only 0.03–0.06% of the β-glucan content was found in the hulls, while it ranged from 4.07% to 5.33% in the whole-grain oat flour samples. The absence of anthocyanins and proanthocyanidins suggests that these coloured bioactive compounds are not where the oat variants' colour comes from. The *in vitro* digestibility of brown whole-grain flour was the highest (48.24%), followed by black (44.72%) and yellow oat flour (44.54%). The degradability of the ground oat hulls was significantly lower, considering that the *in vitro* digestibility ranged from 12.02% in the black genotype to 16.69% in the brown genotype. The examined oat genotypes manifested a significant potential for use as high-quality food and feed ingredients with nutritional and health-promoting advantages.

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Carotenoid profile of the pulp of selected musk gourd varieties

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Musk gourd (*Cucurbita moschata* Duch. ex Poir.) is a well-known vegetable, valued mainly for its high content of carotenoids and the presence of a number of beneficial chemoprotective substances.In our work, we analyzed the carotenoid profile in the fruit pulp of 6 varieties of musk gourd (´Liscia´, ´Matilda´, ´Orange´, ´Serpentine´, ´UG 205´ F1 and ´Waltham´). For the determination, we used HPLC technology based on the different polarity of carotenoids according to the relevant methodological procedures. Polar carotenoids containing epoxy, hydroxyl and oxo functional groups were eluted from the C18 stationary phase approximately in the order of their relative polarities as measured by their partitioning into 6 flavoxanthin, 5,6,5´,6´-diepoxy-β-carotene, phytofluene, α- cryptoxathin and β-cryptoxanthin. Most of the research works are focused on the identification of the total content of carotenoids, mainly α-carotene, β-carotene, lutein and less violaxanthin. Table 1 shows the values of the content of selected carotenoids found in the flesh of the fruits of selected varieties of musk gourd.set at 2.5 cm (as used in this template). Analysis of carotenoid content showed high variability between individual varieties of pumpkins. All analyzed musk gourd varieties contained α-carotene, violaxanthin and lutein, except for the ´Orange´ variety, in which the numerical value of the violaxanthin content could not be clearly determined. We determined the highest content of α-carotene and β-carotene in the ´Orange´ variety. The flesh of the variety ´UG 205´ F1 is the richest in violaxanthin, as well as in other types of carotenoids (α-cryptoxanthin, luteoxanthin, β-carotene 5,6-epoxide), and contains a high level of lutein. We found that the genotype (variety) is an important factor that affects the content of chemoprotective substances in the pulp of the observed vegetables.

Table 1. Carotenoid profile of monitored varieties of musk gourd [mg.100g^{-1} DM]

Variety	α-carotene	β-carotene	violaxanthin	lutein	other	Total content of carotenoids
´Liscia´	4.77	17.21	11.54	14.82	14.91	63.25
´Matilda´	0.97	19.44	7.43	9.22	15.60	52.66
´Orange´	26.61	48.93	-	4.06	11.73	91.32
´Serpentine´	9.07	17.68*	4.68	11.93	6.30	49.66
´UG 205´ F1	2.79	16.12*	14.05	14.52	17.60	65.08
´Waltham´	6.24	16.80*	9.94	15.33	14.96	63.27

Note: DM – dry matter, * β-carotene 5,6-epoxide

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Quality differences among tomato varieties

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Introduction

Tomatoes can be used for eating fresh and processing, and more than half of tomatoes produced are consumed fresh [1]. In Japan, “Momotaro” is the most widely produced and consumed fresh tomato variety [2], and its strong sweetness is one of the reasons for its popularity. However, in recent years, other varieties of fresh-eating tomatoes have been produced and marketed, in addition to “Momotaro,” and although the “Momotaro” type tomatoes currently have a high market share, and other types of tomatoes except Momotaro are not well-known [3], there is a high possibility that they will appear in the market in the future. Therefore, this study investigated and compared the quality of “Momotaro” and currently marketed fresh eating tomatoes.

Materials and Methods

Five tomato varieties were used as follows: Momotaro Hope (TAKIIi & CO., LTD.), Reika (Sakata Seed Corporation), Saturn (Sakata Seed Corporation), Rinka 409 (Sakata Seed Corporation), and Animo (Asahi AGRIA CO., LTD. and Musashino Seed CO.,LTD.). Sugar content was determined using a sugar meter, and acidity was measured with a pH meter. Lycopene and **β**-carotene were measured spectrophotometrically, and the absorbance was calculated from the following formulae: lycopene = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$; β-carotene = $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$. Ascorbic acid was determined through HPLC(high performance liquid chromatography), and the number of viable bacteria was determined using the standard agar medium method. Glutamic acid was measured via HPLC.

Result and Discussion

Momotaro Hope was found to have the highest lycopene content (1.2 mg/100 g), which affects the reddish color of the fruit, in terms of appearance color; Rinka 409 had the highest glutamic acid content (4.4 mg/g), associated with flavor; and Reika and Saturn had the highest Brix values (7.3 % and 7.8 %) for sweetness.

Table 1 Measurements of quality assessment parameters of each tomato

	Acidity (pH)	Sugar content (Brix%)	Lycopene (mg/100 g)	**β**-Carotene (mg/100 g)	Ascorbic acid (mg/g)	Glutamic acid (mg/g)	Number of viable bacteria (CFU/g)
Momotaro Hope	4.4 (0.041)	6.4 (0.045)	1.2 (0.38)	0.54 (0.18)	0.15 (0.0085)	1.5 (0.26)	1.2×10^2 (0.96)
Rinka 409	4.4 (0.051)	5.7 (0.055)	0.69 (0.17)	0.59 (0.14)	0.094 (0.011)	4.4 (0.35)	5.8×10 (0.50)
Reika	4.3 (0.016)	7.3 (0.045)	0.95 (0.27)	0.43 (0.27)	0.11 (0.0067)	3.7 (0.54)	1.1×10^2 (0.50)
+++++Saturn	4.6 (0.14)	7.8 (0.071)	0.93 (0.13)	0.51 (0.14)	0.092 (0.0063)	3.7 (0.078)	2.0×10^3 (0.96)
Animo	4.3 (0.047)	5.0 (0.084)	0.22 (0.075)	0.20 (0.020)	0.058 (0.0052)	2.0 (0.21)	1.2×10^3 (0.82)

The tests were conducted based on n = 9. Parentheses indicate standard deviation.

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Comparison of the nutritional composition of different cultivars of the edible variety of *Jatropha curcas* (L.) - An untapped protein source

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In the context of sustainability and growing environmental awareness, plant-based protein sources are increasingly catching the attention of the food industry as alternatives to animal-based proteins. The kernels of the edible variety of *Jatropha curcas* (L.), also known as Xuta, are a high-quality, plant-derived protein source and have recently been classified as safe for human consumption by the European Food Safety Authority [1]. Xuta has been cultivated and used in traditional dishes in Mexico for several decades.

Depending on the variety, Xuta kernels have a fat content of over 55%. After defatting, the kernel flour has a protein content of over 55% [2]. Contrary to its non-edible variety, phorbol esters are absent in Xuta. However, certain amounts of anti-nutritional constituents such as trypsin inhibitors, lectins, and phytic acid have been detected in Xuta [3,4]. Until now, the varieties of this untapped alternative protein source have not been characterised in detail, Therefore, the aim of this study is to examine the kernels of three elite Xuta cultivars for their chemical composition in order to determine the most nutritionally valuable cultivar.

Prior to the analysis, the kernels of each cultivar were hydrothermally treated (120 **°C for 40** min at 100% steam) according to EFSA recommendations. A complete nutritional profile of macronutrients (crude protein, fat, fibre, carbohydrate) was assessed for each cultivar. The total phenolic content was analysed by the Folin-Ciocalteu assay. In addition, the extraction efficiency of polyphenols using different solvents were tested. Furthermore, the amino acid composition of the protein fraction was determined using HPLC fluorescence detection and derivatisation with *ortho*-phthalaldehyde after defatting with *n*-hexane and chemical hydrolysis with hydrochloric acid.

This first characterisation reveals that some of the Xuta cultivars showed significant compositional differences, but were in the range of reported values in previous studies [2]. The crude protein content differs up to 3% significantly between the cultivars. In accordance with the literature, the crude fat content ranged between 56-63% in the dry matter of the different cultivars. Such variation could be caused by different soil properties or growing conditions in terms of fertilisation and different climatic conditions during the growing season. The content of phenolic components in Xuta kernels is comparable to those reported in [2] (0.364-0.570 g gallic acid equivalent/ 100g defatted kernels). When comparing different solvents, the highest total phenolic content was found in aqueous Xuta extracts, showing that the phenolic compounds found in Xuta are water-soluble. The amino acid composition of the analysed Xuta varieties is in general nutritionally favourable but some heat-sensitive amino acids for example serine could not be detected in the hydrothermally-treated kernels. This effect is of particular interest with regard to the development of products from Xuta, as for example the content of leucine or phenylalanine, two essential amino acids, was reduced by hydrothermal treatment of the kernels. In future, these results could help to establish Xuta as a novel plant protein source and provide a basic understanding of the chemical composition of the kernels of the improved cultivars.

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Study on the phenolic composition and antioxidant properties of white, yellow and black corn (*Zea mays* L.) foodstuffs.

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Corn, also known as maize (*Zea mays* L.), is one of the major food sources in the world. Since corn is gluten-free in its natural form, in the last few years there has been a growing interest of people with celiac disease on it. Commonly, corn products are made from white and yellow varieties. However, there is a growing interest in purchasing colored corn foods because of their nutrition related characteristics, which are mainly attributed to their higher content in antioxidants. Corn food products require processing from the raw grain to the consumer final product. However, the potent antioxidant effect of colored corns, can be largely lost during food processing. Among the antioxidant compounds reported in colored corns, it is important to highlight certain phenolics, carotenoids and particularly anthocyanins, which are regarded as very potent antioxidants[1]. Cyanidin-3-*O*-glucoside is the major anthocyanin in black corn, accounting for approximately 75% of the total anthocyanin content[1].

The present study was undertaken with the aim of reducing the losses of antioxidants, mainly anthocyanins, in black corn food products. With this aim, three different color corns (*Zea mays* L.) were used: white, yellow and black (*Millo corvo*). All of them were cultivated and supplied by growers from the same area (Galicia, Spain) during 2021. We evaluated the antioxidant content of two different black, yellow or white corn-based products (ie, tortillas and cookies) subject to moderate processing. The tortilla making process consisted of mixing flour, warm water in a 1:1 (v:v) ratio, and salt. The mixture was heated from 50°C to 90°C progressively until a thick mass was obtained. Then, the dough was cooked on a hot plate at low temperature (120°C) for 5 min on each side. The cookies were prepared by mixing flour, vegetable margarine, eggs and salt. Once prepared, the dough was rolled out into discs 6 cm in diameter and 8 mm in height, and baked in a rotary oven at 183 ºC for 25 min.

Polyphenols were extracted from raw flour, tortillas and cookies prepared from white corn, yellow corn and *Millo* corvo, respectively. For the experiments, 3 g of each sample were used. Extractions were carried out by adding methanol:water (70:30, w:w). The content of phenolic acids and flavonoids in tortillas and cookies made from white corn, yellow corn and *Millo corvo* flours were determined and quantified by HPLC-QTOF-MS analysis. Additionally, total phenol content was determined in all samples. The method applied was based on that described in the literature with slight modifications[2]. Total anthocyanin content was quantified in all samples using the pH differential method elsewhere reported[3]. Antioxidant activity was studied by DPPH$^{\bullet}$ radical scavenging assay[4] with slight modifications and photochemiluminiscence (PCL) antiradical scavenging assay[5].

Raw *Millo corvo* flour exhibited higher content of phenolic acids, flavonoids and, particularly, anthocyanins than white and yellow flours. Phenolic acids decreased in cookies but they did not in tortillas; flavonoids did not exhibit a clear tendency and anthocyanins were always preserved. Antioxidant activity obtained for *Millo corvo* samples was twice as high as the value measured in white and yellow corns in terms of DPPH radical scavenging activity. The difference in AA was even more remarkable in terms of (PCL) antiradical activity. The conditions used during the cookie-making process enabled the natural antioxidant characteristics of *Millo corvo* to be preserved. However, the conditions applied to prepare tortillas resulted in major losses.

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Rediscovered fruit quince (*Cydonia oblonga* Mill.) as a food source of biologically valuable substances

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Quince (*Cydonia oblonga* Mill.) is considered a health-promoting fruit, although it is mainly consumed in processed form. Quince is known by its many therapeutic effects that include antioxidant, anti-inflammatory, antimicrobial, anti-ulcerative, and anticancer actions [1]. Thermally stable polyphenols are responsible for most of their beneficial effects. The study is focused on the content of chlorogenic acids (neochlorogenic, chlorogenic, cryptochlorogenic and 3,5-dicaffeoylquinic acid) and rutin, which were identified by the HPLC method. It also deals with the total polyphenol content and antioxidant activity in the skin and pulp of five cultivars of quince (Bereckeho, Morava, Aurelia, Vranje, Izobilnaja) grown in the same territory of the Czech Republic. Antioxidant activity (DPPH and FRAP) and total polyphenol content were determined spectrophotometrically. The content of chlorogenic acids (Table 1) in all observed varieties was higher in the skin of the fruit than in the pulp of the fruit. The content of chlorogenic acids in the pulp decreased in the order: chlorogenic acid,> neochlorogenic acid > cryptochlorogenic acid > 3,5-dicaffeoylquinic acid. 3,5-dicaffeoylquinic acid was present in the pulp of only two varieties (Aurelia, Vranja). Unlike our results Stojanović et al. [2] recorded the neochlorogenic acid to be the most represented phenolic acid in quince fruit. The values of chlorogenic acids in samples of quince fruit determined by Blanda et al. [3] were lower than those in our samples. It could be caused by the using of the quince fruits which were not at the stage of full maturity. In addition to chlorogenic acids, the peels also contained rutin, the values of which were in the range of 4564.01 (Vranja) – 7477.92 (Aurelia) µg/g DW. Rutin was most abundant in the peels, while it was not at all present in the pulp. Also Stojanović et al. [2] confirmed rutin to be present in higher amounts in quince peels compared to chlorogenic acids. The total polyphenol content in the peels (13707.27 (Vranja) – 16856.65 (Morava) mg GAE/g DW) was several times higher than in the pulp (2367.75 (Izobilnaja) – 10134.39 (Aurelia) mg GAE/g DW). The values of DPPH antioxidant activity in the peels ranged from 9.30 (Aurelia) to 10.77 µmol TE/g DW (Izobilnaya) and in the pulp from 7.06 (Izobilnaja) to 13.36 µmol TE/g DW (Bereckeho). The antioxidant activity of FRAP was in the range of 69.48 – 89.31 µmol TE/g DW in the peel and the pulp in the range of 40.06 – 92.34 µmol TE/g DW. Sut et al. [4] also found that the content of chlorogenic acids, total polyphenol content as well as antioxidant activity were significantly higher in the peel compared to the pulp of quince fruit. Our results confirmed that the content of bioactive substances in quince is influenced by the cultivar. At the same time, in this study the potential of this fruit in the development of functional foods was confirmed.

Table 1. Phenolic acid content [µg/g DW]

Variety	Neochlorogenic acid		Chlorogenic acid		Cryptochlorogenic acid		3,5- di-caffeoylquinic acid		Rutin
	peel	pulp	peel	pulp	peel	pulp	peel	pulp	peel
Bereckeho	2148.18	1491.04	4633.91	1413.21	317.35	212.21	38.83	-	4849.45
Morava	2021.68	1281.15	5588.92	1554.17	446.41	176.60	54.60	-	4973.10
Aurelia	1873.84	1607.67	4391.09	1652.05	375.51	214.79	92.02	20.27	7477.92
Vranja	1996.38	1490.03	4694.85	1437.74	394.12	210.24	46.30	10.78	4564.01
Izobilnaja	812.91	710.98	3346.75	636.42	22.56	106.36	26.54	-	6117.20

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Effect of blackcurrant skin ingredients on the physicochemical properties of pork meatballs

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Testing the possibility of using plant origin ingredients in meat products has become a very popular research topic during the last years [1]. Such ingredients may play different roles in meat products, e.g., for partial replacement of expensive animal proteins, inhibition of microbiological and oxidative processes during storage, and increase of health benefits. Small fruit (commonly called berries) ingredients have been tested in meat mainly for their antioxidant potential and high content of bioactive compounds [2]. Moreover, berry processing by-products such as juice pressing pomace have also attracted attention as a source of cheap health beneficial substances, which may be used in meat products [3]. Most recently we applied black currant seed ingredients in meatballs and evaluated their effects on various quality characteristics [4]. Considering chemical and physical heterogenicity of pomace, which consists of seeds, skins and pulp residues, in this study we used the skins, which were mechanically separated from the seeds and afterwards processed into different ingredients for their testing in meatballs. The main hypothesis of this study is based on the possibility to control oxidative and other undesirable processes, which occur during storage and processing of meat and meat products and may have negative influence on their quality. It is well-known that such undesirable changes can be controlled by using antioxidant-rich plant origin ingredients. Blackcurrants are also rich in polyphenolic antioxidants and other bioactive compounds.

The following blackcurrant skin ingredients were tested in meatballs by adding 2% of each: dried and milled raw (RS), defatted by the extraction with supercritical CO_2 (DF), insoluble residues after extracting defatted skins with ethanol and water (IR), combined extract of defatted residues consecutively isolated with EtOH and H_2O (EHE).Pork meatballs were packed under modified atmosphere (70% N_2 and 30% CO_2) and stored for 7 days at 4 °C. Due to the presence of high content of red-dark coloured anthocyanin pigments, all added ingredients decreased the lightness (*L**) and yellowness (*b**) of meatballs, while their redness (*a**) significantly increased. There were no remarkable changes in pH-values during the 7 days storage period; however, all the additives except the fibre-rich IR, decreased the pH of meatballs at the day of preparation, most likely due to the addition of black currant organic and other acids. The lowest cooking loss was achieved for the products with RS and IR, due to their high content of insoluble fibres; while the addition of EHE resulted in the highest cooking loss, as it contained only soluble components. The meatballs with rich in antioxidant polyphenols DF and EHE ingredients demonstrated the highest 2,2 diphenyl 1 picrylhydrazyl free radical (DPPH•) scavenging capacity values, while the products with IR gave similar results to control. The effects of blackcurrant ingredients on the composition of volatile compounds were analyzed by head space solid phase microextraction gas chromatography with time-of-flight mass spectrometry (HS-SPME-GS-TOF).

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Determination of total acidity of fruit juice

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Background: Fruit juice is a non-fermented but fermentable product obtained from the eatable parts of one or more types of fruit mixed together, which is healthy, ripe, fresh or chilled or frozen. The color, aroma and taste of the fruit juice should be characteristic of the type of fruit from which the fruit juice was produced.

Fruit juices are characterized by their complex chemical composition. The nutritional value, hygienic quality and organoleptic properties of the fruit juice will depend on this composition. Fruit juice in its composition, in addition to the fruit from which it is produced, may contain added sugars, honey, vitamins and minerals as well as juice or concentrated lemon juice. They are added to improve the quality and taste of fruit juices. The variety of fruit juices also includes a large number of acids. These can be citric, malic, ascorbic and tartaric acids. The acidity of fruit juices can be represented by the content of these individually present acids or as total (titrated) acidity (1,2). The total acidity of fruit juices can be expressed (g/L) as the content of citric acid.

Aim: The aim of this study was to provide information about the total acid contain in commercially available clear fruit juices, using simple and cost-effective method of potentiometric acid-base titration as well as acid-base titration with application of phenolphthalein indicator for determination the end point titration.

Materials and Methods: In this paper, 15 samples of different fruit juices (apple juice, pear juice, peach juice, grape juice, apricot juice) in total were analysed. The solution for analysis, contained 10 mL of selected fruit juice. Acid-base titration was used to determine total acidity of investigated fruit juices expressed as content of citric acid in samples. Standard solution of sodium hydroxide (0.1 M) was used for titrations. Titrations with application of phenolphthalein indicator for determination of end point titration were performed until pink colour of the indicator appears in solution. In aim to perform statistical data analysis each titration was repeated seven times.

Results: Acids content in the fruit juices is very important because it affects their taste and in addition have the role of preservatives. Composition and the total content of acids depend on fruit quality, climatic conditions as well as the process of juices preparation. According the obtained results, titratable acidity of the juice samples was ranged from 1.4 g/L to 15.1 g/L, depending of the type of fruit juices.

Conclusion: Different studies suggested that the total acid level is considered as more important than pH level in assessing the erosive potential of these drinks; they assume that total acid will give the actual hydrogen ion available to interact with teeth enamel. Therefore it is an important parameter that should be monitored with purpose to raise the awareness of the public health. The method of acid-base titrations with application of phenolphthalein indicator for determination the end point titration are suitable for determination of clear fruit juice's total acidity content, as cost-effective and simple method for acidity determination. However in the case of coloured and cloud samples potentiometric titration should be applied.

Key words: fruit juices, total acidity, acid-base titrations

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Characterization of physicochemical properties of a Portuguese miso

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Miso is a traditional fermented paste from Japan. Although *miso* can be used as a *seasoning*, *miso* soup is the most well-known and widely used way to consume this unique paste, with umami *flavour* and aroma capable of providing complexity to different types of food. Commonly, *miso* is produced by fermenting soybean with *koji* (steamed rice inoculated with *Aspergillus oryzae*), water and salt. However, yeasts and lactic acid bacteria (LAB) are also involved in miso production, contributing to its organoleptic proprieties and health benefits, *e.g.* reduction of gastric and heart diseases, as well as intestinal inflammation and improvement of nutrients digestion and absorption [1;2].
The purpose of this scientific project is to produce and characterize nutritious, healthy and innovative *miso*. Traditional Portuguese pulses – chickpea, lupin, and cowpea – were used instead of soybean, as well as specific fermentative microorganisms: *A. oryzae*, yeasts and lactic acid LAB. To ensure the quality and safety of *miso* its production will be carried out under controlled conditions and its fermentation monitored over time.
Misos were produced by mixing cooked and crushed Portuguese pulses, with *koji*, water and two different concentrations of salt: 3% and 12% (w/w). After obtaining a homogenous paste, unpasteurized miso, yeasts and LAB were inoculated. The fermentation process was followed by physicochemical (pH, total soluble sugar content (TSSC), soluble phenolic compounds, texture, linear viscoelastic behaviour, sugar consumption and metabolite production and colour) and microbiological (cell viability) analyses. Potential health properties, like antioxidant activity and bioaccessibility, will also be evaluated at the end of fermentation (final *misos*).
After 15 and 30 days of fermentation, *misos* were analysed and compared with the unfermented paste (beginning of fermentation: 0 day). All *misos* showed a decrease in pH, and an increase in TSSC and phenolic compounds. Regarding to texture a decrease of firmness was observed (Figure 1). Over the first 30 days of fermentation, chickpea and cowpea *misos* (3% salt), clearly showed a more pronounced decrease (18 to 9.9 N and 18.6 to 10.5 N, respectively). Lupin *misos* (3% and 12% salt) had a less noticeable decrease (9.9 to 6.4 N and 8.2 to 7.4 N). As *A. oryzae* produces high levels of extracellular enzymes capable of hydrolysing polysaccharides – present in the miso raw materials – starch is converted into free sugars and smaller peptides, promoting the fermentation activity of LAB, which produces organic acids [2]. The combined action of *A. oryzae* and LAB resulted in a decrease in pH and firmness, and an increase in TSSC and phenolic compounds, mainly in the *misos* made from starch-rich pulses, chickpea and cowpea.
Miso production using Traditional Portuguese pulses, will contribute to a more diversified, nutritious and healthy diet, and to a more sustainable and eco-friendly food production.

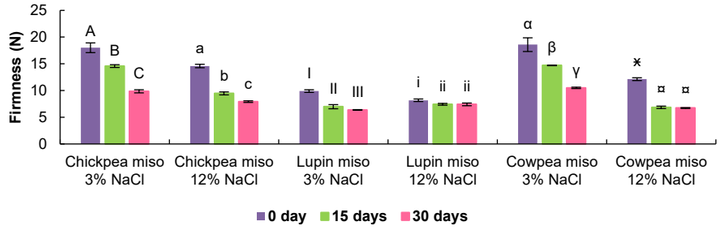

Fig.1. Firmness evolution of the *misos* at the beginning, after 15 and 30 days of fermentation process. Results presented as mean ± standard deviation. Equal letters, numbers and symbols correspond to non-significant differences in the Tukey test result (α = 0.05).

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Role of soybean - millet intercropping and bio-fertilizer in managing potential bio-availability of essential elements

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The potential bio-availability of minerals from food, i.e. agricultural products, is mainly subjected to the concentration of anti-nutrients, due to its high affinity to bind elements and make them unavailable for humans from digestive tract. Thus, increasing the mineral concentration in grain is just one point in the string that can improve food quality, but reduction in concentration of anti-nutrients, such as phytates, is of great importance, too. [1,2] As intercropping and use of bio-fertilizer represent integrative part of sustainable agriculture which influence nutrient use efficiency [3,4], their combination seems to be a good way to manage nutrients uptake and accumulation, and anti-nutrients concentration in grain. Therefore, this research aimed to examine the impact of soybean - common millet arrangement in intercropping, together with bio-fertilizer, on potential bioavailability of essential elements in grain. A two-year field experiment was conducted with soybean and common millet. Mono-crops (T1 - soybean, T2 - millet), as well as three planting patterns of intercrop (T3 - alternating rows of soybean and common millet; T4 - alternating strips of two rows of soybean and two rows of millet and T5 - alternating strips of two rows of soybean and four rows of millet) were set up in 2018 and 2020. The bio-fertilizer Coveron (BF) (containing mycorrhizal fungi, Trichoderma and plant growth-promoting rhizobacteria) was also included in same combinations, as a subplots, as well as variant without BF (BFΘ). After determination of concentrations in grains, the molar ratios between phytic acid (Phy) and magnesium (Mg), calcium (Ca), iron (Fe) and zinc (Zn) were evaluated. Results showed that intercropping and bio-fertilizer significantly affected molar ratios between phytic acid and essential elements. Regarding to the soybean, all 4 ratios showed smaller values in intercropping comparing with mono-crops (both in plots with and without fertilizer). Intercrops + BFΘ decreased Phy/Ca, Phy/Mg and Phy/Fe ratios down to the 0.31 (T4 and T5), 0.16 (T4) and 14.03 (T4), respectively, while intercrops + BF decreased Phy/Zn ratio down to the 25.25 in T3 + BF. These lowest values could be related to lower accumulation of Phy and greater accumulation of minerals in intercropped soybean, due to the presence of cereal (millet) and its ability to excrete phytosiderophores, which promotes mineral uptake [5]. Nevertheless, situation for common millet was different. Ratios of Phy/Ca, Phy/Mg and Phy/Zn had the lowest values in mono-crops (both in BF and BFΘ variants), while the value of Phy/Fe was the lowest in T3 + BF (23.88). Such results suggest soybean - common millet intercropping as a good sustainable agricultural practice to enhance bio-availability of essential elements in grain of soybean. On the other side, positive impact of BF was pronounced in millet, enhancing potential bio-availability of examined minerals in grain by lowering values of all 4 ratios. These findings can be connected to beneficial effect of microbes on nutrients uptake by cereals [6], highlighting the tested combination of fungi and plant growth-promoting rhizobacteria as a sustainable strategy to increase grain quality. However, although this research proved positive effects of soybean - common millet intercropping and bio-fertilizer on potential bio-availability of essential elements, further research is needed to determine the most suitable combination for increased quality of both crops.

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Phytochemical composition of hydro-ethanolic extracts from *Cucumis metuliferus* E. Mey. fruit peels

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According to recent research, a variety of inedible portions of fruits and vegetables, as well as food waste, are excellent sources of phytochemicals that may be isolated and reintroduced as natural food additives into the food chain [1]. Besides the food industry, natural product from non-edible parts of the fruits of Cucurbitaceae family can be used in other industries such as cosmetic and pharmaceutical and the fact that the plants in this family are also used medicinally makes them excellent research subjects for the study of plants with high therapeutic potential [2]. The focus of our study was the solid/liquid extraction of lyophilized fruit peels of *Cucumis metuliferus* E. Mey. (Cucurbitaceae) under different extraction conditions such as extraction time (min), ethanol/water ratio (%) and power of ultrasonic bath (%). It was aimed as well to determine the phytochemical composition of 25 different ethanolic and hydro-ethanolic extracts by performing UHPLC-QToF-MS analysis. The **Figure 1** shows the fruit of Horned Melon (*C. metuliferus*) and the exocarp as part of fruit used for extraction. Under different extraction conditions percentage yields of the dry extracts varied between 26.37% - 43.98%, obtaining the optimal extraction conditions as follows: EtOH (%): 50; Amplitude (%): 40; Time (min): 30. Furthermore, chemical profiling of the extracts revealed the presence of the following compounds: *p*-hydroxybenzoic acid, dihydroxybenzoic acid, galloyl pentoside, dihydroxybenzoyl pentoside, hydroxybenzoyl hexoside, vanilloyl hexoside and hydroxybenzoyl rhamnosyl hexoside. This is the first study that highlights the optimization of extraction conditions of the *C. metuliferus* peels, as well as chemical identification of bioactive compounds present in the extracts. Our future observations will be oriented towards exploring bioactive properties of the obtained extracts and further incorporations in food matrices.

Fig.1. Horned Melon fruit

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Changes of volatiles, free fatty acids and antioxidant profiles in gluten-free sponge cakes with the powdered cocoa bean shell (CBS)

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Nowadays, the value of agricultural by-products has received increasing attention due to economic reasons and the environmental problem with organic wastes. According to the statistics, more than 250 thousand tons of cocoa bean shell (CBS) is produced in Europe [1], and on a global scale, the amount of CBS is even higher. Our study aimed to assess the impact of CBS powder on the functional properties of a gluten-free sponge cake as an innovative approach to cocoa by-product valorisation. For this purpose, the profile of volatile compounds, free fatty acids, and antioxidant properties of gluten-free sponge cakes with 5, 10, 20 and 30% addition of CBS powders of different particle sizes (100 and 200 μm) was evaluated (Fig. 1). The solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) was used to study the profile of volatile compounds, whereas Photochem® kit (Analytic Jena, Germany) was devoted to analyse the lipid-soluble antioxidants ability to scavenge against superoxide anion radicals ($O_2^{\cdot-}$). The free fatty acid composition was determined from lipids extracted by the Folch method. Fatty acid methyl esters were separated using the gas chromatograph equipped with a flame ionization detector (GC-FID). Several volatile components were identified in the sponge cakes with CBS addition. They belong to the chemical classes of alcohols, aldehydes, furfural and pyrazine derivatives (e.g. 2,3- and 2,6- dimetylpyrazine, methyl-, trimethyl-, tetramethylpyrazine). The occurrence of pyrazines, which possess a nutty and sweet odour, in these bakery products might influence positively the aroma perception and potentially increase their acceptance among consumers. The *cis*/ *trans*-oleic acid, palmitic acid, and linoleic acid were the three domain fatty acids in the studied samples. Their content was identified at the range of 49.42- 53.71, 15.78-20.49, and 18.72-14.55%, respectively. Furthermore, the highest antioxidative activity was observed in sponge cakes with 30% CBS addition . Irrespective of the particle size of CBS powders, the antioxidant activity determined in the sponge cakes with CBS100 and CBS200 ((7.67±0.24 and 7.25±0.09 Trolox equiv. mmol/L, respectively) was almost 3-times higher in comparison to the control (2.72±0.11 TE mmol/L). In conclusion, the addition of CBS to gluten-free sponge cakes is a promising ingredient to achieve a product of higher biological activity and pleasant flavour.

Fig.1. Images of cocoa bean shell (CBS) and gluten-free sponge cakes with 5% of CBS addition with 100 μm (on the left) and 200 μm (on the right).

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The influence of integral and organic growing systems on sugar content in selected tomato types and cultivars

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Interest in production of protected crops has grown significantly over the past decade. Different cultivation systems (conventional, integral, and organic) affect the biological activity, which is ultimately reflected in the high quality of the fruits of different vegetable crops. In accordance with the requirements for health-safe products without residues of harmful substances in fruits, there is an increasing need for the production of tomatoes in integral and organic production systems. One of the most important features of these vegetables is their high quality and health safety, high nutritional and biological value. Agricultural production systems and growing practices are critical factors in determining the nutritional quality of tomato fruits [1]. In tomato, the sugar content is one of the important factors and one of the most significant parameters from the aspect of food quality [1]. Therefore, the aim of this work is to monitor fluctuations in sugars content, as parameters that determine the nutritional value in tomato cultivars, induced by growing under integral and organic conditions. For this purpose, a set of sixteen samples of four types of tomatoes - beef, grapolo, mini and midi plum, and cherry - was analyzed. Each type of tomato included two varieties, grown in two agricultural systems - integral and organic. The sugars profile was obtained using High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The content of eleven sugar components was determined. Fructose and glucose were the major sugar compounds [2], while the sugar microcomponents were trehalose, arabinose, melibiose, sucrose, isomaltose, gentiobiose, raffinose, maltose, and panose. The results showed differences between samples produced in integral and organic growing systems, primarily in microsugar components. Higher content of trehalose and melibiose was found in samples obtained from organic production.

A) 1a 1b

B)

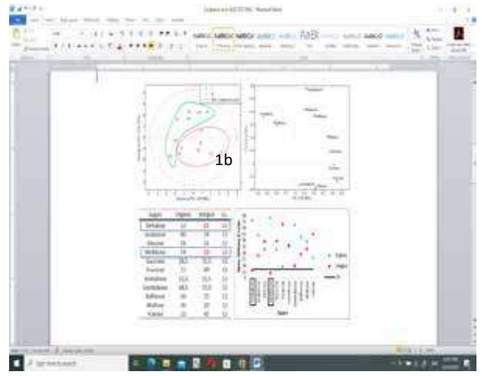

Fig.1. Principal Component Analysis (A)- The difference between integral (I) and organic (O) type of production; score plot (1a) – tomato samples: integral samples (I) 9-14, organic samples (O) 1-8, and loading plot (1b), and results of the Mann-Whitney U test (B) - sugar markers of type of production.

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Experimental design and the desirability function in the estimation of overall food quality

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The overall quality of processed food depends on many processing parameters such as temperature, processing time, mixing speed, pressure, pH value, size of food pieces, type of added ingredients, etc. The goal of food products development is not only to improve the quality of the final product but also to reduce the energy consumption and to improve resources management. Independent evaluation of a single parameter or response is not the best way to set and optimize experiments, because improving one response by controlling one parameter can negatively affect other responses. Experimental design is frequently used for experiment planning when multiple variables should be optimized, while the desirability function allows the determination of operating conditions that provide the “most desirable” response values. The desirability function allows the optimization of several responses simultaneously and the determination of the overall/cumulative/global quality. In this way, it is possible to determine the most suitable conditions for achieving the best overall product quality. In the desirability function, all responses are transformed into dimensionless individual desirability functions (di), which take values from 0 to 1. The value 0 indicates an undesirable response of the system, while the value 1 represents the most desirable response. All values between 0 and 1 indicate more or less desirable responses. There are three different equations for evaluating individual desirability functions depending on whether it is ideal for the response to be maximal, minimal or to have some target value. After calculating the individual desirability functions, they are combined into one global/overall desirability function (D) [1,2]. If the value of this function is different from 0, it means that for all responses, the desired responses were achieved at the same time. If the value of the D is equal to 0, it means that for at least one response the desired response has not been achieved. The overall desirability function represents the geometric mean of individual desirability functions. When applying the experimental design, this means that one composite desirability function is obtained for each experiment. The function D with the highest value represents the experimental conditions under which the most optimal responses of the system are obtained, i.e. the best quality of the final product. Various quality parameters of food products, such as color, taste, odor, the content of minerals, vitamins, fibers, appearance, texture, etc., can be measured as system responses. However, despite the advantages of this approach, the traditional approach that uses the optimization of only one factor and one response is still the most used.

Keywords: desirability function, experimental design, food quality, multiple responses, various parameters

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The sugars content of parental and new perspective descendant strawberry genotypes – potential approach for the future selection process

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Besides being one of the most commercially grown berry fruits, strawberries (*Fragaria x annanassa* Duch.) are known for their nutritional value, richness in polyphenols, and antioxidant capacity. New directions of strawberry breeding and selection are set towards premium fruit quality, high sugars content, and desirable sugars/total acids ratio (sweet index, SI), which represent some of the main prerequisites for selecting new breeding materials [1]. With this intention, a set of 24 strawberry genotypes was cultivated, including 12 parental varieties and 12 of their descendants - perspective candidates obtained by crossing the parental varieties mentioned above. A total of eleven sugars were quantified using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). Results implied that glucose, sorbitol, sucrose, and melibiose content were the most important sugars in separating the fruits of parental varieties and their progeny [2]. Results showed that old, parental varieties had a significantly higher content of glucose and sucrose, while new perspective genotypes had higher sorbitol and melibiose content (Fig.2). PCA analysis confirmed that parental (samples 1-12) and descendant genotypes (samples 13-24), could be discriminated according to sugars profile i.e. that these four sugars have an effect on their differentiation (Fig.1). This was in accordance with Mann-Whitney U test results (Fig.2).

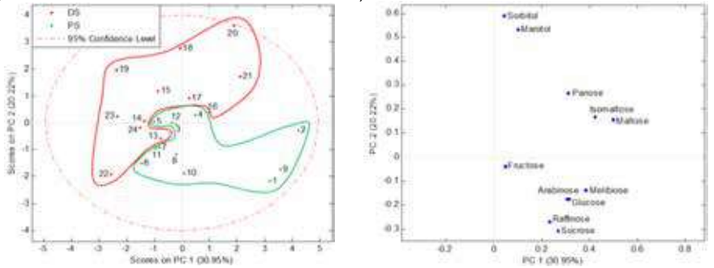

Fig.1. Principal Component Analysis - The difference between parental and descendant genotypes; score plot (A) – parental samples (PS) 1-12, descendant (DS) samples 13-24, and loading plot (B) – sugar components.

Sugar	Parental genotypes (PS)	New perspective genotypes (DS)	U critical value
Glucose	110	**34**	**37**
Fructose	50	94	**37**
Sorbitol	**29**	115	37
Mannitol	44	100	37
Arabinose	85	59	37
Sucrose	120	**23**	37
Melibiose	**13**	131	37
Izomaltoza	86	58	37
Raffinose	85	59	37
Maltose	87	57	37
Panose	57	87	37

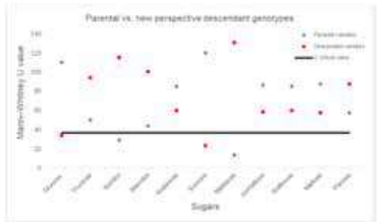

Fig. 2. Mann-Whitney U-test sugar content results - comparison between genotypes

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Phytochemical composition and antioxidant activity peel crude extract of *Cucumis metuliferus* (E. Mey. Ex. Naudin) from Fruška gora

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Cucumis metuliferus E. Mey. Ex. Naudin, also known as horned melon or kiwano is a fruit that belongs to the Curcubitacea family. Even though it is originally from Africa, due to their fast growth and peculiar appearance, it has been expanded worldwide. The kiwano consumption is associated to several health benefits, including antifungal, antimicrobial, antiviral, antihypertensive, antidiabetic, and antioxidant effects [1, 2]. Furthermore, there have been reports that its crude extracts contain alkaloids, saponins, tannins, flavonoids, steroids, cardiac glycosides and carbohydrates. In traditional African culture whole fruit was being consumed. Nowadays, however only inner parts of fruit are eaten and peel is discarded as a waste material [3]. This accumulation of agro waste presents a huge ecological and economical problem. For that reason many scientist are trying to find sustainable solution for this rising problem. One of the possible reuses of this discarded waste is a recovery of present bioactive compounds [4]. Therefore, in this study the crude extracts of kiwano's peel were investigated. The sample of kiwano's peel was lyofilised and milled for further analyses. The procedure of extraction was performed according to the method descriebed in work of Šovljanski et al. (2022) [5]. In obtained extract the content of bioactive compounds was determined, more precisely content of polyphenolics and carotenoids. For the evaluation of antioxidant potential of this agro waste, the antioxidant activity was evaluated by three tests: DPPH, ABTS and reducing power [6]. The gained results, represent in Table 1, show abundance of bioactive compounds as well as observated antioxidant activity which confirms its potential use as a source of valuable secondary metabolites.

Table 1. The results of phytochemical composition and antioxidant activity of Kiwano's peel extract

Analyses		Units	Kiwano's peel sample
Phytochemical composition	TPh	mg GAE/100g	6,40 ± 0,30
	TCar	**µg β-car/100g**	97,76 ± 3,10
Antioxidant activity	DPPH	mM TEAC/100g	630,95 ± 26,02
	ABTS	mM TEAC/100g	1664,22 ± 24,36
	RP	mM TEAC/100g	564,46 ± 29,03

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Mints producing thymol isomers – New chemotypes in five Hungarian *Mentha longifolia* (L.) L. accessions involved to experimental cultivation

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Keywords: *Mentha longifolia* (L.) L.; essential oil; thymol; carvacrol; chemotypes

Mentha longifolia (L.) L. is considered to be the most widespread wild-growing mint taxon of the Earth [1]. In the present work, Essential oil (EO) yield and chemical diversity of five Hungarian accessions were studied. These were involved to a cultivation experiment as a part of the first phytochemical evaluation of the species in Europe. The goal is to establish the cultivation of this species as a source of preservative agents (polyphenols) and flavoring agents (terpenic volatiles, EO).

Accessions annotated as HOR-1, HOR-2, EGR3, DOM and KBT were selected from 36 wild-growing populations. Cultivation sites were Eger (N 47.906834°; E 20.388889°) and Budapest (N 47.398820, E 19.149270). Both sites were sampled for investigations of essential oil in full bloom in 2019 and 2020. Air-dried samples were processed in a Clevenger apparatus according Pharmacopeia Hungarica Editio VII. Components were identified via GC-MS using standards, retention indices and spectral libraries.

EO yield (1–2 mL/100 g dry weight) was dependent on accession and year. Principal component analysis (PCA) also demonstrated the strong separation of groups in the essential oil samples associated to the products of three main monoterpene metabolic pathways (limonene-3-oxo derivatives, limonene-2-oxo derivatives and cymyl compounds).

HOR-1 is assumed to be a new chemotype in the species, dominated by carvacrol (19.28–20.56%), thymol (13.36–13.90%), carvacryl acetate (8.81–10.40%), *para*-cymene (7.24–8.01%) 1,8-cineole (14.87–17.45%). Concentration of these showed minor fluctuations. However, a turn of composition was observed in EO of HOR-2, as one batch was based on thymol (19.79%) and 1,8-cineole (14.93%), while the others were rich in dihydrocarvones (up to 69%). EO of EGR, DOM and KBT based on limonene-3-oxo metabolites and sesquiterpenes which are typical for the species [3]. However, EGR3 also may represent an unknown chemotype of *Mentha longifolia* as it bears an unusual combination of high cis-piperitone epoxide contents and sesquiterpenes.

The γ-terpinene pathway in the genus *Mentha* is less known and may be rare [3]. We demonstrated that chemotypes of horsemint might also differ in phenotypic appearance of chemical traits influenced by habitat or development. Our results led to the conclusion that any statement on chemotype needs detailed examinations.

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HPTLC—MS/MS analyses of phenolic compounds in bee pollen botanically originated from *Hedera helix*

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Hedera helix L. (ivy) is an evergreen plant which blooms from September to November [1]. Although honeybees commonly collect flower pollen during the spring season, *H. helix* flowers serve them a valuable food source before winter. Beekeepers may collect bee pollen for human usage. Bee pollen was known as 'life-giving dust' in ancient times because of its valuable constituents including proteins, fats and carbohydrates. The proportion of these constituents well fit with the dietetic recommendations, so a human can live healthy only by eating bee pollen [2]. Additionally, it has a wide range of phenolic compounds which are responsible for its bioactivity as antioxidants as well as for its anti-inflammatory and antimicrobial properties etc.

This is the first report on HPTLC-MS/MS analyses of phenolic compounds in bee pollen that botanically originated from *Hedera helix*. It was found that pre-development of the plate was crucial for MS analyses to overcome the issues related to ion suppression. Therefore, HPTLC-MS/MS analyses were performed on twice pre-developed HPTLC silica gel plates F_{254} that were developed up to 7 cm with EtOAc-HCOOH-CH_3COOH-H_2O (10:1.1:1.1:2.6, v/v) [3] as a developing solvent in a saturated twin trough chamber. Natural product detection reagent was applied for post-chromatographic derivatization of one narrow part of the chromatographic zones that supported appropriate positioning of the elution head of the TLC-MS interface that was used to transfer the compounds from the chromatographic zones into the MS detector. The full MS spectra were scanned in the range of 100-2000 *m/z*. The ions which gave the most intensive signals were fragmented with 35% collision energy. The investigated bee pollen samples were obtained from Slovenia (Hrastnik) and Türkiye (Ordu). The analyses confirmed similar chemical profiles of the main phenolic compounds discovered in both bee pollen samples.

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Biocompounds from mushroom aqueous and polysaccharide extracts

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The application of mushrooms for medicinal purposes has a long history, primarily due to its therapeutic properties. Today, mushrooms are often used as functional food or natural sources in the development of various nutraceuticals. Using advanced instrumental techniques, it was shown that mushrooms are a good source of highly valuable polysaccharides (i.e., glucans), sterols (i.e., ergosterol), different antioxidants, proteins and peptides. However, due to the great diversity of fungi, additional research in this area should be performed. The aim of this study is to analyze biocompounds from polysaccharides and aqueous extracts of two different mushrooms (*A. bisporus* and *A. aegerita*). Mushroom extracts were prepared according to procedure previously desribed by Popović Minić (2023)[1]. Lyophilised mushroom powder was extracted with 80% methanol containing 0.1% HCl, after which the suspension was filtered through 0.45μm filters and used for further chromatographic analysis by UHPLC-QToF-MS. Chemical characterization of mushroom biomolecules was performed using exact mass (*m/z*) and MS^2 fragment ions of each detected compound and their retention times. The identified compounds represented four structurally distinct groups: 1) organic acids and their derivatives (7 compounds); 2) phenolic acids and their derivatives (11 compounds); 3) esters (28 compounds); and 4) other organic compounds (Gibberellin A1). Based on the obtained results, the differences between the tested samples can be clearly observed. In *A.bisposrus* and *A.aegerita* polysaccharide extracts only few organic acids and esters were detected, while phenolics and majority of esters were not recorded. On the other hand, the presence of organic acids, phenolic acids, esters and their derivatives was confirmed in both aqueous extracts. The highest number of detected compounds (as many as 41 compounds) was detected in the aqueous extract of *A. aegerita*. Among organic acids, fumaric, malic and citric acids were detected in all the mushroom extracts, whereas *p*-hydroxybenzoic acid, *m*-hydroxy-hydrocinnamic acid, sinapic acid, 2-(pentanoyloxy)benzoate, and 3-(11-hydroxyundecoxy) benzoate were detected among phenolic acids and their derivatives in aqueous extracts of both mushrooms. Regarding detected esters, following compounds were identified in the tested samples: 8-carboxyoctanoate, 3-(octyloxy)-3-oxopropanoate, 9,12,13-trihydroxyoctadecenoate, 13-hydroxy-9,11-octadecadienoate. The estimated profiles of biocompounds present in mushroom extracts can contribute to the further understanding of their antioxidant and biological properties.

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Some antioxidants and dietary fibre in various small grains (cereals)

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Small grain cereals are an important source of numerous nutrients. They are rich in vitamins, minerals, dietary fibre, and antioxidants, and easy to be incorporated in various diets, thus promoting health and wellbeing. Antioxidants and dietary fibre from small grains are important for immunity, in prevention of metabolic syndrome, cardiovascular diseases and many other diseases.

The aim of the study was to determine concentration of some non-enzymatic antioxidants: soluble phenolics, phytic acid (expressed as phytic P), total glutathione (GSH), carotenoids and capacity to reduce DPPH radical, as well as beta-glucan and arabinoxylan in grain of 20 genotypes from Maize Research Institute gene-bank: bread wheat, durum wheat, rye, triticale, barley, oats, and ancient grains, such as spelt and emmer wheat.

Results indicate that barley genotypes are the richest source of soluble phenolics and GSH (up to 1361.2 μg g^{-1} and 607.3 nmol g^{-1}, respectively; Table 1), while durum and bread wheat are low in phenolics and GSH. ccordingly, barley genotypes had the highest values of reduction capacity of DPPH radical, which were followed by triticale and ancient grains, i.e. emmer and spelt wheat. Lower variability among tested genotypes was present in concentration of carotenoids and phytic P, with greater values in grain of spelt wheat (5.0 μg g^{-1} and 5.2 mg g^{-1}, respectively). Besides to its antioxidative properties, phytate is also an important antinutrient, binding mineral elements, so its lower concentration in grains is desirable trait. Hence, oats are low in this antinutrient, what with greater concentration of beta-glucan and arabinoxylan, as an beneficial dietary fibre, makes them recommendable part of healthy diet. When dietary fibre was considered, the highest concentration of beta-glucan was detected in barley (up to 4.6%). Considering variability of examined antioxidants and dietary fibre, the greatest variability of phytic P was detected in grains of spelt and emmer wheat, greater variability of reduction capacity of DPPH radical was in barley genotypes, as well as variability of arabinoxylan concentration was in rye and oats genotypes. One of the tested barley genotypes is the greatest source of variability in GSH, phenolics and beta-glucan.

This study proved that barley, rye, oats and ancient grains are great source of non-enzymatic antioxidants. Besides, barley and oats could be considered as the main sources of beta-glucan and arabinoxylan, what with low concentration of phytic acid make them valuable part of human diet.

Table 1. The concentration of phenolics, glutathione (GSH), phytic phosphorus, carotenoids, beta-glucan and arabinoxylan, and reduction capacity of DPPH radical in grains of examined small grain genotypes

Genotypes	Phenolics (μg g^{-1})	GSH (nmol g^{-1})	Phytic P(mg g^{-1})	Carot.(μg g^{-1})	DPPH (%)	Beta-gluc. (%)	Arabinoxy. (%)
Durum wh.	178.4-311.8	77.9-85.1	4.5-4.8	2.7-4.7	62.6-74.2	0.11-0.16	4.8-5.6
Bread wh.	179.9-211.4	107.4-130.6	4.5-4.7	3.2-3.4	75.8-80.2	0.19-0.20	6.2-6.7
Spelt wh.	368.8	207.6	5.2	5.0	87.3	0.38	0.5
Emmer wh.	256.3-398.8	135.4-155.7	4.5-4.8	2.6-3.7	79.2-89.7	0.13-0.17	3.8-4.8
Triticale	218.9-314.8	169.3-216.8	4.6-4.9	2.5-2.9	81.2-94.0	0.12-0.28	6.3-6.5
Rye	538.2-641.6	360.2-382.1	4.0-4.2	4.0-4.2	75.2-80.7	0.57-0.85	7.2-7.6
Barley	889.0-1361.2	364.6-607.3	4.3-4.7	3.6-4.2	89.8-97.9	3.34-4.59	3.3-4.6
Oats	500.7-782.5	466.4-471.2	3.5-4.1	2.8-4.8	60.2-75.7	3.05-3.70	10.6-12.7

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POSTER PRESENTATIONS

T2

Food Sustainability, including byproducts valorization

Chickpea flours as a high nutritional quality ingredient for healthy bakery innovation

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The growing demand from citizens, including those with gluten intolerance, for higher quality breads for daily consumption, justifies the industry's interest in developing new bakery products using flours obtained from vegetable matrices of geographical proximity, more sustainable from the environmental point of view, and with high nutritional quality and health-promoting properties.

Pulses are edible seeds of plants belonging to the legume family that occupy an important place in the diet worldwide. Among pulses, chickpea flours are vegetable matrices that offer extraordinary opportunities to elaborate innovative breads made with mixes of flours, which would be healthier that those frequently consumed made exclusively with wheat flours (1).

The objective of this study was the characterization of the nutritional and bioactive quality of five chickpea flours from Spain (Murcia, Cádiz, Zamora and Albacete) and Germany and the development of mother doughs with the aim of investigating their potential as a healthy an innovative ingredient for bread making.

The protein content of chickpea flours was quantified by the Dumas method, and the total starch and phytic acid content were determined by using two commercial kits from Megazyme: Total starch assay kit, AA/AM (K-TSTA) and Phytic acid assay kit (K-PHYT), respectively, and the fatty acid profile was measured by gas chromatography with flame ionization detector. In addition, the content of free phenolic compounds was determined with the Fast Blue BB reagent, after extraction in acidified methanol (2) and the antioxidant activity by the ORAC method (2). Finally, two types of spontaneously fermented mother doughs, daily back slopped until their fermentative maturity, were prepared with wheat flours combined with chickpea flours from Zamora at 30% (MDG1) and 50% (MDG2). The indigenous microbiota of each MDG was analysed with metagenetic techniques and breads were obtained with MDG1.

Chickpea flours showed similar nutritional quality, regardless of their origin, although differences were observed in the content of certain nutrients and bioactive compounds. Protein (22-25%), starch (33-37%), and phytic acid (2.3-2.6%) contents were very similar in all flours, with the exception of flours from Murcia and Albacete, which had higher protein and phytic acid contents, respectively. The content of phenolic compounds varied between 27-51 mg gallic acid equivalents (GAE)/100 g, while the antioxidant activity ranged from 14 to 21 μmoles Trolox equivalents/g. Oleic and linoleic acids were the major fatty acids present in chickpea flours. The flours also proved to be suitable matrices for the growth of autochthonous LAB and yeast species. Breads developed with 30% of MDG1 showed good sensory properties.

In conclusion, the good nutritional properties of chickpea flours, together with their good suitability for the production of mother doughs, make these flours a very attractive ingredient for the production of new bakery products.

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On the valorization of coffee by-products: functionality of lignin from silverskin and parchment for nanoparticle production

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Due to growing concerns about sustainability, practices related to the reduction of the use of non-renewable resources and the recovery of agri-food waste are increasing. The coffee industry generates huge amounts of waste with added value with the potential to be upgraded [1]. On the one hand, parchment is a strong fibrous endocarp that covers both hemispheres of the coffee seed and separates them from each other. It represents 5.8% of coffee berry dry weight and contains up to 35% lignin. On the other one, coffee silverskin constitutes a thin tegument of the outer layer of the two beans forming the green coffee seed and represents 4.2% of the coffee berry, being also rich in lignin. It is obtained as a by-product of the roasting process. Lignin nanoparticles are materials that have excellent properties, such as biodegradability and non-toxicity, and have great potential as chelating agents, antimicrobials agents, UV protectors, nanofillers, adsorbents, catalysts, supercapacitors, emulsion stabilizers, delivered systems, drugs, and gene carriers [2]. The objectives of the present work are (1) to extract lignin from coffee parchment and coffee silverskin using natural deep eutectic solvents (NADES) and (2) to evaluate the functionality of these lignins to be structured into nanoparticles.

Lignin was extracted (155 ºC/2 h) using NADES composed of acetic acid (AA) or lactic acid (LA) with choline chloride at 2:1 and 10:1 molar ratio, respectively. Samples are designated as AAP or LAP (for parchment-extracted lignins) and AAS or LAS (for silverskin-extracted lignins). Lignin extraction yield was calculated by gravimetric method. The composition of the isolated lignins was determined by using the Laboratory Analytical Procedures for biomass analysis provided by the National Renewable Energies Laboratory standard analytical methods, using the protocol NREL/TP-510-42618. Also, FTIR spectra were recorded. Lignin nanoparticles (LNP) were prepared by an anti-solvent method, for that the isolated lignins were previously dissolved in acetone and then added drop-by-drop to distilled water. LNP were characterized according to their production yield (gravimetric method), morphology (TEM microscopy), hydrodynamic properties (size, z-potential and polydispersity index, by DLS) and hydrodynamic stability after 10 days at 5ºC. LNP prepared from a commercial kraft-type lignin (LC) were used as control for all analyses.

The amount of biomass extracted was between 10-12%, representing up to 50% of the initial lignin content in the coffee by-products. In general, all lignin samples presented a high purity, showing a high acid-insoluble lignin content, but also acid soluble lignin and some carbohydrates impurities. Values for coffee-isolated lignins were comparable to those of the commercial one. FTIR spectra showed in all samples the typical lignin bands according to literature [3-4]. In commercial and parchment-derived lignins (LC, LAP and AAP) the S units prevailed, whereas silverskin-derived ones (LAS, AAS) presented a higher G/S lignin ratio.

LC and AAP showed a good functionality for the production of nanoparticles, that were spherical in shape, ≈100 nm in diameter and showed a z-potential of −25 mV and +35 mv, respectively. In the case of LC, they tended to aggregate. The nanoparticles production yield was ≈60% for both lignins. From this results it can be deduced that AAP lignin, as well as that from commercial origin, was the only able to form nanoparticles. After 10 days of chilled storage, LC and AAP nanoparticles maintained their size, polydispersity and z-potential in the same range than when fresh.

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The use of natural edible coatings to preserve chestnuts

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The chestnut tree (*Castanea sativa* Mill.), belonging to the genus *Castanea* and the family *Fagaceae*, is a species of environmental and food importance in Europe [1], mainly in the Trás-os-Montes region, in Portugal, where the orchards are more prevalent [2]. Its chestnuts have a short shelf-life, showing high product loss during storage due to the loss of weight and microbial degradation [3]. Therefore, there is a strong need to ensure the extension of chestnut´s shelf-life, guaranteeing the product´s quality [4]. The present work intended to understand the impact of several natural coatings for chestnuts, made from wax, chitosan, chitosan+rosemary, chitosan+wax, chitosan+wax+reosemary, and comparing them to a commercial coating. Individual fatty acids (using GC-FID) and the antioxidant activity (measured by the ability to inhibit the formation of thiobarbituric acid reactive substances) were analysed for three different storage times, namely 0 (T0), 7 (T1) and 21 (T3) days. Regarding the fatty acids, at T0, the wax coating revealed the highest contents of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, respectively: 1.61± 0.06 and 1.75 ± 0.007 g 100g dw; compared to the levels obtained by the commercial coating (1.39 ± 0.06 and 1.59 ± 0.02 /100g dw, respectively). For T1 and T3, the coating made with chitosan + wax showed more effective results than the commercial one (1.70 ± 0.005 g/100g dw and 0.65 ± 0.03 g 100g; 1.19 ± 0.01 and 1.94 ± 0.02 g/100g dw, respectively). The coating with chitosan + rosemary, showed the best antioxidant activity also for both T0 and T1, protecting the chestnut from oxidation, revealing the strongest EC_{50} values obtained (0.71 ± 0.01 mg/mL and 0.35 ± 0.03 mg/mL, respectively). In relation to T3, for the same coating, it was possible to observe an increase in the EC_{50} value (1.48 **± 0,05** mg/mL). Therefore, it can be concluded that all chitosan-based coatings were more effective after seven days. However, further studies are needed to determine which coating is best suited to preserve the other characteristics of chestnuts, namely the organoleptic ones.

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Application of *Cucumis melo* L. peel flour in bakery products

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Cucumis melo L. is a very consumed fruit all over the world because it has excellent sensory and nutritional qualities, being a good source of bioactive compounds for humans. However, the peel and seeds are usually discarded [1,2,3]. Food waste is considered a major problem with a direct impact on the economy, society and environment. The valorisation of these by-products could be an advantageous approach to face the increase in food waste since it can compromise the implementation of the concept of food sustainability. On the other hand, this valorisation would allow the development of new food products with beneficial properties for the health of the population.

The aim of this study was to develop two formulations based on *C. melo* L. peel flour and to evaluate their nutritional composition, total phenolic content and antioxidant potential.

In 2021, the *C. melo* L. samples were collected from melon production and distribution companies, located in Torres Vedras and Rio Maior (Portugal). The nutritional composition was analytically determined. The energy value and available carbohydrates were calculated. Antioxidant activity was determined using two different methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and ferric reducing antioxidant power (FRAP). Total phenolic content was also assessed by spectrophotometry and the results were expressed as gallic acid equivalents (GAE).

According to the results, dietary fibre and available carbohydrates are the main constituents of the *C. melo* L. peel flour, 50 and 24 g/100 g, respectively. For DPPH•, 26 mg trolox equivalents/100 g were observed, while for FRAP, the value obtained was 863 mg trolox equivalents/100 g. *C. melo* L. peel flour presented a content of 249 mg GAE/100 g, for total phenolic compounds.

The incorporation of *C. melo* L. peel flour allowed the development of a biscuit and a muffin with 15 and 13 g/100 g of dietary fibre, respectively. According to Regulation (EC) No 1924/2006 on nutrition and health claims, it is possible to say that the products developed can be considered high in fibre (> 6 g/100 g). In the case of the biscuit developed, the use of *C. melo* L. peel flour, allowed to have a content of total phenolic compounds of 250 mg GAE/100 g. Concerning the developed muffin, a total phenolic compounds content of 254 mg GAE/100 g, and 23 and 892 mg trolox equivalents/100 g for the DPPH• and FRAP method, respectively, were obtained.

In conclusion, *C. melo* L. peel flour can be considered a good source of dietary fibre and total phenolic compounds, allowing the development of two formulations.

The dietary fibre and phenolic compounds contents present in the developed food products can contribute to the valorization of *C. melo* L. peel flour since it is possible to nutritionally enrich different foods, reduce food waste, reduce the environmental impact and contribute to improving public health.

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Valorization of *Ficus carica* L. orchards subproducts: evaluation of antioxidant properties of fig tree leaves obtained by different green extraction approaches

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Figs' production has a high economic impact in several countries of Europe [1]. These fruits are consumed fresh or in the form of sweets and jams. However, from figs production results valuable subproducts, such as fig tree leaves, that are rich in bioactive compounds that can be used to increase figs' products functional value, in a sustainable, circular economy context.
In this work, five different varieties of figs tree leaves (*Ficus carica* L.) were supplied by a national producer – Quinta da Mó (Sesimbra, Portugal). The fig tree leaves were oven dried (40 °C), and grinded to obtain a fine powder. The analysis were conducted with a mixture of the five different figs varieties in equal proportions, responding to Quinta da Mó reality in terms of subproducts that are produced. Three different green extraction approaches were conducted to obtain antioxidant compounds from fig tree leaves, namely microwave assisted extraction (MAE), pulsed electric field-assisted extraction (PEF), and ultrasound assisted extraction (UAE). The solvents of extraction were ethanol and water in variable amounts with different times and temperatures depending on methodology. The antioxidant potential was determined by the ferric reducing antioxidant potential (FRAP), 2,2-diphenyl-1- picryl-hydrazyl-hydrate (DPPH) radical scavenging potential and the total phenolic content (TPC) evaluated by the Folin-Ciocalteu method.
The extracts obtained by MAE presented the highest polyphenolic content and the highest scavenging potential. The phenolic content ranged from 58 ± 2.19 to 100.55 ± 8.40 mg gallic acid equivalents per gram of extract, being the highest value obtained with 50:50 ethanol:water at 70 °C and 20 min of extraction. The DPPH radical scavenging potential ranged from 100.35 ± 1.82 to 351.20 ± 1.95 mM Trolox equivalents per gram of extract. The most active extract was obtained at 100 °C, during 20 min with 20% ethanol. Concerning the FRAP, the values ranged from 852.61 ± 34.59 to 2164.39 ± 79.97 μM $FeSO_4$ equivalents per gram of extract, being the highest obtained with 20 min of extraction, at 70 °C with 50:50 ethanol:water.
The results suggest that MAE is the most efficient methodology to extract antioxidant compounds from fig three leaves. This neglected subproduct revealed to be rich in antioxidant compounds, that can be further used to increase the entire figs chain products value, including the development of healthier functional products, within a circular economy context.

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Ficus carica L. 'Dauphine' leaves as source of antimicrobial compounds

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With worlds' population increase, it is necessary to develop sustainable strategies to increase food production maintaining security and quality standards. It is mandatory to create smart strategies to reduce food waste, but also to add value to currently neglected by-products [1]. Fruit is in the basis of a healthy diet, being mostly consumed fresh or in the form of sweets and jams. Figs are consumed worldwide, having a high economic relevance in several European countries [2]. This work aimed to add value to subproducts resulting from figs tree pruning – fig three leaves, by evaluating their antimicrobial potential. Fig tree leaves (*Ficus carica* L.) Dauphine variety were supplied by a national producer – Quinta da Mó de Cima (Sesimbra, Portugal). Fig tree leaves were oven dried (40 °C), grinded to obtain a fine powder, and subjected to microwave assisted extraction (MAE). The extraction conditions varied in time of extraction (10, 20 min), temperature (40, 80 and 100 °C) and different proportions of ethanol and water (20%, 80% ethanol). After extraction, the extracts were dried under rotary evaporation (< 40 °C) and resuspended in DMSO at 100 mg/mL to perform the antimicrobial studies.
All extracts were evaluated for their potential to inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The antimicrobial activity was determined by spectrophotometric analysis (OD 600nm) and the anti-biofilm properties were evaluated by the crystal violet staining method.
Extracts obtained with 80% ethanol presented the highest inhibitory activity against all studied microorganisms, inhibiting about 30% *P. aeruginosa* and *C. albicans* growth, and 20% *S. aureus* growth.
Several extracts presented anti-biofilm properties, however, the extract obtained with 80% ethanol, 20 min at 80 °C showed the highest potential to inhibit *C. albicans* and *P. aeruginosa* biofilms (≈ 50% and 40% inhibition, respectively, at 125 μg/mL).
These results reveal that fig tree leaves contain valuable bioactive compounds with antimicrobial properties against Gram-positive, Gram-negative bacteria and fungi, that can be further explored as natural food preservers.

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Biovalorisation of agricultural by-products obtained through green extraction methodology

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The world's population, determined by the United Nations, is currently around 8 billion people, with an expected growth of another 2 billion by the end of 2100, resulting in a necessary increase of approximately 80% of world food production to meet the population's needs [1, 2]. The result of this increase in production is the intensification of food waste and loss, which negatively affects the environment [3]. Worldwide, the rate of wasted food reaches 17%, of which roots, fruits, vegetables, and oilseeds represent circa 40 – 50% [3]. Therefore, many studies have been conducted to find methodologies capable of adding commercial value to waste products [4]. Residues and by-products have several bioactive ingredients, such as phenolic compounds, carotenoids and vitamins [5].

The present work aims to optimise, in terms of the overall extraction yield, the extraction conditions using a sustainable and green technology, SFE-CO_2 (supercritical fluid extraction using carbon dioxide) [6], for agricultural by-products of olive pomace, olive leaf, white onion and collard. The extracts bioactivities, including antioxidant, anti-inflammatory, and cytotoxicity are evaluated. These results are of interest for the nutraceutical and food industries since they can be related to the bioactive compounds extraction amount with functional properties [7].

Extractions were carried out using a 1L extractor with a pressure range of 80, 90 and 100 bar, at constant temperature (50 °C) and for 2 h. The chemical composition of the extracts was evaluated by liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), and toxicity evaluated using a non-tumour cell line.

In general, higher pressures result in higher extraction yields, which favours the extraction of the active compounds. Concerning the chemical composition determined by GC-MS, palmitic acid, linolenic acid (compound only present in white onion), linoleic acid, stearic acid, oleic acid, squalene and vitamin E are the main compounds present.

Antioxidant (EC50, TBARS), anti-inflammatory (IC50, RAW 264.7 rat macrophages) and cytotoxicity (GI50, non-tumoral pig liver and renal cells) activities were determined with specific methodologies. The results show interesting antioxidant activity for white onion and collard extracts (33.4 ± 1.4 and 10.7 ± 0.2 mg/mL, respectively) and anti-inflammatory results for olive pomace extract (390 ± 13 μg/mL). Additionally, except for the olive pomace, the obtained SFE-CO2 extracts did not show cytotoxicity. As future work, the extracts can be incorporated (in free or stabilised forms) into food matrices to increase the bioactive compound content and thus produce fortified fruits and vegetable products with natural functional properties.

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Recovery of phenolic compounds from grape pomace after different defatting processes

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The global wine production generates enormous quantities of waste (grape pomace). It is at an inflection point today, since the sustainable development of industry is the major challenge globally. The extraction of valuable organic compounds from the pomace could be the best way to fulfil the demands of circulatory economy. Nevertheless, processes for making white and red wine are not identical; consequently, chemical compositions of pomaces left after winemaking are subtly different [1].

The aim of the present study was to explore the differences between white and red pomace regarding total polyphenol content, ascorbic acid content and antioxidant potential. In addition, the impact of defatting process on these parameters was assessed.

For that purpose, samples obtained after making white and red wine were lyophilized and then defatted by chloroform using conventional Soxhlet method and by supercritical CO_2. Folin-Ciocalteu spectrophotometric assay was used for estimation of total phenolics [2]. Ascorbic acid content was determined by 2,4-dinitrophenylhydrazine method [3]. Four different tests (FRAP, DPPH, ABTS and CUPRAC) were done with intention to get the most comprehensive information about antioxidant activity [4].

Total phenolic content ranged between 16.51 and 21.07 mg GAE/g dry weight. The most potent was red pomace left after Soxhlet method for isolating oil, followed by white pomace defatted using the same protocol (20.04 mg GAE/g dry weight). Contrary, samples processed with supercritical CO2 had higher ascorbic acid content in comparison with Soxhlet method (183.07 and 146.92 versus 68.15 and 126.94 mg/100g of dry weight, for white and red pomace, respectively). All four antioxidant assays derived similar results and were strongly correlated with TPC ($p<0.05$), while the correlation with ascorbic acid content was absent.

Finally, it can be concluded that defatting process has a strong impact on investigated parameters unrelated to grape pomace color. Meanwhile, Soxhlet method resulted in higher oil yield, lefting behind defatted pomace rich in polyphenols. Oppositely, supercritical CO2 managed to extract less oil, but the pomace had higher ascorbic acid content, probably because this process does not require high temperatures on which vitamin C can be deactivated.

Keywords: *grape pomace, Soxhlet extraction, supercritical CO_2 extraction, total phenolic content, ascorbic acid content, antioxidant activity*

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Fig Residue as a Novel Source of Bioactive Molecules: A Sustainable Integrated Project

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The residue of fig production is a rich source of bioactive molecules that could be used for several purposes in the food, pharmaceutical and cosmetic industries. The production of bioactive molecules from these residues could lead to a wide variety of novel products. The application of these products to various industries could contribute significantly towards circular economy. The development of a project to evaluate the potential of the use of fig production residue as a source of commercial products would therefore be beneficial for the development of a country's economic well-being. The “100% Figo” project was conceived with this subject in mind strongly inspired by the principles of the circular economy.

Tagusvalley TGV - Association for the Promotion and Development of the Tejo Valley Technopole and BLC3 - Technology and Innovation Campus, both private R&D facilities, are part of the 100% Fig Project. Additionally, there are two public institutions of higher education—the Polytechnic Institute of Bragança and the Polytechnic Institute of Leiria —as well as the private company Quinta da Mó de Cima, which currently cultivates the fig varieties Bourjassote Noire, Pastelliere, Longue d'Aout, Dauphine, and Pastelliere (Fig.1). The project's main objective is to use bioresidues (leaves, peels, and damaged fruits) from the production and processing of figs to create new, wholesome food products while advancing towards sustainability contributing to circular economy. The first phase is the identification of putative preservative and bioactive compounds contained in the bioresidues of the five fig species using high performance chromatographic methods. To determine the ideal extraction conditions, these molecules will be recovered using pulsed electric fields, high hydrostatic pressure, ultrasound, and microwave-assisted extraction. After possible toxicity has been ruled out, the molecules and extracts will be used to preserve a variety of foods, including jams and other sweets made from fruit. This will allow researchers to look at the stability over a shelf-life in terms of chemical and physical properties, were stability, preservative, healthfulness (functional ingredients and sugar reduction), and sensory assessments are included.

Fig.1. Leaves and fruits of the studied varieties

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Chemical and functional characterization of clean-label food emulsion with microalgae added to the aqueous phase

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Functional foods have been specifically designed to provide health benefits beyond their basic nutritional value. They are often fortified with additional nutrients, or may contain natural compounds with potential, positive effects on health. Following this new trend, in recent years, food manufacturers began developing new products fortified with additional nutrients or containing natural bioactive compounds that may promote general health and wellbeing. Numerous scientific publications evaluated the potential of microalgae to be used as ingredients in this context [1]. This strategy makes sense as microalgae biomass is rich in bioactive compounds, namely as proteins, long-chain polyunsaturated fatty acids, vitamins, minerals, pigments, and phenolic compounds, as well as many others.

The present study focuses on creating an innovative formulation of vegan and clean-label emulsion from chickpea protein and *Chlorella vulgaris* (C. *vulgaris*). These emulsions display better nutritional/functional qualities, and their demand is ever growing in the food industry due to the popularization of healthier, plant-based alternative products [2, 3]. In the current work, the emulsions were prepared with either 4 % of organic *Chlorella* (autotrophic-dark green), or the heterotrophic Hon-ey and White *Chlorella* variants. The microalgae biomass was produced and supplied by Allmicroalgae (Portugal), derived from either autotrophic or heterotrophic culture processes, and differ in pigmentation and biochemical composition. The different culturing methods applied to *C. vulgaris* had an impact on the appearance and bioactive composition of the emulsion. Beyond the desirable development of different colors, promoted by the presence of pigment in microalgae biomasses, which turned them into intense green-yellow tonalities, an enhancement of the nutritional profile is also expected. The latter conclusion is based on previous works, developed by the same team and by using the same microalgae biomass. In some cases, the content of protein, calcium and iron was higher enough to fully meet Reg. EC 1924/2006 requirements for nutrition claims made on foodstuffs [4]. Additionally, these emulsions would represent a sustainable alpha-linolenic acid (18:3 ω-3; ALA) source, suitable for vegetarians, vegans and non-fish eaters. Additionally, the authors have also determined an interesting amount of vitamin B12 in the aforementioned biomasses, namely around 200-990 μg/100 g of sample. Moreover, changes in textural and rheological properties are expected, considering the presence of a substantial amount of protein and polysaccharide in the microalgae’s biomass.

The use of these emulsions as carriers for healthy ingredients, such as colorings, with antioxidant and other beneficial properties, is therefore a subject of significant interest.

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Lipid profile of fish by-product oils obtained by ultrasound-assisted extraction

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The fishing industry produces large quantities of fish by-products every year, currently little explored by the agri-food sector. However, the upcycling of these natural resources has been promoted by the generally high lipid content they have and by the emergence of more sustainable extraction techniques, but still little explored to obtain lipid fractions from fish by-products [1].

Therefore, this work aimed to obtain oils from fish by-products by ultrasound-assisted extraction (UAE) and to characterize their lipid profile. Category 3 fish by-products supplied and considered standard by the processing industry (ETSA Group) were freeze-dried and reduced to a fine powder. Five samples were processed by ultrasound-assisted extraction at different powers (125 to 500 W) for 5 min. The oil yield was determined gravimetrically and the fatty acid profile was analyzed by gas chromatography with flame ionization detection (GC-FID), after a derivatization process performed to obtain fatty acid methyl esters.

The lipid profile consisted mostly of monounsaturated fatty acids, due to the high levels of oleic acid. It also present high amounts of the polyunsaturated eicosapentaenoic acid (EPA) and of the unsaturated palmitic acid. These lipophilic constituents were not significantly affected by the different UAE powers applied to the fish by-product samples. These oils will be of interest for animal feed formulation.

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Development of an ice cream enriched in derivatives of winemaking by-products

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The wine industry is one of the main agro-industries in the world. Due to its worldwide dimension, this activity results in the production of a large amount of organic by-products. As Europe is the region responsible for most of the world's wine production, it is estimated that this sector, in this region, generates about 14.5 million tons of by-products annually [1]. In recent years, within the framework of a circular economy, science has sought to respond to the need to reduce food waste through the valorization of by-products, with little or no commercial value. Oenological by-products are no exception and their potential as a source of functional ingredients is evident due to their phytochemical profile and associated properties. Grape pomace is the main by-product of wine production, consisting of seeds, skins, stems and sometimes even pulp, representing a waste of around 214 thousand tons annually in Portugal [2]. This by-product, despite the steps of maceration/pressing and subsequent fermentation of the grapes in the winemaking process is still a source of phenolic compounds, associated with color properties, antioxidant [3] and antimicrobial [4] potential. On other side, ice cream is a dairy product worldwide consumed and is, generally, low in natural polyphenols and antioxidants. Moreover, there is a current demand from food companies for natural sources of colored compounds, antioxidants and antimicrobials, meeting consumer demands. In this context, the aim of this work was the reuse of red grape pomace, through the preparation of phenolic fractions and study of the impact of its incorporation on the physicochemical properties of ice cream formulations, guaranteeing commercial viability.

Samples of grape pomace from Vinhão grape variety were harvested in 4 geographic locations and screened for their potential to serve as functional food ingredient. Hydroethanolic extracts of each sample were estimated regarding the content of total phenolic compounds (TPC-Folin-Ciocalteu method), total monomeric anthocyanins (TMA-differential pH method) and antioxidant potential (DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ scavenging). The most promising extract, obtained from the pomace sample of grapes harvested in Lousada, presented a TPC content of 67.0±3.9 GAE (gallic acid equivalents) mg/g, a TMA content of 18.0±0.4 ME (malvidin equivalents) mg/g, and antioxidant potential of 7.8±0.6 TE (trolox equivalents) mg/g and 10.2±2.7 TE mg/g of dry extract, for DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ scavenging, respectively. This extract was studied in terms of their individual phenolic compounds by HPLC analysis and further purified using Molecular Imprinted Polymers (MIP's). The extract, as well as its MIP's purified fractions, were used as ingredients in ice cream formulations, at different concentrations.

The use of the extract as ingredient, in various concentrations, interfered in the physical and chemical properties of the ice creams. In particular, the addition of the extract, both freeze-dried or liquid, led to a decrease in the overrun and an increase in the melting rate. The acidification of the ice cream matrix caused by the addition of the liquid extract, improved the stability of the anthocyanins, which resulted in a greater intensification of the red and blue tones, according to the CIELAB color scale, compared to the addition of the freeze dried extract. The richness of the extract in phenolic compounds resulted in the increase of the antioxidant properties of the ice cream, which makes these grape pomace derivatives potential functional ingredients and preservatives for application in the ice cream industry.

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POSTER PRESENTATIONS

T3

Novels foods

Impact of 15% *Arthrospira platensis* (Spirulina) inclusion combined with/without enzymes on breast's meat quality in broilers

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The urgent need of finding novel protein feed sources to fulfil the worldwide necessities makes microalgae a good alternative to common feed sources like soybean [1]. Notwithstanding, its recalcitrant cell wall hinders the nutrients digestibility [2]. Thus, the use of pre-treatments, like enzymatic ones, could be a good option to improve that digestibility and accessibility [3,4]. In this *in vivo* trial, we studied the effects of 15% inclusion of *Arthrospira platensis* (Spirulina) combined or not with enzymes in broilers´ **diet for 35 days** on breast meat quality. From d 7 to 35, the chickens were fed four different diets: a corn-soybean basal diet (control diet), a basal diet containing 15% of *A. platensis* (SP), a basal diet containing 15% of *A. platensis* with 0.025% of a commercial enzyme, VemoZyme P (SPV), and a basal diet containing 15% of *A. platensis* with 0.10% of a recombinant enzyme, porcine pancreatin (SPP). Breast meat of broilers fed *A. platensis* with or without combination of enzymes showed higher values of total carotenoids ($P < 0.001$). Also, chlorophyll *a* significantly increased ($P = 0.033$) when the chickens were fed *A. platensis* in combination with commercial enzyme, whereas chlorophyll *b* ($P = 0.001$) was higher when *A. platensis* was given to animals, with or without enzymes. The incorporation of microalga significantly decreased total lipid content ($P = 0.001$), polyunsaturated fatty acids (PUFA) ($P = 0.001$) and *n*-6:*n*-3 ratio ($P < 0.001$), in comparison with control diet. The *n*–3 PUFA significantly increased ($P < 0.001$) with *the* incorporation of microalga, especially C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3, whereas *n*-6 PUFA significantly decreased ($P < 0.001$), mostly C18:2*n*-6. Concerning, saturated fatty acid (SFA), C16:0 was increased ($P < 0.001$) with the inclusion of *A. platensis*. Nevertheless, total cholesterol and α–tocopherol significantly decreased ($P < 0.001$) with incorporation of *A. platensis*. For oxidative lipid stability, after 8 days of breast meat storage at 4 ºC, there were no significant differences between the control and microalga groups ($P > 0.05$). In conclusion, the incorporation of 15% *A. platensis* in broiler diets, improved breast meat nutritional quality in terms of total carotenoids and *n*-3 PUFA, although further research is needed to enhance the efficiency of enzymatic feed supplementation to disrupt the cell wall and release some bioactive compounds.

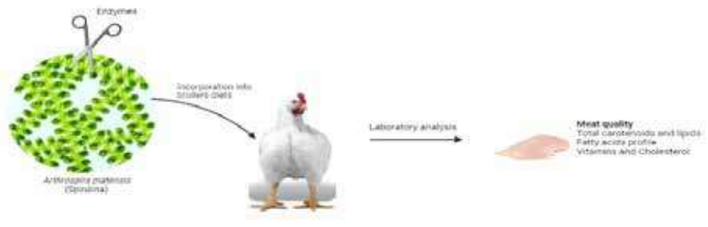

Figure 1. Impact of feeding 15% *Arthrospira platensis* combined or not with enzymes on breast meat quality in broilers (Created in BioRender.com).

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Effect of alginate-based edible coatings enriched with *Origanum vulgare* L. essential oil on the shelf-life of biological apples

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Food waste is a major concern of the food industry and strategies are continuously being developed to respond to this problem by increasing the shelf life of products. On the other hand, it is also crucial to propose the valorization of agri-food by-products and residues through the development and implementation of different methods for extracting active substances at an industrial level, given their value in the agri-food chain as new functional ingredients.

In this context, edible coatings constitute continuous matrices having the potential to increase the shelf-life of foods. They can be formed by proteins, polysaccharides, and/or lipids which will protect the food from changes in moisture, exchange of oxygen, and loss of color, flavor, and texture. Moreover, these edible coatings can be applied incorporating bioactive compounds such as those contained in some essential oils to provide microbiological stability contributing to the extension of the product shelf-life by reducing the growth risk of pathogenic bacteria and spoilage flora on food surfaces [1].

Within the different essential oils that can be used, this work focused on the use of *Origanum vulgare* L. (oregano) as a source of essential oil with bioactive properties to be incorporated in alginate-based edible coatings in apples. Two concentrations of oregano essential oil (OEO) were used in the edible coating formulations (0.50% and 0.75%) as these were the conditions that yielded better preliminary results. The apples are being monitored in terms of visual appearance (firmness and color features) [2] and aromatic profile.

Regarding the visual analysis, the edible coating formulation with 0.50% OEO seems to better preserve the apples than those treated with 0.75% OEO which started to show brown spots. The results obtained so far seem to indicate a putative impact on the aromatic perception of the apples, since some aromatic compounds from OEO were already detected in the apple flesh. This will be confirmed in the end of the study by a sensory panel evaluation.

This work is included in the BIOMA Project – "Bioeconomy integrated solutions for the mobilization of the Agro-food market" brings together a broad consortium of national entities in the agri-food sector that intends to move companies from the agri-food value chain to more competitive and sustainable levels, promoting strategies and an environment that enhance the adoption of integrated Bioeconomic solutions.

Fig.1. Biological apples with alginate-based edible coatings enriched with *Origanum vulgare* L. essential oil.

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Nisin-Loaded Fucoidan Particles: Preparation and Characterization

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Nisin is a cationic 3.5 kDa peptide produced by *Lactococcus lactis* subsp. *lactis* and used in the food industry against Gram-positive bacteria. Sensitivity to environmental stresses, susceptibility to proteolysis, and undesirable interactions with food components can decrease the biological activity of nisin in food products. To overcome those drawbacks and ensure antimicrobial activity, nisin can be encapsulated using lipids, proteins, or polysaccharides [1]. In this study, fucoidan was used for the synthesis of particles. Fucoidan is an anionic, sulfated polysaccharide found in brown seaweed (e.g., *Fucus vesiculosus*, *Macrocystis pyrifera*, *Laminaria japonica*). Due to its antioxidant, antibacterial, antiviral, antiobesity, antiallergy, anticancer and anticoagulant properties, the use of fucoidan has increased in various medicinal and biological fields [2, 3].

Nisin-fucoidan particles were synthesized by a simple and cost-efficient complexation method at different pH values ranging from 4.0 to 7.0 (Fig. 1).The concentration of nisin ranged from 0 to 1 mg/mL and the fucoidan concentration was 0.4 mg/mL. FTIR, UV, and DLS methods were used to confirm the interaction of components. Studies of the particle stability were based on the measurement of particle size and loading efficiency during the storage.

The resulting particle size was in the range of 200–600 nm at all pH values, when the concentration of encapsulated nisin varied from 0 to 0.6 mg/mL. A sharp increase in the size and the agglomeration of the particles was observed when the concentration of nisin was further increased. After the storage of particles for 4 weeks at +4 °C, the encapsulation efficiency and particle size changed insignificantly. It can be concluded that the particles are stable. The antimicrobial efficiency of nisin-loaded fucoidan particles was evaluated against model Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) and Gram-positive (*Bacillus subtilis* and *Listeria innocua)* bacteria using agar-diffusion method as well as viability assay. The highest antimicrobial activity was observed against *B. subtilis*. *S. typhimurium* was more resistant to the action of functionalized particles. The antimicrobial effect of nisin-fucoidan particles was the lowest against *E. coli* and *L. innocua* bacteria. The developed complexes have the potential for the application as biopreservatives in the food industry.

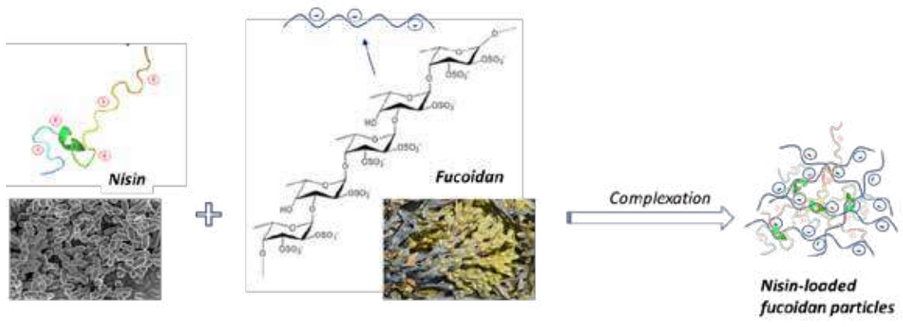

Fig.1. The scheme of nisin-loaded fucoidan particles formation

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Production and functional characteristics of low-sodium high-potassium soy protein for the development of healthy soy-based foods

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The plant-based products that are mainly produced by soy protein isolate (SPI) present significantly higher sodium (Na) content than the corresponding animal-based products. Accordingly, the production of low-sodium soy protein ingredients becomes a challenging task. For this purpose, alternative soy fractionation processes were investigated, and the use of KOH as the replacement for NaOH has been established to produce soy protein fractions (SPFs). The obtained MF-K contained 0.2 mg sodium and 24 mg potassium per 100 g of fraction, which was 3 % of the sodium content in the SPI, and the potassium content was over 10 times higher than SPI. Besides, using KOH increased the protein content of SPFs by almost 7 %, as well as their water holding capacity (WHC) and thermal stability; however, the yields of SPFs were dropped by around 4–8 % while the protein solubility of SPFs was reduced companied with the application of KOH. The fractionation processes mainly affected the protein composition, powder morphology, and viscosity of SPFs, while the sodium and potassium content showed limited impacts on the variations. Overall, the application of KOH during different fractionation procedures provided the possibility to produce low-sodium high-potassium soy protein ingredients for the development of healthy soy-based foods.

Cellulose/saccharide delivery systems of raspberry phenolics

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Raspberries are fruits known for their pleasant flavour and significant amount of bioactive compounds [1]. Such compounds include polyphenols with proven health benefits that need to be protected from extreme environmental conditions (high temperatures, the presence of light and oxygen). In order to achieve polyphenols stability, different polysaccharides are utilized as carriers of polyphenols [2]. In this work, cellulose was used as a carrier of raspberry polyphenols in combination with glucose, maltose, sucrose or trehalose with the aim to examine saccharides impact on encapsulation of polyphenols. The samples were prepared by freeze-drying and different ratios of cellulose:saccharide were used (1:0.5 or 1:1) for complexation while the amount of polyphenols source was constant. The spectrophotometric methods for evaluation of total polyphenols content and antioxidant activity were performed as well as HPLC (high-performance liquid chromatography) method for identification and quantification of individual polyphenols. The results of total polyphenols content showed that the sample with cellulose:glucose 1:0.5 ratio had the highest values (1376.23 mg/kg), while sample with cellulose:trehalose 1:1 ratio had the lowest values (1191.65 mg/kg). The same trend was observed for antioxidant activity values obtained by DPPH, ABTS and FRAP methods. HPLC results showed presence of three anthocyanins in samples (cyanidin-3-sophoroside, cyanidin-3-glucoside and cyanidin-3-rutinoside) as well as quercetin, ellagic acid and its derivates. Results of spectrophotometric and HPLC analysis showed that type of saccharide and its ratio to cellulose had impact on encapsulation of raspberry polyphenols. The FTIR-ATR analysis was conducted to confirm the complexation of polyphenols with applied carbohydrates. The results of this study are significant for the food industry and the development of novel food ingredients enriched with bioactive compounds.

Fig.1. Graphical abstract

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Microencapsulation of volatile compounds from chokeberry juice into alginate/pullulan hydrogel beads

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Microencapsulation is a rapidly evolving technology that is defined as a process of isolation of an active ingredient from the external environment using a liquid or a solid barrier. This process is employed to treat volatile compounds to impart some degree of protection against evaporation, reaction or migration in foods [1]. In addition to altering the organoleptic properties of the food in which they are found, volatile compounds also possess remarkable biological activities that make them attractive for the food, cosmetic and pharmaceutical industries. Production, storage, packaging materials, and the presence of oxygen, light, moderate temperature or other ingredients in the products can cause changes in the composition of volatile compounds including a complete loss of the product's flavour. The abovementioned obstacles can be overcome by the process of microencapsulation i.e. preparation of hydrogel beads. One of the commercially available encapsulators in the production of hydrogel beads is BÜCHI Encapsulator B-390 [2]. In the present study, chokeberry juice was used as a source of volatile compounds and alginate (3.75%) or alginate (3.75%) with pullulan (0.5%, 1%, 1.5% and 2%) were used as wall materials for the preparation of hydrogel beads by encapsulator under constant conditions (1000 **µ**m vibrating nozzle, pressure 200 mbar, frequency 200 Hz, electrode 1000 V). Volatile compounds were determined using GC-MS analysis. Detected volatile compounds were divided into 5 groups including acids, alcohols, carbonyl compounds, terpenes and esters among which alcohols were present in the highest concentration in all prepared samples. Samples with the addition of 1.5% of pullulan in the wall material had the highest concentration of terpenes (94.78 **µ**g/100 g) such as β-citronellol, and also the highest concentrations of carbonyl compounds (102.20 **µ**g/100 g) and esters (56.93 **µ**g/100 g). The results showed that the composition of hydrogel beads depended on the wall material composition.

Fig.1. Graphical abstract.

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Application of organic sunflower cake to composite flour and effect on the properties of the dough and the fiber content of the bread

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Enriching bakery products is a promising way to increase their nutritional value and make them more attractive to consumers [1]. Daily fibre intake should range from 17g (children aged 7-11) to 26g (men aged 19-34) [2], however bread made from wheat flour, or wheat rye flour is poorer in fibre content [3]. As part of the research, homogenized organic sunflower cakes obtained as a secondary residue after pressing sunflower oil were applied to wheat rye flour. It is one of the options for the recovery of by-products and the sustainability of food production [4]. The effect of the addition of 5%, 10% and 15% on the rheological properties of the dough (Mixolab) and objective (Volscan) and sensory properties of the experimental bread was evaluated. The addition of sunflower cake reduced water absorption of composite flour and dough yield, but extended dough development time, which must be considered when setting the kneading mode in the technological process. The addition of sunflower cake also affected the rate of gelatinization of starch and weakened the protein structure in composite flours. The fermentation activity of the dough was not fundamentally affected by the additions, which we perceive positively. The amount of sunflower cake added negatively affected the volume of experimental breads, however, breads with an addition of up to 10% were sensorily acceptable. The consumption of bread (10% sunflower cake in composite flour) at a rate of 150 g per day (recommended amount) [5] covered the daily fibre requirement at 26.5% to 38.3% (Fig. 1). In addition to its contribution from the point of view of the circular economy, the application of by-products of the food industry is also potentially beneficial from a nutritional point of view and from the point of view of providing sufficient sources of valuable substances, e.g., fibre, in the nutrition of the population.

Fig.1. Ensuring the daily fibre requirement by consuming bread with the addition of sunflower cake of 10%

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Obtaining of FOS by controlled hydrolysis of inulin with *Aspergillus welwitschiae* FAW1 endoinulinase

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Most of the functional oligosaccharides (OS) consist of monomers, present in varying degrees of polymerization (DP) ranging between 3 and 10 units [1]. DP of inulin-type fructooligosaccharides has a great impact on fermentability and their utilization by probiotic bacteria such is Bifidobacteria, thus they have a great impact on their health-promoting effect [2]. Technological properties of fructooligosaccharides (FOS) can improve the physicochemical and sensory characteristics of food products, leading to their increased application in the food industry [3,4]. It has been found that microbial endoinulinase plays an important role in production of inulin-type fructooligosaccharides. *Aspergillus welwitschiae* FAW1 strain has proven to be non-toxigenic with the absence of biosynthetic gene clusters for mycotoxins (ochratoxins and fumonisins) and therefore safe for use in food production [5]. Growing on the natural substrate, triticale (*Triticosecale* sp) FAW1 strain produced inulinase complex from which endoinulinase (InuA) was purified by chromatographic techniques. FOS was prepared by time-controlled hydrolysis of inulin. Monitoring kinetics and determining the amount of obtained FOS by TLC and HPLC methods led to a conclusion that FOS production by hydrolysis of inulin is kinetic dependent reaction. Depending on the reaction time, FOS with different compositions are obtained.The largest amount of produced FOS (DP 2-6) has been in 15-20 minutes of the reaction, where the resulting mixture contains small amount of mono- and disaccharides. The obtained FOS were characterized on antioxidant capacity. Produced FOS showed significant antioxidant potential according to ORAC method which classifies them as potent candidate as additives in functional food. Endoinulinase (InuA) form *A. welvitscihae* FAW1 considered as key enzyme in FOS preparation. The composition and lenght of the produced FOS can be varied by controlling the reaction time, depending on the needs of of the market and their eventual application.

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Use of oil-seed proteins for the microencapsulation of chokeberry and sea-buckthorn pomace extracts

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Anthocyanin-rich chokeberry pomace from wine production and polyphenol and carotenoid-rich sea-buckthorn pomace from juice production are valuable raw materials with high potential as natural pigments with several health benefits. Microencapsulation is commonly used for entrapping bioactive compounds (core material) within specific wall material forming microcapsules. This process protects the core material from degradation and oxidation, enhances its stability, and ameliorates its release and bioavailability. Thanks to their interesting functional properties, proteins of animal origin (whey proteins, casein, gelatin) are widely used as wall material for encapsulation. To respond to the increasing demand for more sustainable plant-based products, plant proteins (soy and pea protein) have recently found use as wall materials as well.

This study aims to investigate the use of protein concentrates recovered from the oil-seed cake of hemp, rape, and flax together with maltodextrin (MD) as wall material for the microencapsulation of chokeberry and sea-buckthorn extract using spray-drying. First, we evaluated the functional properties of different protein concentrates by measuring solubility, emulsifying, and foaming properties. Results showed that flax seed protein had the highest emulsifying capacity, while rape seed protein exhibited excellent foaming capacity. These three oil seed proteins and whey protein were used as wall material with MD. Pomace extracts (60 mL, 1.3 g dry matter) were mixed with a protein solution (80 mL, 2 – 3 g dry matter) and MD (3 g), homogenized and spray-dried at 190°C, 13% pumping speed, and 50% drying airflow. Anthocyanins and total polyphenols encapsulation yield and efficiency, particle size distribution, particle morphology, and colour of the obtained powders were analysed. Results showed that adding oil seed proteins to MD resulted in a high encapsulation yield and efficiency comparable to the results obtained with whey protein. Particle size distribution analysis showed a difference in the median particle size of powders obtained with chokeberry (Dv50 between 2.8 – 7.1 µm) and sea-buckthorn (Dv50 between 5.8 – 17.3 µm) extracts. We also observed that the hemp and rape seeds protein mixture with MD resulted in a relatively higher median particle size distribution value than the flax and whey protein mixture. Further experiments are conducted to determine encapsulated chokeberry and sea-buckthorn extract storage stability.

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Effect of 15% Spirulina incorporation with commercial peptidases supplementation on colour and sensory breast meat profile in broilers

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Spirulina (*Arthrospira platensis)* is a microalga, particularly rich in proteins, lipids, minerals and pigments [1]. It is a good alternative to conventional feedstuffs, such as soybean meal [2]. Spirulina has a recalcitrant peptidoglycan cell wall that is mostly indigestible by poultry, therefore reducing nutrients' digestibility [3]. The use of exogenous peptidases represents a promising strategy for disruption of microalgae cell walls [4]. The objective of the current study was to investigate the effects of Spirulina as feed ingredient in broiler diets, combined or not with commercial peptidases in order to improve meat quality. Birds were fed 4 different diets from d 7 to d 35, as follows: a corn-soybean basal diet (control group), a basal diet containing 15% of Spirulina (SP), a basal diet containing 15% of Spirulina plus 0.025% VemoZyme P (SPV), and a basal diet containing 15% of Spirulina plus 0.10% porcine pancreatin (SPP). In what results concern, the yellowness (b*) score presented higher values in breast from birds fed Spirulina diets ($P < 0.001$). In addition, Spirulina promoted a significant increase on the accumulation of carotenoids in breast meat, which was associated with yellow colour development. In contrast, the pH value on the control group was higher compared to the Spirulina group. Off-flavour was not affected by dietary treatments. Meat tenderness from birds fed SP and SPV diets was less intense than the one observed for control birds ($P <0.001$). The juiciness of breast meat decreased ($P < 0.001$) with microalgae treatments, relative to control. All breast meat displayed positive scores (> 4) in overall appreciation. However, meat from non-supplemented birds exhibited higher overall appreciation (see Table 1). In conclusion, the incorporation of 15% Spirulina in broiler diets, individually or combined with exogenous peptidases, decreased pH, caused a modification in meat colour, from pinkish to yellowish, and did not meat sensory analysis scores.

Table 1. Meat quality and carcass traits of broilers' breast.

Breast	**Control**	**SP**	**SPV**	**SPP**	**SEM**	**P-value**
pH 24h	5.81^{a}	5.57^{b}	5.55^{b}	5.56^{b}	0.034	<0.001
Colour						
Lightness (L*)	59.8	57.7	58.2	58.9	0.983	0.453
Redness (a*)	4.40	4.99	4.86	5.03	0.360	0.592
Yellowness (b*)	9.20^{b}	20.9^{a}	21.4^{a}	22.9^{a}	0.730	<0.001
Sensorial traits						
Tenderness	6.04^{a}	5.00^{b}	5.31^{b}	5.63^{ab}	0.186	<0.001
Juiciness	5.45^{a}	4.56^{b}	4.44^{b}	4.61^{b}	0.157	<0.001
Flavour	6.01^{a}	5.48^{b}	5.59^{ab}	5.69^{ab}	0.121	0.013
Off-flavour	0.096	0.196	0.151	0.201	0.071	0.684
Overall appreciation	5.85^{a}	4.92^{b}	5.06^{b}	5.31^{ab}	0.153	<0.001

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Highly active endo-pectinase from *Aspergillus tubingensis*: A novel enzyme for fruit processing

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Pectinases are a type of enzymes frequently used in the food industry to clarify, liquefy, and stabilize fruit juices [1]. The main challenge in fruit juice production is the cloudiness of the juice, which is largely caused by the presence of pectic polysaccharides. Endo-pectinases are enzymes that hydrolyze the glycosidic bonds in pectic polymers [2]. Commercial pectinolytic enzymes are typically produced by fungi, with *Aspergillus* spp. being the most commonly used [3].

The aim of this research was the production and characterization of a novel endo-pectinase from the *Aspergillus tubingensis* strain for use in liquefying and clarifying different types of fruit juice. To accomplish this, solid-state fermentation was conducted on agricultural waste, such as sugar beet pulp and wheat bran, to produce pectinolytic enzymes. The resulting crude extract was concentrated *via* ultrafiltration and used to isolate the endo-pectinase *via* ammonium sulfate and ethanol precipitation methods. Ion-exchange chromatography technique on DEAE Sephadex A-25 matrix was used for further purification of the endo-pectinase.

The purified enzyme was characterized by the determination of total pectinolytic activity, specific pectinolytic activity, and SDS-PAA gel electrophoresis. The activity of the endo-pectinase was confirmed by a diffusion test and zymography with Ruthenium Red visualization. The resulting enzyme was used to liquefy apricot, banana, apple, quince, strawberry, and orange pulp, with juice yields ranging from 71% to 83%, depending on the fruit used. The juices treated with endo-pectinase showed much higher clarification compared to untreated juices. Additionally, the treated juices demonstrated more pronounced antioxidant properties, as determined through the DPPH assay.

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Highly active xylanase used in juice clarification

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Xylan makes a significant part of cereals and fruits, which are used in the food industry. Therefore, enzymes that hydrolyze xylan (xylanases) have found application in the modification of cereal-based food, improving the digestibility of animal feed, and improving the texture of bakery products[1]. In the juice industry, the main problems are turbidity, viscosity, and sedimentation during standing, which are caused by polysaccharides present in fruit (pectins, cellulose, and hemicellulose (xylan))[2]. Pineapple, apple, orange, and tomato have a high content of hemicellulose, so xylanases are suitable for improving the properties of these juices[3,4]. The *Aspergillus tubingensis* FAT 35 strain (considered safe for use in the food industry) growing on SSF medium composed of corn cob produced a high level of xylanase enzyme (4.03 U⁄mL) and not that high pectinase (1.02 U⁄mL) and cellulase (1.43 U⁄mL) activities at pH 3 which is pH of freshly prepared apple, pineapple and organge juice.. The fermentation extract was used for clarification of pineapple, apple, and orange juice and for increasing the filtration rate and yield of these juices. Results indicate that *A. tubigensis* xylanase could be used for clarification and improvement of properties of juices of fruits that contain hemicellulose in high proportion.

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Valorisation of sauerkraut processing by-products

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During the production of sauerkraut, cabbage juice is released almost immediately after the addition of salt, and can sum up to 30% till the end of the process. Yet it is also a valuable source of various bioactive compounds and functional metabolites. The research into development of new technologies and value-added applications of sauerkraut juice would contribute to sustainable, residue - free technology in processing plants as well as create high value-added products [1]. The aim of this research was to evaluate addition of dehydrated sauerkraut juice to the bread, meat and salad dressing quality.

Sauerkraut juice was spray dried using starch as a wall material. Salad dressings, bread and meat were prepared using various concentrations of dehydrated sauerkraut juice (DSJ). Nutritional composition, volatile profile and sensory properties were determined for developed products.

Dehydrated sauerkraut juice was tested in food applications as a salt alternative, and its possible reduction in experimental salad dressings with olive oil and sour cream. Nor salt nor dehydrated sauerkraut juice dissolves in oil bringing the salty sensation mouthfeel in the experimental samples with olive oil. Overall, experimental samples with DSJ were liked more than the control sample with salt. This leads to conclusion that DSJ could be used in salad dressings as salt alternative, reducing the consumed salt amount and enriching the food with minerals and bioactive compounds [2]. Supplementing wheat bread with DSJ increases the TPC content. Although the TPC was higher in the Bread DSJ, the bioaccessibility index was lower. Sauerkraut juice and its products have a very distinct flavor and aroma that is composed of various volatile compounds, like aldehydes, alcohols, sulfur compounds, esters, ketons, terpenes, furans etc. and can be metabolized from bioactive compounds, delivering health promoting attributes. There were 9 volatile compounds detected in the bread samples, 8 of them exceeded 5 %. The highest peak area was for benzaldehyde, giving volatile oil of almond odour and it was higher with the DSJ addition. A very distinct nuance – a caraway-like odour – in the bread sample with the DSJ addition was detected. There is 1% of caraway added in the production process of sauerkraut in Ltd. 'Dimdiņi' (Latvia), and this volatile compound is so strong to remain through the spray-drying process and the bread baking. The TPC content and antiradical activity by ABTS+ in the meat DSJ sample is higher by 64% and 51% accordingly. The bioaccessibility index for TPC of the meat samples are significantly higher than 1 and thus the compounds are available for absorbption. The combination of proteins and phenolic compounds can affect the bio accessibility of TPC, and is influenced by the specific compound interactions. There were 8 volatile compounds detected in meat samples. The highest peaks for the Meat C sample were hexanal, mostly formed by oxidation of linoleic acid, and 3-furaldehyde, giving fruity, green grass and almond like odor, also volatile oil-of-almond. [3].

Dehydrated and concentrated sauerkraut juice improves the nutritional value of the food products but sensory properties are not always acceptable.

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Potential application of green extracts rich in phenolics for innovative functional foods: Natural deep eutectic solvents as medium for isolation of biocompounds from berries

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Natural Deep Eutectic Solvents (NADES) are novel and promising solvents for green extraction of phytochemicals from food and agricultural products. NADES are made of natural origin compounds that are connected via hydrogen bond and have unique tuneable properties[1]. Numerous studies investigated the extraction of bioactive compounds using NADES resulted in better extraction efficiency compared to conventional solvents, suggesting large potential of these solvents[2,3]. Low-toxicity, natural origin and affordability of NADES-based extracts enables their application as a preservative in the food industry or functional ingredient of food supplements. The aim of this study was to develop an efficient eco-friendly method for the extraction of phenolic compounds from berries, blueberry, chokeberry and black goji berry, using NADES. This goal will be achieved through investigation of extraction efficiency of applied NADES based on three different approaches, a) chromatographic, through high-performance thin layer chromatography (HPTLC) fingerprint analysis, ultra-high-performance liquid chromatography with a diode array detector and a triple-quadrupole mass spectrometer (UHPLC-DAD-MS/MS) target analysis and bioautography, b) spectroscopic, through quality control parameters, total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity (RSA), and c) microbiological, through well diffusion method and minimum inhibitory concentration (MIC).

In this study, 36 NADES mixtures, prepared from primary plant metabolites, were tested as green alternatives for extraction of phenolics from three berries. Different hydrogen-bond acceptors (HBA), such as choline chloride, L-proline, L-glycine, and L-lysine were mixed with various hydrogen-bond donors (HBD), four organic acids, two sugars, glycerol and urea, at various molar ratios for NADES preparation. Guided by the fact that extraction efficiency depends on polarity, viscosity and dissolving ability of solvents[4], the influence of HBA, HBD and water content were investigated. Methanol, as conventional solvent, was used as contrastive solvent.

Choline chloride-based NADES in combination with malic acid, glycerol or urea showed as most efficient. All groups of studied NADES showed various extraction capabilities for phenolic compounds based on chromatographic evaluation and spectrophotometric tests. These results indicate great power of NADES solvents towards selective extraction of phenolics and shows that NADES are designer solvents. Appropriate selection of components for NADES preparation can result in selective extraction of specific phenolic compounds, regardless of how similar structures of targeted compounds are or how complex the matrix is, which gives a significant advantage for application of NADES.

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Edible flowers: a novel antioxidant source to enhance food stability

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One of the main quality parameters that affects food quality is lipid peroxidation, once it leads to deterioration of nutrition and organoleptic characteristics [1]. To prevent this chemical reaction is important to find natural alternatives, such as antioxidant systems [1]. Since ancient times, edible flowers have been used in human diet and culinary preparations, with an increasing demand from the consumers for their health benefits, nutritional value, and bioactive properties [2,3]. Recent studies have demonstrated that edible flowers present antioxidant properties which are commonly attributed to their phenolic composition, being considered as key drivers for food industry [2,3]. This study intends to assess the phenolic content and antioxidant capacity through spectrophotometric methodologies (total phenol, *ortho*-diphenols, and flavonoids and ABTS•$^{+}$, DPPH•, and FRAP assays, respectively) and phenolic profile by High Performance Liquid Chromatography – Diode Array Detector (HPLC-DAD) methodology of five different edible flowers, namely *Viola tricolor*, *Rosa damascena* Mill.*, Pelargonium graveolens*, and two different species of *Calendula officinalis* L.. The total phenols content, *ortho*-diphenols, and flavonoids ranged from 12.28 ± 0.29 to 82.06 ± 1.28 mg Gallic Acid (GA)/g; between 0.89 ± 0.00 and 222.67 ± 0.02 mg GA/g and from 5.53 ± 0.38 to 12.97 ± 0.71 mg Catechin (CAT)/g, respectively. For the antioxidant capacity, *Rosa damascena* Mill., and *Pelargonium graveolens* were the flowers that presented the highest antioxidant capacity for the three methods. Concerning, the individual phenolic profile determined by HPLC-DAD, flavonoids (flavonols and anthocyanins) and non-flavonoids (phenolic acids) were identified. This study contributes to the valorisation of edible flowers, with economic and/or industrial interest, enhancing their possible application in food industries as novel sources of antioxidants to improve food stability.

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Stable, environmentally friendly and inexpensive biocatalysts for obtaining important ingredients applicable in the food industry

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Clays are naturally occurring, environmentally friendly, chemically inert, thermostable, inexpensive resources that are easily modified into materials with tailored properties. As such, they can be used as suitable supports for enzyme immobilization and application in the food industry. Natural polysaccharides starch, xylan, pullulan, and its derivatives obtained by the action of enzymes, have numerous potentials for food industrial applications. In this work the enzyme supports were prepared from bentonite from Coal mine "Bogovina", Serbia by acid activation (AA), pillaring (P), and pillaring followed by acid activation (PAA). The characterization of the obtained materials included chemical and phase composition, surface acidity, and textural properties. After characterization, a-amylase from Bacillus paralicheniformis (BliAmy), commercial xylanase from Sigma-Aldrich (Xyl), and pullulanase from B. paralicheniformis (BliPull) were immobilized on bentonite based supports by 24 h adsorption at 25 °C. The obtained biocatalysts BliAmy-AA (106 IU/g), Xyl-P (74 IU/g), and BliPull-PAA (45 IU/g) showed very good storage stability with the activity preserved after 4 weeks of testing. Products of hydrolysis were detected by TLC and indicate a promising application in the food industry.

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Hydroxyapatite Pickering emulsions loaded with olive leaf extract as an innovative alternative to traditional mayonnaise-like food sauces

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The production of innovative food products is an emergence of the food industry to satisfy consumers' needs and trends, namely healthy, fat-reduced, and bioactive-based functional products [1, 2]. In this context, Pickering emulsions, which are stabilised by solid particles, have been increasingly studied due to their high physical stability, mainly to coalescence, Ostwald ripening, and their ability to create functional and label-friendly solutions [1]. Additionally, agro-food residues are a source of complex carbohydrates, fibres, proteins, lipids, vitamins, and phytochemicals (phenolics, carotenoids) with potential health benefits [3]. The present work aims to combine these two current topics in food research by developing (1) Pickering emulsions stabilised with nano-hydroxyapatite (n-HAp) particles, and (2) functional Pickering emulsions through the incorporation of olive leaf extract. Thus, in this perspective, the main goal of this study is to create a differentiated emulsion formulation with functional and vegan characteristics to replace traditional mayonnaise sauces.

The n-HAp aqueous paste (nanoXIM-HApCare, supplied by Fluidinova S.A.), sunflower oil, and olive leaf extract were used as aqueous and oil phases, respectively. For the oil-in-water (O/W) Pickering emulsions production, a mixing system comprised of a rotor-stator homogeniser (Miccra D-9) and a peristaltic pump was used. Briefly, the oil phase was injected into the aqueous phase using the peristaltic pump at ~43 mL/min, and the homogenising device was set at 11 000 rpm to ensure a prompt dispersion. The olive leaf extract was obtained using supercritical fluid extraction using carbon dioxide as solvent (SFE-CO2) for 2 h, 90 bar and 50 °C. This methodology, a sustainable and green technology, was used to increase the bioactive compound's selectivity and preservation. This study addresses the effect of n-HAp solid particles concentration (5-15 wt.%) and oil/water ratio (oil phase ranging from 50% to 80%) on emulsion stability. The morphology, droplet size distribution, phase inversion, rheological properties, and oxidative stability of the produced Pickering emulsions were monitored using an optical microscope, laser diffraction, droplet test, rheometer, and a Rancimat, respectively.

The results indicate that the produced Pickering emulsions have good stability for the tested period of 30 days, except the 80/20 oil/water Pickering emulsion that revealed phase separation after a few days from production. Additionally, the Pickering emulsions produced with higher n-HAp or oil concentrations have a semi-solid structure making them attractive options to mimic the traditional mayonnaise texture. Regarding oxidative stability, the Pickering emulsions showed considerably improved stability compared to commercial mayonnaise (traditional and light products), which suggests higher resistance to the peroxidation by-products and a longer shelf life for the n-HAp Pickering emulsions. The n-HAp Pickering emulsions can offer advantages for olive leaf extract encapsulation. Overall, this approach can also provide a promising perspective to develop innovative food-like products with low-fat, eggless and functional properties as replacements for traditional ones.

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Chemical and functional characterization of innovative and healthy fermented plant-based products from sustainable land and sea vegetables

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Today, consumers look for products able to promote health, while at, the same time, valuing the sustainability and flavour of the products. So, to guarantee healthy and tasty products, while minimizing food loss and waste, are the main challenges faced by the food industry. Food fermentation has become an important mild-processing technology that takes advantages of the nutritional and health benefits resulting from microorganisms' metabolism to develop innovative, sustainable, clean-label, safe, and appealing food products [1].

Attentive to consumer demands, the ***FermentedVegAlgae*** project, a partnership between the Instituto Superior de Agronomia, Mendes Gonçalves and SONAE MC companies, blends innovation, environmental and health concerns, adding value to surplus vegetables (that would otherwise be discarded) and less-used nutritionally-rich plant sources (macroalgae), through the process of food fermentation.

Up to now eight fermented plant-based prototypes (sauerkraut-like and spreads) were developed: six from surplus vegetables (with white or red cabbage together with other vegetables like carrot, ginger, garlic and chilli), three from macroalgae (*Palmaria palmata* and *Alaria esculenta*), and three from mixtures of vegetables and macroalgae. Fermentation performance was evaluated by measuring pH, acidity and the total soluble solids, until stability.

The evolution of the fermentation process and centesimal composition of the final fermented products were performed. For the **vegetable fermentations**, 1% added NaCl and a consortium of 4 lactic acid bacteria (LAB) were used. After the fermentation processes have been completed, the products are pasteurised, and probiotic bacteria *(Bacillus coagulans)* were added to increase even more the probiotic potential of the products. After 21 days of fermentation, stable and safe pH and titrable acidity were achieved (3.37-3.54 and 1.35-1.76% lactic acid). For **macroalgae**, macroalgae were previously treated by ultrasounds, mixed with 0.3% added NaCl, and fermented by a consortium of the same LAB and *Debaryomyces hansenii* (a yeast that produces high levels of riboflavin and antimicrobial compounds). Through the fermentation process, pH dropped and titrable acidity increased, reaching food-safety values after 5-7 days (3.72-3.95 and 2.2%-3.6% lactic acid, for *A. esculenta* and *P. palmata*, respectively). In both, **vegetables and macroalgae** fermented products, firmness and consistency decreased. The reduction was more pronounced in the fermented vegetables, with white cabbage and chilli sauerkraut-like product registering the highest decrease in both parameters in comparison with the non-fermented product (77.6 % and 77.3% reduction in firmness and consistency, respectively).

The evaluation of their potential health benefits (antioxidant potential, minerals, content in vitamin B6 and B12, total phenols and flavonoids and mineral bioaccessibility) is being accessed. Due to the action of both LAB and *D. hansenii*, producers of fitases and oxalases that will help the release of iron and calcium, it is expected an increase in the bioaccessibility of these minerals.

Fermentation of vegetables and macroalgae can be considered a way for tailoring the technological and health-related functionality of food products while increasing their sustainability and market value.

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Synergistic action of ZnO nanoparticles and essential oil on the antimicrobial and functional properties of biopolymer films

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Nanoparticles are particles within the range of 1 to 100 nm, which exhibit enhanced properties such as heightened reactivity, size, sensitivity, surface area, stability, and strength compared to materials with similar composition. To overcome the limitations of essential oils, various nanomaterials including gold, silver, platinum, iron, copper, chitosan, and zinc have been employed to create nanoparticles loaded with essential oils that possess antimicrobial properties. The utilization of nanoparticles in conjunction with essential oils safeguard them from degradation caused by heat and UV rays, resulting in a greater degree of stability, flavour retention, and functional effectiveness, which in turn increases the longevity of the final product [1].

Zinc oxide (ZnO), a commonly used inorganic nanoparticle that possesses diverse functions [2], is a metal nanoparticle approved by the FDA for its antimicrobial properties. ZnO nanoparticles exhibit exceptional biocompatibility and thermal stability, and they are non-toxic. Research has demonstrated that ZnO nanoparticles exhibit potent and meaningful antibacterial activity against both gram-positive and gram-negative bacteria [3]. ZnO nanoparticles can be synthesized through various methods, resulting in different shapes and structures, having the potential to reduce the risk of food contamination and prolong the shelf life of food products by inhibiting the growth of bacteria and fungi [2].

Essential oils (EOs) are volatile functional compounds that are derived from the different parts of aromatic plants which contain a variety of terpenoids, terpenes, aromatics, and aliphatic compounds, resulting in their broad range of applications. Along with their primary antimicrobial and antioxidant properties, these compounds can be used as potential substitute for synthetic preservatives in the food industry, but also for obtaining various active packaging films due to their improved functional characteristics [3-4].

The mechanism of antimicrobial activity depends on the type of essential oil or microorganism strain utilized. Essential oils have an easier time penetrating gram-positive bacteria compared to gram-negative bacteria, likely due to the presence of lipoteichoic acid which facilitates the entry of essential oils into the gram-positive microbial cells. Research studies have shown that the bioactive constituents present in essential oils bind to the surface of cells and permeate the phospholipid bilayer of the cell membrane, leading to membrane impairment that negatively affects cellular metabolic processes and results in cell death. Alteration of the integrity of the cell membrane causes the release of vital intracellular components such as proteins, reducing sugars, ATP, and DNA, obstruction of ATP synthesis and related enzymes, leading to electrolyte leakage. Clove, cinnamon, oregano, pimento, rosemary, and thyme essential oils exhibited potent antibacterial effects against *Staphylococcus aureus, Salmonella typhi*, and *Pseudomonas aeruginosa* [1].

The simultaneous incorporation of essential oil-loaded nanoparticles exhibits a synergistic antimicrobial effect by improving the diffusion of essential oils through biological membranes [1]. Moreover, the combined addition of ZnO nanoparticles and essential oil significantly lowered the water vapor permeability (WVP) of the films while enhancing their flexibility and strength [4]. The research studies indicated that incorporating ZnO nanoparticles and essential oil into biocomposite films enhances their barrier, mechanical and antioxidant properties, as well as their thermal stability. The addition of combined ZnO-NPs and EO can alter the moisture content, solubility, water-absorption capacity, water vapor and oxygen permeability, elongation at break, thickness, tensile strength, and Young's modulus of the films [2].

Keywords: antimicrobial activity, essential oils, food industry, functional properties, synergic effect, ZnO nanoparticles

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Development of functional Flavoured water through the incorporation of chestnut Flower Extract: Evaluating Bioactive Potential and Stability

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The incorporation of natural extracts rich in phytochemicals into beverages offers the potential for functional enrichment. The use of agro-industrial by-products as a source for such bioactive molecules can be a valuable strategy to reduce the environmental impact and produce high-added-value ingredients. In this study, a polyphenol-rich chestnut flower extract was incorporated into lemon-flavored, non-carbonated water aiming to enhance its composition of bioactive compounds and its antioxidant properties. Moreover, the antioxidant stability of these samples was investigated. Control and treatment water samples with 400 mg/L of chestnut flower extract were produced by SuperBock™ using their standard formulations and processes. The polyphenol composition of water samples was analyzed by HPLC-DAD-MS/MS and their antioxidant capacity was assessed by using spectrophotometric assays after the processing. In addition, an accelerated stability experiment was conducted by subjecting the water samples to a temperature of 50°C for nine days, with the antioxidant capacity being monitored every third day. The flavored water containing the extract presented the same polyphenol profile as the chestnut extract incorporated, being chestanin the main compound found, indicating the absence of chemical changes due to the processing conditions or interaction with the formulation. The water supplemented with chestnut flower extract also exhibited superior antioxidant activity than the control in the DPPH assay. It can be highlighted that the antioxidant activity of the water incorporated with the chestnut flower extract was maintained and largely exceeded that of the control sample consistently throughout the period of the accelerated experiment. In summary, the incorporation of the chestnut flower extract into lemon-flavored water effectively enhanced its composition in phytochemicals and imparted antioxidant capacity to this product. The antioxidant stability of the treatment sample under extreme conditions of temperature highlights the potential for the inclusion of chestnut flower extract as a functional ingredient in lemon-flavored water improving its quality over the shelf-life of the product.

Additional ongoing investigations include evaluating the cellular antioxidant and anti-inflammatory activity of the functionalized waters.

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Oxidative stability of fish by-product oil added of bioactive acorn extract during accelerated storage conditions

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Oxidation is a principal cause of quality deterioration in oils and fats during processing and storage, resulting in the production of rancid odors, unpleasant flavors, color changes, and reduction of nutritional value [1,2]. This renders them unsuitable for consumption. Antioxidant compounds play an important role as protective agents, capable of delaying or inhibiting lipid oxidation by impeding the initiation or propagation of oxidizing chain reactions [2]. Given the legal restrictions in different countries and the reported carcinogenic effects associated with artificial antioxidants, there is an urgent need for natural alternatives. Furthermore, the growing consumer preference for more natural products has sparked interest within the food industry to develop natural antioxidant-based ingredients to reduce or avoid lipid oxidation and preserve organoleptic quality attributes of food products. Therefore, the objective of this study was to assess the efficacy of a natural acorn extract in preventing oxidative reactions and preserving the quality of fish oil during accelerated shelf-life. The acorn extract was initially characterized for its phenolic compound profile by HPLC-DAD-ESI/MS and antioxidant activity through two cell-based *in vitro* assays. Subsequently, fish oil was extracted from fish by-products using a Soxhlet apparatus [3]; a portion of the oil was mixed with acorn extract, while another portion was used as a control. Both samples were analyzed at time 0 and after 7, 14, and 21 days of accelerated storage at 60 °C. The oxidative stability was assessed by evaluating color parameters, fatty acid profile by GC-FID, peroxide value, titratable acidity, and *p*-anisidine value. The acorn extract was rich in ellagic acid and catechin derivatives and had an antioxidant activity comparable to that of Trolox, which was used as a positive control in the *in vitro* assays. Regarding fish by-product oil, oleic, palmitic, and linoleic acids were abundant fatty acids. The accelerated shelf-life study showed that the acorn extract is a promising natural antioxidant to preserve fish oil quality attributes, evidenced by better retention of color and titratable acidity, *p*-anisidine and peroxide values. Overall, the suitability of acorn extract for preventing lipid oxidation of fish by-product oil was demonstrated. These findings pave the way for novel approaches to maintaining the quality of edible oils.

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POSTER PRESENTATIONS

T4

Food and health, functional foods and ingredients

Chromium Nanoparticles Support the Pro-Healthy Regulation of Liver Lipid Metabolism and Inflammation in Obese Rats when Combined with the Abandonment of High-Fat/Low-Fiber Diet

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Chromium (III) (Cr) is a key microelement involved in the metabolism of carbohydrates, proteins, and fats in humans and animals. Reports have also indicated that chromium(III) is involved in the metabolism of nucleic acids and favorably stimulates the immune response and disease resistance [1]. Because of these properties of chromium, particularly its ability to regulate carbohydrate-lipid metabolism and reduce body weight, it is popularly used as a factor supporting the treatment of type 2 diabetes and as a component of supplements used in slimming (anti-obesity) treatments. However, because of the relatively low bioavailability of chromium picolinate, other forms of this element are sought that could be better utilized by the body. Thus, researchers are increasingly interested in complexes of Cr with amino acids as well as inorganic chromium nanoparticles (NPs) [1,2]. NPs tend to exhibit different properties than larger particles of the same element. Nevertheless, the essentiality of chromium (Cr) has been questioned in some recent studies [3]. Although some studies have suggested that chromium supplementation decreases insulin levels, improves glucose disposal rates and lipid profiles, and beneficially reduces body weight in obese individuals [4], in other studies, Cr supplements to diabetic or healthy subjects did not indicate beneficial effects on glucose metabolism and diabetes [5]. Therefore, the aim of the study was to investigate whether metabolic disturbances in the liver tissue associated with chronic intake of an obesogenic diet could be subsequently alleviated through dietary supplementation with various forms of chromium and/or switching to a low-fat diet. We hypothesized that switching from high-fat dietary habits combined with a pharmacologically relevant dose of chromium supplementation (commonly used picolinate or novel form as nanoparticles; 0.3 mg/kg body weight) would benefit physiological responses in the hepatic status.

The study on Wistar rats was conducted to investigate the effects of a pharmacologically relevant dose 0.3 mg/kg body weight of chromium supplementation (commonly used picolinate or novel form as nanoparticles) and switching away from obesogenic dietary habits on the parameters of lipid metabolism, inflammation, and oxidative stress in liver and plasma. The feeding period consisted of an initial 9 wk and an experimental 9 wk period. During the initial period, C rats were fed a standard low-fat diet (diet C), while the remaining groups (M, F, MP, FP, MN, FN) were subjected to an obesogenic high-fat diet (diet F). The dietary treatments used in the experimental period: Group C, control fed a C-diet; M, fed a C-diet; F, fed an F-diet; MP, fed a C-diet with Cr-Pic supplementation; FP, fed an F-diet with Cr-Pic; MN, fed a C-diet with Cr-NP; FN, fed a F-diet with Cr-NP.

The disorders induced by the obesogenic diet were effectively mitigated when the diet was switched to the standard (low fat) diet. Supplementation of the standard diet with chromium nanoparticles considerably enhanced favorable effects against the development of fatty liver disorders. This combination exerted the strongest reduction in fat content and cholesterol in the liver. In this group, a favorable antioxidative effect was also observed through elevation of GSH/GSSG in the liver as well as reduction of ALT activity in plasma and level of IL-6 in the liver. The mechanisms involved in the regulation of lipid metabolism, oxidative stress, and development of inflammation might be associated with lower expression of COX-2, HIF-1α, and LOX-1 and upregulation of PPARα. In the group where the obesogenic diet was not switched to the standard diet, supplementation with chromium nanoparticles exerted similar favorable effects against obesity-related disorders but was not as efficient as that observed in the group with the standard diet. Thus, the findings of the present study indicate that switching from obesogenic dietary habits together with chromium nanoparticle supplementation benefit physiological responses in the hepatic status, increasing regulatory effects against obesity-related disorders.

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Mentha × piperita L. var. *officinalis* forma *rubescens* Camus antimicrobial activity *in vitro* and *in situ*

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The three botanical groups (also known as Medicinal and Aromatic Plants, or MAPs) that are most commonly used as herbs are Lamiaceae, Asteraceae, and Rosaceae [1]. The Lamiaceae family includes the genus mint (*Mentha*), which has 42 species, 15 hybrids, and hundreds of subspecies, varieties, and cultivars [2]. *Mentha x piperita* L. var. *officinalis* forma *rubescens* (Camus), also known as black or English *Mentha x piperita* (MP), which has purple stems and leaves, is a popularly grown variety of MP [3,4]. The goal of this research was to assess the *in vitro* and *in situ* antimicrobial, activity of commercial black MP essential oil (BMPEO), which was obtained by steam distillation of the flowering clematis. The major composition of the BMPEO by the statement of producer were menthol 38 %, menthone, and isomenthone. The disk diffusion method was used to evaluate the antibacterial effects on food-borne pathogenic and food spoilage bacteria (*Bacillus cereus* CCM 2010, *Listeria monocytogenes* CCM 4699, *Staphylococus aureus subsp. aureus CCM 2461, Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, and *Pseudomonas aeruginosa* CCM 1959). The effect of the vapor phase of BMPEO against all bacteria was observed in *in situ* **analysis using carrots** at concentrations of 500, 250, 125, and 62.5 **µL/L**. BMPEO exhibits diverse antimicrobial activity, and the zones of inhibition for gram-positive ranged from 9.67±0.58 to 22.33±0.58 mm and gram-negative bacteria from 9.33±0.58 to 18.67**±0.58** mm. *In situ* antibacterial analysis on carrots suggests that the vapor phase of BMOEO can inhibit the development of gram-positive and gram-negative bacteria. At the lowest concentration of the BMPEO (62.5 µL/L), *B. cereus* **was the most strongly inhibited** bacteria (47.88%). According to the findings, BMPEO may one day be used to prolong the shelf life of carrots and may also be useful in vegetable storage. Due to the existence of the main bioactive constituents, the commercial essential oil from black MP exhibits potential antimicrobial effects against specific food-borne and food spoilage bacteria. This investigated essential oil may be promising as natural substitutes for use in food preservation in order to slow or inhibit bacterial development, ensure food safety, and increase the shelf life of the food products. However, it is necessary to assess whether this essential oil has an organoleptic effect on meals and whether its antimicrobial effectiveness has been confirmed.

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Anticancer activity of hydrolysed wheat bran mediated through macrophages stimulation

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths[1]. Due to scientific evidence, new dietary interventions are needed to decrease its incidence and mortality[2]. Among other compounds, wheat bran (WB) contains high biological value dietary fiber and polyphenols with recognized anti-tumour and immunomodulatory activities.[3] In particular, ferulic acid (FA) possesses anti-inflammatory and anti-cancer properties against several types of cancer. However, its low solubility and bioavailability in aqueous medium hinder its widespread application.[4] Recently, our group has applied processing routes to develop a wheat bran hydrolysate (HYD) with antioxidant and anti-inflammatory properties through dietary fiber and ferulic acid solubilization.[5]

The current study aimed to explore the immunostimulatory effect of HYD and a mousse enriched with HYD (MH) before and after *in vitro* digestion on macrophages. The antiproliferative activity of the harvested macrophage supernatants on CRC cells was also analyzed. MH showed significantly ($p < 0.05$) higher content in soluble poly- and oligosaccharides, as well as total soluble phenolic compounds (TSPC), than control mousse (M). Although *in vitro* gastrointestinal digestion slightly reduced the TSPC bioaccessibility of MH, ferulic acid levels remained stable. HYD showed the highest antioxidant activity followed by MH, which demonstrated a greater antioxidant activity before and after digestion as compared to M. Macrophages released the highest amounts of pro-inflammatory cytokines after being treated with 0.5 mg/mL of digested WB samples. Treatment with digested HYD-stimulated RAW264.7 supernatant for 96 h showed the most anticancer effect and spent medium reduced cancer cell colonies more than direct WB sample treatments. Although a lack of inner mitochondrial membrane potential alteration was found, increased Bax:Bcl-2 ratio and caspase-3 expression suggested activation of the mitochondrial apoptotic pathway when CRC cells were treated with macrophage supernatants. Intracellular reactive oxygen species (ROS) were positively correlated with the cell viability in CRC cells exposed to RAW264.7 supernatants ($r = 0.78$, $p < 0.05$), but was not correlated in CRC cells treated with THP-1 conditioned media. Supernatant from WB-stimulated THP-1 cells may be able to stimulate ROS production in HT-29 cells leading to a decrease of viable cells in a time-dependent manner.

Therefore, this study provides knowledge insight of a novel anti-tumour mechanism of HYD through the stimulation of cytokine production in macrophages and the indirect inhibition of cell proliferation, colony formation, and activation of pro-apoptotic proteins expression in CRC cells.

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Diet with raspberry polyphenols and prebiotics enhances liver lipid metabolism and regulates the synthesis of bile acids in obese rats.

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Some of the diseases occurring in economically developed countries are the result of fixed, faulty eating habits. It has been proven that a high-fat, low-fiber diet significantly increases the risk of obesity, cardiovascular disease, and gastrointestinal disease. One of the factors that increases the risk of cancer and inflammation in the gastrointestinal tract is the excessive content of secondary bile acids in the intestines, e.g., deoxycholic (DCA) and lithocholic (LCA). The concentration of these acids is influenced by two mechanisms: 1) synthesis of primary bile acids (cholic acid (CA) and chenodeoxycholic acid (CDCA)) in the liver; and 2) microbial metabolism of primary to secondary bile acids in the gastrointestinal tract. Previous nutritional studies have shown that the dietary combination of polyphenols with prebiotic fructooligosaccharides (FOS) has a positive effect on the activity and profile of the gut microbiota by increasing the growth of Bifidobacterium and Lactobacillus. These bacteria can participate in the conversion of secondary bile acids to less cytotoxic forms and increase the concentration of polyphenol metabolites, which reach the liver and then regulate the mechanisms responsible for lipid metabolism (Fotschki et al., 2022). Therefore, the aim of the experiment was to investigate the effect of supplementation with raspberry polyphenols and FOS on the synthesis and profile of bile acids in Wistar rats fed an obesogenic diet.

The feeding experiment was performed on Wistar rats divided into 4 groups of 8 animals each, i.e., a group with a standard diet for laboratory rodents (C), a group with a high-fat diet with a reduced content of dietary fiber (HF), a group with an HF diet supplemented with raspberry polyphenolic preparation (HF+PP), and the group with the HF+PP diet supplemented with FOS (HF+PP+F).

After 8 weeks of feeding in the HF group, it was observed a considerably higher concentration of bile acids (mainly DCA and muricholic acid-α and -β), activity of the bacterial β-glucuronidase in the cecum, increased levels of cholesterol and triglycerides, and accumulation of fat in the liver. In this group, the analyses of mRNA expression showed lower levels of FXR (farnesoid X receptor) and SHP (small heterodimer partner) in the liver. These molecular factors are responsible for lipid metabolism and the regulation of cholesterol conversion to primary bile acids. Supplementation with a polyphenolic preparation mitigated the effects of the HF diet by lowering the concentration of bile acids, β-glucuronidase activity in the cecum, and reducing the concentration of cholesterol and fat accumulation in the liver. The hepatic effect could be associated with an increased expression level of FXR. The effects of the polyphenolic preparation were enhanced when FOS was added to the HF+PP diet. In the group of animals fed a diet with FOS, it was observed that the lowest concentration of secondary bile acids (in particular, DCA and muricholic acid-α and –β) and the highest concentration of CA and hyodeoxycholic acid were in the cecum. Moreover, the activity of the β-glucuronidase enzyme in the caecum was the lowest among all examined groups. This microbial enzyme is able to convert harmful substances and secondary bile acids conjugated in the liver back into more toxic forms, causing intestinal disorders. The dietary combination of the polyphenolic preparation with FOS had the strongest regulatory effect on cholesterol, triglycerides, and fat accumulation in the liver. Beneficial changes in the liver lipid metabolism could be partially linked with the downregulation of AHR (aryl hydrocarbon receptor) and the upregulation of the FXR receptor and the cytochrome CYP8B1 responsible for the conversion of hepatic cholesterol into CA.

To sum up, the combination of PP and FOS enhances beneficial effects against disorders induced by the obesogenic diet through regulation of mechanisms of liver lipid metabolism and reduction of secondary bile acids in the gastrointestinal tract.

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Health Effects and bioavailability of Omega-3 fatty acids

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Long-chain omega-3 polyunsaturated fatty acids (LC (n−3) PUFAs) play crucial roles in various physiological processes in the human body. Among these, docosahexaenoic acid (DHA, 22:6(n−3)) is particularly essential for proper development and function of the brain and retina throughout life. Despite its importance, DHA deficiency has been widely reported worldwide due to the low intake of DHA and its low absorption. Recent research has shown that the distribution of fatty acids (FAs) within triacylglycerols (TAGs) affects lipid absorption.

With recent advances in the synthesis and determination of enantiospecific structured TAGs, this study was able to determine if dietary TAGs possessing DHA either in *sn*-1, *sn*-2, or *sn*-3 position and two palmitic acid residues in the remaining *sn*-positions, [*sn*-22:6((n−3)-16:0-16:0, *sn*-16:0- 22:6((n−3)-16:0 or *sn*-16:0-16:0-22:6((n−3)] would lead to the difference in FAs content or composition of visceral fat in rats. The study was conducted in male rats and the intervention time was 4 weeks, and by using tripalmitin, *n*−3 deficiency and normal feed as controls. The study focused on the extraction of lipids from visceral fat samples, followed by the analysis of FA methyl esters using gas chromatography. Variations in the FAs content of the lipids in visceral fat were observed between groups that were fed with DHA.

DHA showed a higher absorption on the *sn*-3 position of TAGs in visceral fat when compared with the DHA located on the *sn*-1 and 2 positions. Moreover, DHA located on the *sn*-3 position of structured TAGs had a higher content of FAs 20:1(*n*−9) and 20:4(*n*−6), and total (*n*−3) PUFAs in visceral fat TAGs compared with the *sn*-1 position. However, When DHA was situated at the *sn*-2 position as opposed to the *sn*-1 and *sn*-3 positions, there were no discernible disparities in the fatty acid composition of visceral fat were discovered. This study showed the different bioavailability of DHA in different positions of TAGs.

Chokeberry and red cabbage anthocyanins selectively cross the blood-cerebrospinal fluid barrier

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The aim of this study was to identify the ability of anthocyanins and their phase II metabolites to penetrate the blood-cerebrospinal fluid barrier (blood-CSF barrier) in a model study with sheep as well as to determine changes in the profile and concentration of these compounds in physiological fluids of sheep (blood plasma, urine, and cerebrospinal fluid (CSF)) after intravenous administration of a mixture of cyanidin 3-galactoside (Cy3gal, the main anthocyanin compound of chokeberry) and cyanidin 3-diglucoside 5-glucoside (Cy3diG5G, the main anthocyanin compound of red cabbage) enabling to neglect the influence of absorption processes.

The study involved the analysis of anthocyanins and their phase II metabolites in physiological fluids of sheep (n = 16), performed after a single intravenous administration of a mixture of Cy3gal and Cy3diG5G at a dose of 1 mg of each compound/kg of body weight. The micro-HPLC MS/MS analysis demonstrated that the anthocyanins did penetrate the blood-CSF barrier after intravenous administration, and that the permeation potential of Cy3gal derivatives was higher than that of Cy3diG5G derivatives. The obtained results indicate that anthocyanins may compete for the same carrier responsible for their transport through the blood-CSF barrier, and that the differences in the penetration of these compounds are due to the type and number of substituents, as well as the molecule size.

In conclusion, the results obtained in this study provide novel, important knowledge indicating that chokeberry and red cabbage anthocyanins (except acylated derivatives) and their phase II metabolites cross the blood-CSF barrier. Thus, the obtained results indicate that anthocyanins accumulating in the CSF in their native and conjugated forms may beneficial affect the processes occurring in the environment of the central nervous system that is particularly susceptible to oxidative damage, due to their multiple properties, including a strong antioxidant potential. Tracking the biological fate of anthocyanins after their intake (from absorption into the blood to penetration into the CSF) may facilitate the design of further experimental procedures allowing to determine the biological properties of these colour phenolic compounds, including their neuroprotective efficacy. However, further research is needed to fully elucidate the mechanism by which anthocyanins cross the brain barrier and to determine whether the concentration of anthocyanins detected in CSF, up to several hundred nmol/L, may beneficially influence the processes occurring around nerve cells. This is of particular importance considering that diseases of the central nervous system pose a severe problem in the contemporary population, and that the identification of preventive behaviours, including the nutritional ones, that can minimize their incidence or mitigate their course may be one of the possible and important solutions.

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The use of β-cyclodextrin for decreasing the cholesterol content in cereal products

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Cardiovascular diseases (CVD) are the leading cause of mortality in the world, and the most frequently proposed mechanism for CVD consists of increased blood lipid content, especially total cholesterol. It is well known that the main sources of dietary cholesterol are foods of animal origin, for example, meat, eggs, or milk products [1]. However, as these materials are also added to several cereal products, they can also serve as a source of cholesterol in our blood. Cereals and cereal products are staple foods in most human diets and their global use is projected to increase from 2.7 billion t to 3 billion t by 2030, mainly due to higher feed use (+163 Mt), followed by food use (+146 Mt) [2,3]. Their formulation can include several ingredients such as flour, fat, sugars, milk powder, egg products, butter, etc. Therefore, they are a good source of dietary cholesterol and also cholesterol oxidation products [4]. Methods to reduce cholesterol content in food materials can be based on enzymatic conversion of cholesterol, steam distillation, adsorption on various sorbents, supercritical extraction, or lipid removal using liquid solvents [5]. The most selective method is based on the adsorption of cholesterol molecules onto a cyclodextrin (CD) cavity. Thus, there are several articles that describe the production of low-cholesterol milk products such as butter, cheese, or cream [6]. However, no articles also include application to cereal products. Thus, this study aims to monitor cholesterol content in various cereal products using the validated HPLC-UV-DAD method and the application of low-cholesterol ingredients (milk, butter, egg melange) prepared by treatment with β-CD to cereal products, such as biscuits, pasta, or muffins. The validation parameters, such as linearity, recovery, or repeatability of the purpose method, were satisfied with a limit of detection (LOD) of 3.2 mg/kg. The cholesterol content varied between 27.23 and 1112.88 mg/kg, while the highest percentage of cholesterol was found in products including also egg products, such as pasta. Similar results were described by Islam et al. [7], with the range from 27.6 to 1142.6 mg/kg, depending on the recipe. The next step was the elimination of cholesterol from the ingredients, and it was observed that by the application of β-CD, it was possible to eliminate up to 95% of cholesterol from butter and 92% from egg melange. Therefore, the cholesterol content in the final products also decreased, for example, in biscuits from 339.97 to 22.92 mg/kg or in pasta from 1086.33 to 120.91 mg/kg. In our study, we also monitored the colour and texture properties of the final products, and no differences were observed compared to the original ones. In conclusion, it can be stated that cereal products can also play a role in increasing the incidence of CVD, and therefore the elimination of cholesterol content from ingredients of animal origin can serve as a useful tool to reduce cholesterol intake from these products without changing their sensory or texture properties.

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Antimicrobial activity of red wine phenolic and non-phenolic fractions

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In wine-producing regions, wine is frequently used for seasoning meat and meat products, particularly dry-cured ones. Its use in preparing meat products confers an undeniable sensory effect on its colour and aroma, and it might contribute to the control of pathogens [1]. The antimicrobial effect of wine is expectedly due to the low pH and the respective presence of organic acids, ethanol and phenolic compounds. The antimicrobial effect of wine is probably due to the synergistic effect of these three groups of compounds. Several studies have shown the antimicrobial activity of wine, particularly against some gastrointestinal pathogens such as *Escherichia coli*, *Salmonella*, *Listeria*, *and Shigella* [2]. This work aimed to evaluate the antimicrobial activity of wine phenolic and non-phenolic fractions against pathogens of concern in the meat products industry. The wine was filtered (Whatman n.4) and dealcoholized in a roto evaporator (30ºC, vacuum). The sample was diluted (1:4), and the pH was adjusted to 7 with NaOH 1M. The wine was fractionated by solid phase extraction (SPE) using a reversed stationary phase (C18). The stationary phase was previously activated with methanol and equilibrated with water at pH 7. The treated wine was applied, and the column was washed with an equal volume of water at pH 7. The retained phenolic compounds were fractionated using sequential elution with ethyl acetate (fraction 1) and methanol (fraction 2). The retained fractions were adjusted to pH 2 and applied to another SPE column previously equilibrated with HCl 0.1M. The column was washed with an equal volume of HCl 0.1M (fraction 3). The retained phenolic compounds were eluted with methanol (fraction 4). The four fractions were concentrated in a roto evaporator under vacuum and lyophilized. The procedure was made in triplicate. The four fractions were analyzed by HPLC (Ultimate 3000, Dionex Corporation) equipped with a photodiode array detector. The column was a C18 inverse phase (25 cm long; 4.5 mm diameter; 5 µm). The eluent was 5% aqueous formic acid and methanol. The flow rate was 1 ml/min, and the column temperature remained at 35°C. Detection was performed at 200 – 650 nm with an injection volume of 50 µl. The identification of the compounds was carried out considering the literature, the order of elution, the retention times and the UV and visible spectra presented in the literature. The chromatograms for phenolic acids and catechin were obtained at 280 and 325 nm and for monomeric anthocyanins at 525 nm [3]. Quantification was performed as described in [4]. Analyses were performed in duplicate.

The disk diffusion technique was used to detect the antimicrobial activity of wine and its different fractions. This activity was evaluated in triplicate on *Salmonella* (ATCC 49214), *L. monocytogenes* (ATCC 35152), *Cl. sporogenes* (DSM 767) and *S. aureus* (ATCC 25923). Cl. sporogenes was used as a surrogate of Cl. botulinum. The concentration of the extract was adjusted to 1 mg/ml. The microdilution method in wells was used to evaluate the minimum inhibitory concentration. Besides the ATCC strain, for Salmonella and L. monocytogenes, indigenous strains isolated from meat products or their production environment were tested.

The F1 presented coumaric esters, flavonoids and non-flavonoids; F2 was rich in anthocyanins, mainly malvidin-3-glucoside; F3 and F4 included hydroxycinnamic acids. In F3 it was possible to observe caftaric acid, p-coumaric acid esterified with tartaric acid and fertaric acid, while in fraction 4 it had caffeic acid and ferulic acid. It should also be noted that fraction 4 did not contain flavonoids or anthocyanins.

It was observed that Salmonella was not inhibited by the wine nor by any of the fractions tested. Cl. sporogenes was also not inhibited by the wine, but the F2 showed a robust inhibition . L. monocytogenes and S. aureus were very sensitive to all the tested conditions. The F1 showed a low MIC of 100 mg/l against L monocytogenes. Considering that L. monocytogenes is a severe concern in the dry-cured meat products industry, mainly when green label products are produced without chemical preservatives, namely nitrite, the adequate use of wine on seasoning the products might be an important hurdle to control that pathogen.

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IgE recognition and structural analysis on disulfide bonds cross-linked allergens aggregation in roasted peanut

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Abstract

Peanut, as a nutritional food and widely used food ingredient, can unfortunately cause serious allergic symptoms sometimes. Roasting changed structure and allergenicity of peanut allergens[1], expounding their structural information is necessary for explaining the allergenicity alternation. Here, attention was focused on the allergen aggregation (AA) formed in roasted peanut. The IgE recognition capability were assessed by western blot (WB). Disulfide bonds (DBs) rearrangement and specific modification in aggregation was analyzed by combining mass spectrum with bioinformatic analysis software. Results showed that, the AA was strongly recognized by IgE and crosslinked mainly by disulfide bonds. Disulfide bonds can stabilize the native conformation of protein, however, it could take place rearrangement during heat process[2]. The DBs rearrangement in AA containing interprotein (98 pairs), intraprotein (22 pairs) and loop-linked (6 pairs) types. Ara h 2 and Ara h 6 showed the most active cysteine among allergens. Maillard reaction can affect the potential allergenicity of allergens[3]. For chemical modification, Ara h 1 and Ara h 3 were predominant in the aspect of the advanced glycosylation end products. And these chemical modifications changed the electrostatic status of local structure. In conclusion, in roasted peanut, the AA strongly recognized by IgE and was formed from the allergen fragments crosslinked by DBs, as well as happened chemical modifications. Some newly formed DB might change the conformational structure of allergens, while others do not. Many linear epitopes were involved in crosslinking totally or partly. Modification prefers to alter local structural property of protein. These reactions would change the structures of proteins, which effects the IgE recognition.

Keywords: peanut protein; roast; aggregation; disulfide bond rearrangement; modification

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Microbiological and chemical composition of some Portuguese hazelnuts

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Hazelnut is one of the most popular dried fruit all over the world, because of its unique organoleptic characteristics and also due to its nutritional composition [1]. The characteristics of the different hazelnut varieties that exist worldwide are dependent of several factors, such as, the genotype, agricultural and post-harvest practices, climatic conditions and also the geographical location where they are cultivated. Moreover, hazelnut quality is also affected by its chemical composition [2]. The aim of this study was to access the microbiological quality and also the chemical composition of three hazelnut varieties (Grada de Viseu, Tonda de Giffoni and Butler), cultivated in the Viseu region, Portugal. Therefore, hazelnut kernels were analysed for moisture, water activity, fat, protein, fibre and ash. Furthermore, there also quantified the microorganisms at 30ºC and the moulds and yeasts at 25ºC. All analysis were performed in triplicate. According to the results, fat was the major chemical component for all varieties, varying from 64.38±1.67 g/100 g (var. Grada) to 78.16±1.71 g/100 g (var. Tonda), with statistically significant differences between the varieties ($p<0.0005$). Moisture content was higher for the var. Butler (6.02±0.37 g/100 g) and lowest for the var. Tonda (4.86±0.33 g/100 g), with statistically significant differences between the three varieties under study ($p=0.013$). According to the recommendations of the European Union, moisture content of hazelnut kernels should not exceed 6.0% [3]. As for the water activity, the values ranged from 0.54±0.01 (var. Butler) to 0.56±0.01 (var. Grada), but in this case with no statistically significant differences between the varieties. The var. Butler presented the fruits with the higher ash content (2.31±0.18 g/100 g), while var. Grada was the one with the lowest value (1.69±0.16 g/100 g), again with statistically significant differences ($p=0.012$). The var. Grada presented the higher fibre (6.13±0.03 g/100 g) and protein content (22.84±0.18 g/100 g), with statistically significant differences among the varieties in the two cases. The results also showed (Table 1), that according to the limits established for the count of microorganisms at 30ºC and mould and yeast by the National Health Institute Doutor Ricardo Jorge [4] ($<10^6$ CFU/g for microorganisms at 30ºC, $<10^5$ CFU/g for yeast and $<5x10^2$ CFU/g for moulds), all the varieties presented a satisfactory microbiological quality, with statistically significant differences between the varieties in the case of the quantification of microorganisms at 30ºC. Moreover, as it can be observed in Table 1, var. Butler presented the highest values for the microorganisms at 30ºC and also for the moulds and yeast at 25ºC, which may be explained by its higher moisture content. The results of this study are very important to characterize the microbiological quality and also some chemical properties of the three most representative hazelnut varieties cultivated in Portugal.

Table 1. Mean count (log CFU/g±standard deviation) of total microorganisms at 30°C and moulds and yeasts at 25°C of the samples under study (n=3).

Sample	Microorganism at 30ºC[1]	Mould and Yeasts at 25ºC[1]
Grada de Viseu	2.84**±0.03**[b]	2.40**±0.07**
Tonda de Giffoni	2.61**±0.03**[a]	2.46**±0.04**
Butler	2.90**±0.01**[c]	2.47**±0.03**
p-value	<0.005	0.059

[1]Mean values in the same column with the same letter are not statistically different ($p>0.05$)

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Chemical characterization of Cistus ladanifer L. phenolic extract and its antifungal activity against Botrytis cinerea

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Botrytis bunch rot, caused by *Botrytis cinerea*, is an important disease of grapevines in temperate climates worldwide. It can cause extensive economic losses through grape desiccation, rot, and biochemical changes that reduce wine quality. Therefore, it is crucial to identify new compounds, especially natural ones, that are active against *B. cinerea*. Biofungicides from plant origin have been recommended to reduce viticulture's dependence upon synthetic fungicides for the last decades [1]. Cistus (*Cistus ladanifer* L.) is a perennial shrub from the Cistaceae family that can be found in abundance in the Mediterranean's marginal fields [2]. Moreover, cistus phenolic extract has been described as a potential antifungal agent, due to its interesting phenolic composition, including ellagitannins and flavonoids [3]. In this sense, the present study evaluated the antifungal potential of a phenolic extract of *C. ladanifer* from Northeastern Portugal, against *B. cinerea*. Dry leaves from cistus were extracted by maceration using ethanol: water (80:20 *v/v*) as solvent. The phenolic extracts were characterized by HPLC-DAD/ESI-MS and evaluated for their capacity to inhibit *B. cinerea* using the microdilution method. Ellagic acid derivatives (24.3 mg/g extract; 83.8%), flavonoids (3.93 mg/g extract; 13.6%), such as flavonols and flavones; phenolic acids, and derivatives (0.76 mg/g extract; 2.6%), were found in the sample (Figure 1). The most abundant group was ellagic acid derivatives in which punicalagin and punicalagin gallate, were found in the highest amounts (13.3±0.9 and 11±2 mg/g extract, respectively), being these results in line with previous studies [3]. The extract revealed an interesting capacity to inhibit the growth of *B. cinerea* at a concentration of 10 mg/mL, which could be related with the high composition in ellagitannins. Considering the bioactivity of cistus phenolic extract, this natural product could be applied as an alternative to synthetic fungicides used in the prevention and treatment of fungal infections in grapevine, minimizing the environmental and health impacts caused by these chemical agents.

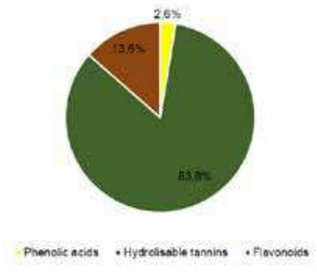

Fig.1. Cistus leaf extract phenolic composition.

Keywords: Grape pathogens; *Botrytis cinerea; Cistus ladanifer* L.; Plant extracts; Biofungicides.

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Fatty acid profile and indices of atherogenicity and thrombogenicity of fish by-product oil for pet food formulations

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The seafood processing industry produces large amounts of fish by-products every year, but only a small portion is directly utilized in animal feed. These by-products, encompassing fish heads, skins, bones, viscera, as well as food-grade pieces resulting from a break in the cold chain or end of shelf-life, contain valuable liposoluble compounds such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1,2]. As a result, the feed manufacturing industry is increasingly interested in extracting these oils for incorporation into pet food formulations. Therefore, this work was carried out to characterize the fatty acid profile and the related atherogenicity and thrombogenicity indices of fish oil obtained from category 3 by-products. A sample representative of the by-products continuously collected by "Grupo ETSA" was received in the lab and immediately lyophilized. The fish by-product oil was extracted using a Soxhlet apparatus and then submitted to a transesterification process for further analyzes of fatty acids by gas chromatograph with flame ionization detection (GC-FID) [3]. The indices of atherogenicity and thrombogenicity were calculated as described in a previous publication [2]. The atherogenicity index (AI) is an indicator of cardiovascular disease risk and expresses the imbalance between atherogenic and antiatherogenic fatty acids, while the thrombogenicity index (TI) translates the tendency for clot formation in the blood vessels [4]. Monounsaturated fatty acids (MUFA) represented the most abundant category of fatty acids in the fish oil given the high relative percentage of oleic acid (36%), followed by the unsaturated palmitic acid (15%) and the polyunsaturated (PUFA) linoleic acid (14%) and the *n*-3 DHA (6.4%). The low atherogenic potential of the fish by-product oil fatty acids was showed by an AI value of 0.4, given the higher percentage of anti-atherogenic fatty acids (MUFA and PUFA) compared to pro-atherogenic fatty acids (C12:0, C14:0, and C16:0). Likewise, a TI value below 0.3 also showed low thrombogenic potential due to the higher proportion of anti-thrombogenic fatty acids (MUFA and *n*-3 PUFA) in relation to pro-thrombogenic fatty acids (C12:0, C14:0, and C16:0). Overall, this study contributes to the valorization of fish by-products as a source of high-quality oil that can be used in the formulation of pet food and other products.

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Determination of selected parameters and compounds in fermented food

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Presently as a result of the multitude of food-microbe combinations, fermented food are thousands of different types of fermented foods and beverages. They have regained popularity as part of Western diets and the fermentation has applied not only to prevent food spoilage but also to produce unique and consumer desired sensory characteristics. Most traditional foods with live bacteria are based on lactic acid bacteria (LAB) fermentation. During fermentation, LAB synthesize vitamins and minerals, and produce biologically active peptides with antioxidant activity. In vegetable fermentations, the growth of LAB enhances conversion of phenolic compounds to biologically active metabolites. Fermentation can also result in new compounds with health-modulating potential. For example, amino acids and derivatives with neurotransmitter and immunomodulatory functions are also synthesized during fermentation [1]. Bousquet et al [2] suggested that consumption of fermented vegetables could be associated with a lower COVID-19 mortality due to their potent antioxidant effect. In summary, fermented food are increasingly appreciated for their properties that reach well-beyond preservation and sensory attributes. Therefore, the systematic and accurate studies of fermented foods quality are very important.
It should be added that consumer trends have shifted towards super foods that not only fulfil basic nutritious requirement, but also exert any number of functional features while being natural without additives. In this context, fermented vegetables and fruits play important role as a source antioxidant compounds, vitamins, peptides and protein derivatives. This type of superfood is becoming more and more popular in Europe, but the number of studies on its quality is still insignificant.
The main goal of project was determination of selected parameters in fermented vegetables and fruits. The samples were purchased from the local health food store and included 13 products. Assay of antioxidant capacity, pH, total acidity, content of total polyphenol, salt, free amino acids and selected biogenic amines were carried out based on the spectroscopic and chromatographic procedures. The obtained results for tested samples were compared by statistical test and in regards of fermented vegetables and fruits quality.

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3.

POSTER PRESENTATIONS

T5

Chemical reactions and interactions of food components

Effect of Fining with Chitosan and k-Carrageenan on Protein Stability, Macromolecular, and Phenolic Composition of White Wines

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White wine protein instability can cause serious economic losses to the wine industry. Wine protein haze formation can occur under high temperatures, throughout storage, or through wine transportation due to the protein self-aggregation phenomena, resulting in light-dispersing particles. This instability needs to be prevented by removing the unstable proteins from the wine, usually by fining, before wine bottling. Bentonite fining is the most effective and used process to avoid protein instability in white wine. However, bentonite fining can affect wine quality, by impacting negatively wine sensory characteristics, and if used in high doses bentonite lees could comprise 5% to 20 % of the wine volume associated with poor settling and presents additional waste disposal challenges [1,2,3]. Therefore, in this work, the effect of fining with sodium bentonite, calcium bentonite, fungal chitosan, and k carrageenan on the chemical composition of the macromolecular fraction, polysaccharides and proteins, phenolic compounds, chromatic characteristics, and protein stability of monovarietal white wines was studied. k-Carrageenan reduced the content of pathogen related proteins (chitinases and thaumatin-like proteins) and the wine protein instability, and it was more efficient than sodium and calcium bentonites. Fungal chitosan was unable to remove pathogen related proteins, chitinases, and/or thaumatin-like proteins, and consequently to heat stabilise the wines. Additionally, fungal chitosan reduced the concentration of wine polysaccharides by 60%. Sodium and calcium bentonite also reduced the concentration of wine polysaccharides though to a lesser amount (16% to 59%). k-Carrageenan did not affect the wine polysaccharide concentration[4]. It was observed, that the application of the different fining agents resulted in a significant but small decrease in the wine total phenols. Overall, the impact of these fining agents on the wine chromatic characteristics was slight. In general, k-carrageenan is appropriate for white wine protein stabilisation, having a more desired impact on the wine macromolecular fraction than the other fining agents, decreasing the quantity of the wine pathogen related proteins without affecting polysaccharide composition.

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Oxidative stability of selenomethionine

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Oxidation of amino acids and proteins can occur in the production of food as well as in various physiological processes, e.g. during ripening and storage, but also in fermentation reactions, e.g. in the production of coffee [1]. In foods, the hydroxyl radical and singlet oxygen are primarily responsible for this, but oxidation by superoxide radical anions and hydroperoxy radicals is also possible. The sulphur-containing amino acids such as methionine are particularly susceptible to oxidation, with the two most important oxidation products being methionine sulphone and methionine sulphoxide [2,3]. Industrial processing of proteins can lead to relative methionine oxidation rates of up to 67% [4, 5]. As a structural analogue, selenomethionine can be incorporated into proteins, so-called selenoproteins. As a trace element, selenium assumes important functions in the human body, e.g., as a component of antioxidant enzymes (e.g. glutathione peroxidase), which protect the organism from cell damage by radicals. Due to its structural similarity, it can be assumed that this analogue of methionine is very susceptible to oxidation. The analogous oxidation products selenomethionine-selenium oxide and dihydroxyselenomethionine have already been detected in biofortified wheat biscuits and in model experiments [6, 7].

In order to assess the influence of different oxidation factors, both methionine and selenomethionine were subjected to different model oxidation systems (e.g., Fenton oxidation, hypochlorite, iodine). Furthermore, the oxidation of Benzoylmethionine and -selenomethionine was performed to ensure the stability of the amino group during the oxidation experiments and to specifically address the reactivity of the side-chains. Quantification of the oxidation products was subsequently performed by UHPLC-MS/MS.

The results of the studies are still pending.

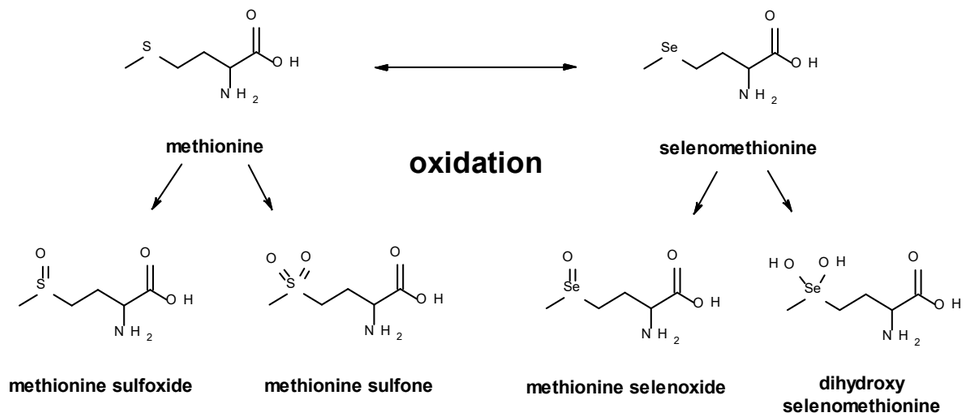

Fig. 1 Oxidation derivatives of methionine and selenomethionine

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POSTER PRESENTATIONS

T6

Chemical changes in food under processing and storage

Functional mango peel powders: what is the impact of different drying methods on their phytochemical composition and antioxidant activity?

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In 2018, mangoes were among the six most-produced fruits worldwide(1). Annually, 15–25 million tons of mango byproducts are generated(2). According to the previous studies, one of the best strategies to valorise mango peels in the food industry is to convert them into powders(2). Hence, this study aims to evaluate the impact of different drying methods on mango peels' nutritional composition, bioactive compounds, and antioxidant activity.

Firstly, mango peels were washed with peracetic acid (PAA) (mango peels to disinfectant solution ratio: 1:1 (kg:L); PAA concentration: 27 mg/mL; disinfection time: 19 min) **(fresh sample (FS))**(1). Then, they were submitted to three different processes: freeze-drying **(freeze-dried samples (FD))**; hot-air drying at 65 ºC for 48 h, with constant air circulation **(hot-air dried samples (HAD))**; and freezing at -20 ºC for 30 days and then hot-air drying at 65 ºC for 48 h, with constant air circulation **(Frozen and hot-air dried samples (FZ+HAD)).** The dry matter, soluble and insoluble fiber, protein, fat, and ash of these four samples were determined according to AOAC methods. Carotenoids were extracted with hexane: acetone (50%:50%; v:v). Moreover, free and bound phenolic compounds were obtained by performing a methanolic extraction and an alkaline and acid hydrolysis, respectively. The main mango peels' carotenoids and phenolic compounds were identified using HPLC analysis. Finally, total free and bound phenolic compounds were quantified through Folin-Ciocalteu method, and their antioxidant activity was evaluated through DPPH and ABTS assays.

Overall, the different drying methods did not impact mango peels' soluble and insoluble fiber, protein, fat, and ash content. Considering the four analysed samples, these parameters ranged between 19.47±0.48 - 20.73±0.44, 19.60±0.62 – 21.04±1.89, 4.93±0.13 – 6.02±0.44, 1.56±0.01 – 1.92±0.03, and 2.05±0.19 – 2.89±0.11, respectively. All of them were expressed in g/100DW. However, drying markedly impacted mango peels' phenolic compounds, carotenoids, and antioxidant activity. All drying methods caused a statistically significant decrease in total free phenolic compounds (FS: 12.76±0.80 mg of gallic acid equivalents (GAEs) / gDW; FD: 10.04±0.17 mg of GAEs/ gDW; HAD: 7.69±0.12 mg of GAEs/ gDW; FZ+HAD: 6.63±0.05 mg of GAEs/ gDW). The main free phenolic compounds identified in all samples were mangiferin (FS: 873.61±71.43; FD: 863.71±6.15; HAD: 647.61±1.86 FZ+HAD: 447.88 ± 5.37), gallic acid (FS: 590±30.93; FD: 650.48±11.85; HAD: 850.29±10.99 FZ+HAD: 523.60 ±10.83), quercetin-3-O-galactoside (FS: 467.20±34.15; FD: 464.31±1.97; HAD: 391.07±4.29 FZ+HAD: 502.41±22.73) and penta-O-galloyl-β-D-glucose (FS: 397.60±22.56; FD: 384.30±5.63; HAD: 362.88±5.03 FZ+HAD: 167.94 ±3.36). Compared with other dried peels, FZ+HAD samples showed a significantly lower amount of these compounds (excluding quercetin-3-O-galactoside). Gallic acid (basic hydrolyse: FS: 476.99±52.51; FD: 42.65±0.94; HAD: 152.66±15.35 FZ+HAD: 207.74±4.23; acid hydrolyse: FS: 83.00±12.98; FD: 12.27±0.16; HAD: 23.25±0.02 FZ+HAD: 28.26±0.76) and 4–hydroxybenzoic acid (basic hydrolyse: FS: 59.26±5.00; FD: 16.69.65±0.69; HAD: 50.39±4.17 FZ+HAD: 46.15±6.23; acid hydrolyse: FS: 23.13±0.75; FD: 2.58±0.14; HAD: 6.12±0.25 FZ+HAD: 5.63±0.50) were the main phenolic compounds obtained in both basic and acid hydrolysis. All phenolic compound amounts were expressed in μg/gDW. Regarding carotenoids, drying also had a negative impact. FS samples contained violaxanthin (2.94±0.05 μg/gDW), lutein (4.83±0.52 μg/gDW), and β-carotene (104.46 ±0.25 μg/gDW), while in dried samples, only lutein (lower than quantification limit) and β-carotene (FD: 36.19 ± 0.53 μg/gDW HAD: 36.06 ± 2.45 μg/gDW; FZ+Oven: 40.65 ± 3.66 μg/gDW) were detected. All drying methods impaired the antioxidant activity of free and bound phenolic compounds. As expected, freeze drying enabled a better preservation of free phenolic compounds' antioxidant activity. No statistically significant differences were found between the antioxidant activity of free phenolic compounds from HAD and FZ+HAD samples. Concerning bound phenolic compounds' antioxidant activity, overall, no significant differences were detected between dried samples. This study showed that mango peel powders had a high amount of fiber, phenolic compounds, and carotenoids, suggesting that they have a high potential to be used as functional ingredients. However, drying processes, namely hot air drying, should be optimized to enable better preservation of mango peels' bioactive compounds and antioxidant activity.

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Effect of High Hydrostatic Pressure on Free Amino Acid and Biogenic Amines in Sausages During Storage

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Sausages are one of the most common meat products that are present on human diets. They have high nutritional value because of their high protein content and other important nutrients. Besides the essential nutrients these products also contain biogenic amines (BA) that may cause food intolerance in sensitive individuals. Novel technologies need to be developed to lower the risk of formation of biogenic amines in high protein containing food products. The aim of this study was to investigate the effect of high hydrostatic pressure (HHP) on the formation of free amino acids (FAA) and biogenic amines (BA) in sausages made from different types of meats (chicken, mangalica, pork, horse, and deer) during storage. The samples were subjected to 600 MPa pressure for 5 minutes before being stored at 12-16 °C for five weeks. Free amino acids and biogenic amines were detected by an Amino Acid Analyzer. HHP treatment had varying effects on FAA and BA content depending on the meat type and storage time. HHP treatment reduced the content of Glu, Ala, Leu, Lys, and 1mHis in chicken sausages while increasing the content of these amino acids in deer and horse sausages. The BA content varied by meat type, with chicken sausage containing the most BA, followed by mangalica, deer, pork, and horse sausages. Putrescine and tyramine were the major amines found in most sausage samples, followed by cadaverine, histamine, spermine, and spermidine. HHP treatment slightly increased the BA content of chicken and mangalica sausages while successfully decreasing the BA content of pork and horse sausages during storage. The effect of HHP treatment on the content of free amino acids and biogenic amines in sausage samples was highly dependent on the type of meat. It is suggested that the effectiveness of the HHP treatment be investigated for each product.

Key words: biogenic amines, high hydrostatic pressure, sausage

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Effect of different drying techniques on the physicochemical and techno-functional properties of sesame protein isolate

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Abstract

Recently, the demand for plant-based proteins has been increasing in terms of use as functional food additives and nutritional supplements. Sesame protein, one of the plant-derived proteins, has an important potential for this purpose. In order to preserve the physicochemical and functional properties of the plant-derived protein, to increase its shelf life and to facilitate its use as an additive, it must be converted into powder form on an industrial scale. For this purpose, different drying techniques are used, which have various effects on protein properties. In this study, the effects of different drying techniques, namely freeze drying, spray drying and oven drying, on structural, chemical and techno-functional properties of sesame protein isolate to converting into powder form. Drying process was carried out after the preparation of protein solution (pH 7.0) about 10% (w/v) total solid in distilled water. Following the drying treatments, the samples were analysed for their particle size, color, protein solubility, free and total –SH groups, emulsifying properties, water and oil holding capacity, foaming properties, electrophoresis (SDS-PAGE) and FTIR. The moisture and protein content of samples obtained through different drying techniques varied from 97.61 to 98.49% and 90.52 to 91.34%, respectively. The lowest particle size was detected in the oven-dried protein powder, while the highest protein solubility (72.62%) was measured in the powders obtained by spray drying. Based on the color properties, spray dried protein has the highest L^* value (83.19) combined with the lowest a^* and b^* values indicating the more creamy white color. Amongst the three protein samples, spray dried sesame protein isolate displayed the highest emulsion activity and stability index values and foaming properties including capacity and stability, which could be attributed to the higher solubility. Also, the spray-dried protein had significantly higher water and oil holding capacities (1.86 g water/g protein and 1.56 g oil/g protein, respectively) than freeze and oven dried protein samples. While the highest free –SH value was obtained for freeze dried sample with a mean of 5.44 µmol/g protein, there was no significant difference in total –SH values among all the drying techniques, ranging from 21.27 to 23.73 µmol/g protein. The SDS-PAGE results indicated that the molecular weights of protein samples did not change depending on drying techniques. Based on the FTIR analysis, partial change was determined in secondary structure of proteins evaluating by α-helix, β-sheet, β-turn and random coil peaks. The present study concluded that the drying techniques used in the conversion of sesame protein solution into powder form had a significant effect on the physicochemical and techno-functional properties of protein.

Keywords: Sesame protein, drying techniques, physicochemical properties, techno-functional properties

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The effect of food processing and packaging of clams on the content of tropomyosin

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In the last several decades, the trend of seafood consumption has significantly increased not only in the countries with a tradition of seafood consumption, but also in other ones [1]. The increase in the world's population and the awareness of healthy food, the globalization of markets, and the development of aquaculture are some of the factors that have led to this trend. The aquaculture of shellfish like clams, mussels, oysters and scallops has been very developed all around the world and the food products based on them have become part of the daily diet for many consumers. In addition, these food products are considered healthy food because of the high content of proteins and essential fatty acids, but their consumption may carry some risks of food allergy. Tropomyosin from shellfish (TPM) is the major allergen responsible for the development of anaphylaxis in persons with food allergy. The content of TPM in shellfish and its bioavailability from food products can have potential influence on the sensitization of consumers to TPM. It is known that food processing can change the bioavailability of food allergens [2]. The main goal of this study was the investigation of how processing and packaging of shellfish samples can affect the content of TPM in them.

For this study, clam *Venerupis philippinarum* was chosen as the species with the highest world aquaculture production [1]. After the purchasing of live clams, the animals were separated into 5 groups for the next treatments: fresh live (control group), freshly removed inner content was kept at +4°C for 3 days (three days` shelf-life), frozen in a plastic bag and kept at -20 °C during 7 days, marinated and kept in a glass jar at room temperature during 8 days and freshly boiled. After processing and packaging of samples, the total protein extracts were prepared in 10 mM sodium phosphate buffer pH 7.4 1M NaCl, 1 mM PMSF and the concentration of total proteins was determined by BCA method. The concentration of TPM in the total protein extracts was determined using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using in-house prepared clams` TPM standard. The content of TPM (µg) in the samples was expressed per mg of extracted soluble proteins, individual animal and grams of soft wet tissue.

The cooked samples have significantly higher TPM content expressed per gram of soft wet tissue compared to all other treatments. Food processing such as freezing, marinating, or extending the shelf-life at 4°C by 3 days has very little effect on the change in TPM content per gram of soft wet tissue compared to the fresh samples. The processing of clams, like cooking or marinating, caused the content of total soluble extracted proteins to be three to four times lower compared to the other three treatments. In these samples the obtained ratio of the total TPM/ total soluble extracted proteins ratio was the highest. This result can be explained by the fact that TPM is thermostable and stays soluble after cooking, while other proteins become insoluble because of denaturation. The lower ratio of TPM/ total soluble extracted proteins was found in marinated samples compared to the cooked samples. The lowest total tropomyosin/ total soluble extracted proteins ratio was found in 3 days' shelf-life.

Table 1. The content of TPM (µg) in clams after processing and packaging of samples. Data are expressed as Mean ± SD of 5 samples in two independent experiments.

Treatment	µg TPM /mg of proteins	µg TPM /individual animal	µg TPM /soft wet tissue
Fresh	4.1±2.1	269.5±67.8	64.9±36.5
Frozen	8.3±6.2	499.1±236.2	109.5±77.4
3 days at +4°C	1.8±0.1	118.3±0.5	31.6±2.5
Cooked	72.3±0.1	992.4±24.1	401.6±74.4
Marinated	11.8±8.0	94.9±78.6	44.3±34.6

Treatments like cooking, marinating and keeping the inner content of the shell at +4°C can significantly affect extractability of proteins, particularly affecting the ratio of major allergen TPM in the total protein extracts. Further studies are needed to examine bioaccessibility of TPM in different biologically relevant fluids (gastric/intestinal) and during digestion in relation to the processing conditions.

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Chemical and colour changes of flavored cold-pressed sunflower oil during long-term storage conditions

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In recent years, flavored vegetable oils have increasingly attracted the attention of consumers but also of researchers because of their oxidative stability. Lipid oxidation, as the main cause of oil deterioration, affects their chemical, nutritional and sensory properties. The rate of oxidative changes is primarily determined by the fatty acid composition and the content of antioxidants (naturally present or added). Since there are doubts about the harmfulness of synthetic antioxidants, there is a growing interest in replacing them with those of natural origin [1]. The use of medicinal herbs and spices for this purpose has gained particular importance since it was confirmed that certain plant species can be used as effective, healthy and environmentally safe substitutes for synthetic antioxidants. In addition to improving oxidative stability, the addition of these plants also improves the sensory properties of common vegetable oils [2].

The main objective of this study was to invetigate the chemical and colour changes of cold-pressed sunflower oil (CPSO) with the addition of essential oils (EOs) of winter savory (*Satureja montana* L.) and basil (*Ocimum basilicum* L.) under long-term storage conditions. The EOs were obtained by hydrodistillation. Samples of CPSO (containing 250, 500 and 1000 ppm EO) and control samples (with 200 ppm BHT and CPSO without any antioxidant) were stored at room temperature for six months. In order to determine a level of oxidative changes, samples were analyzed monthly for peroxide value (PV), anisidine value (AV) and conjugated dienes (CD) and trienes (CT). A computer vision system (CVS) was used for measure the colour [3]. Colour parameters (L^*, a^* and b^*) were used to determine the total colour difference (TCD), yellowness index (YI) and browning index (BI) after addition of EOs and after three and six months of storage. The process of oil aromatization was followed by the sensory evaluation of the samples immediately after addition of EOs.

The results of the oxidative status monitoring showed that the content of primary oxidation products increased faster than the secondary products in all samples. The values of all monitored parameters were the lowest in the sample with BHT, while in the flavored samples they were between the values determined for control samples. It was also found that the winter savory EO was more effective in inhibition the formation of primary oxidation products than the basil EO. From the sensory analysis, it can be concluded that tested flavored oils have excellent sensory quality with an average score ranging from 4.13 (CPSO with 1000 ppm basil EO) to 4.87 (CPSO with 500 ppm basil EO). Odor and taste of the samples with 1000 ppm of both EOs were rated as too intense. Although no colour change was visually detected after the addition of EO, a change between 2.23 and 2.44 was detected by the CVS method, indicating that all samples were different from the control. During the test, the TCD values of all samples increased and the highest values were recorded for the oil samples with the addition of 500 ppm EO (13.29 and 12.99, for the sample with the addition of EO of basil and winter savory, respectively). On the other hand, the values of YI and BI decreased with storage time. As the storage time increased, the L^* and b^* values of the samples increased and the a^* value decreased, resulting in an overall decrease in the values of YI and BI. The unexpected increase of b^* value can be attributed to the formation of the chroman-5,6-quinones which are formed during the partial oxidation of vegetable oils [4].

The results of the monitored parameters show that both primary and secondary oxidation products were formed in all samples, but with different dynamics, and that BHT was the most effective in suppressing the formation of these products. Based on the obtained results, it can be concluded that adequate oxidative stability of the CPSO can be achieved with a higher concentration of EO. On the other hand, the addition of EO is limited by sensory properties of the oil, since only at lower concentration of added EO CPSO, flavored with winter savory and basil EO exhibits excellent sensory properities. Changes in the colour parameters (L^*, a^* and b^*) resulted in an increase in the TCD value and decrease in the YI value. There was no browning of the samples during storage. The results of this study indicate that the used EOs for tested oil and conditions are not sufficiently potent antioxidants to prevent chemical and colour changes. Since basil and winter savory have been proven to have strong antioxidant potential, their use in the form of extracts obtained by various methods could be a method to more effectively stabilize edible oils.

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Effect of stir-frying on the flavor characteristics of oat flour and dough formation

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Abstract: Unlike other grain crops, oats need to be fried in processing, and frying has a direct effect on the flavor characteristics and dough properties of oat flour. Oat flour was divided into raw, semi-cooked and cooked flour according to the degree of frying, and its physicochemical properties and microstructure were studied. The flavor components were determined by electronic nose technology, and the flavor characteristics of oat dough under different treatments were comprehensively evaluated by statistical methods such as principal component analysis, and the formation characteristics of oat dough under the degree of frying and different water temperature and surface were investigated. The results showed that: the straight chain starch content of oat flour increased significantly ($p<0.05$) with the increase of frying degree, and the solubility and swelling tended to increase first and then decrease; the starting temperature, peak temperature and enthalpy of oat flour decreased gradually with the increase of frying time, and frying did not change the crystalline structure of oat starch; the scanning electron micrographs showed that the surface of starch granules of cooked oat flour had more depressions. The cohesiveness of the dough increased with the increase of the mixing water temperature; the aldehydes and alkanes aromatic components of the dough increased significantly in the samples after frying and with hot water. It showed that the fried oat flour had the best processing performance, dough flavor and dough formation characteristics.

The effect of kombucha as a non-conventional starter culture on the chemical composition and free amino acid profile of fresh cheese

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Milk enzymes, starter lactic acid bacteria (LAB), coagulants and non-starter lactic acid bacteria (NSLAB) catalyze the biochemical changes in cheese during its storage [1]. The milk components present in the curd are involved in the enzymatic degradation of proteins. Chymosin or rennet substitutes, starter bacteria and their enzymes, and regular milk enzymes are the major agents involved in the proteolysis of cheese during storage [2]. Kombucha, as a non-nconventional starter culture, has a significant impact on shortening the fermentation time in the production of fresh cheese and increasing its yield. [3,4]. Therefore, changes in chemical composition and free amino acid composition are inevitable.

The aim of this study was to investigate the influence of kombucha inoculum as a non-conventional starter culture on the chemical composition and free amino acid profile of fresh cheese, compared to fresh cheese produced with the traditional starter culture CHN-22 (Chr. Hansen A/S, Denmark). Chemical composition analysis shows that fresh kombucha cheese has higher dry matter, fat, ash, and total protein content compared to traditional fresh cheese (Table 1). After preparation, 15 amino acids were detected in both samples (Fig. 1 a), with the kombucha cheese having a higher content of free amino acids, among which phenylalanine stood out (96.37±0.01 mg/100g). After 30 days of storage, 17 free amino acids were detected in kombucha fresh cheese with a total content of 139.88±0.31 mg/100g (Fig. 1 b), with phenylalanine being the most abundant (84.92±0.05 mg/100g). During storage, the content of most amino acids, with the exception of valine, serine and phenylalanine, increased significantly, especially in kombucha fresh cheese.

Table 1. Chemical composition of fresh cheese made with traditional CHN-22 starter culture and kombucha

Chemical properties	CHN-22	Kombucha
Dry matter (%)	30.36±0.01[a]	48.63±0.01 [b]
Fat (%)	11.5±0.14[a]	23.5±0.00[b]
Ash (%)	0.98±0.01[a]	1.37±0.01[b]
Total proteins (%)	13.52±0.01[a]	23.01±0.725 [b]

* Different letters (a, b) within the same row represent statistically significant difference at significance level P<0.05

a) b)

Fig.1. Free amino acid composition of fresh cheese made with traditional CHN-22 starter culture and kombucha a) after production. b) after thirty days of storage

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Impact of High-Pressure Processing technology on lipid oxidation and antioxidants in Sardines (*Sardina pilchardus*)

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High-pressure processing (HPP) is an innovative non-thermal technology in the food processing sector exhibiting an excellent capacity for microbial inactivation, improving the shelf life of food products and preserving their quality [1]. Sardines is the most relevant pelagic fish of the Mediterranean Sea and a source of multiple bioactive compounds such as ω3 polyunsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, and antioxidants, for instance, carotenoids and tocopherols [1]. However, sardines are prone to microbial spoilage and lipid oxidation during food processing and storage. HPP treatments could be applied to fresh fish to prolong their shelf life. Specifically, sardines, which have been poorly investigated, and the undesired chemical changes such as lipid oxidation that might be induced during the HPP treatments are not well covered in the literature.

The aim of this work is to evaluate the extent of lipid oxidation and bioactive compounds degradation on sardine fillets after different HPP treatments, i.e., samples pressurized at 400 and 600 MPa for 1, 2.5, 5, and 10 minutes, and subsequently stored at (+4°C) for 0, 7, and 14 days. Treated samples and control ones were subjected to lipid extraction at the storage days. All the extracted lipids were analyzed for volatile compounds using headspace solid phase micro-extraction (HS-SPME), together with gas chromatography-mass spectrometry [2]. Carotenoids and tocopherol contents were investigated as well using ultra-high-performance liquid chromatography coupled with a photodiode detector array and fluorescence detectors [3].

The preliminary results showed alterations in the studied lipid dependent on the intensity of pressure and its duration. Volatile secondary oxidation products such as aldehydes and ketones were present in the extracted lipids of HPP-treated sardines at the end of the storage period. Carotenoids were varied to different extents influenced by pressure values of treatment during storage.

HPP conditions seemed to have an impact on the presence of oxidized products while improving the shelf-life of fresh sardines. However, there was no notice of lipid rancidity in the pressurized samples stored for up to 14 days at (+4°C).

Eventually, high-pressure processing is a solution to preserve sardines and maintain their antioxidant content.

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Canned versus home-made: Maillard reaction products in complex food samples

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Maillard reaction products (MRPs) are formed during the non-enzymatic reaction between amino compounds and reducing sugars by thermal processes, storage and preservation of food. MRPs shape the sensory characteristics with regard to taste, scent and color, but are also discussed in the context of the pathogenesis of metabolic diseases [1], culminating in recommendations for a “MRP-free” diet or avoidance of industrial processed food [2]. It is frequently postulated that a consumption of industrially processed foods leads to a higher intake of MRPs [3]. However, little is known about the quantitative relevance of dicarbonyl compounds and glycated amino acids in home-made and/versus industrially processed food.

For the present study, 13 commercially available canned food meals were recreated by home cooking with respect to main ingredients and energy intake. For both meal groups, individual MRPs were analyzed i) after cooking/processing and cooling and ii) after ready-to-eat heating. Quantitation of the dicarbonyl compounds methylglyoxal (MGO), glyoxal (GO), 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal) as chinoxalines after derivatization with o-phenylendiamine and hydroxymethylfurfural (HMF) was perfomed by HPLC-UV [4]. The glycated amino acids (pyrraline, N^6-(5-methyl-4-oxoimidazolin-2-yl)-ornithin (MG-H1), *N*-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine (MG-HCr), N^ε-(carboxyethyl)lysine (CEL), N^ε-(carboxymethyl)lysine (CML)) were analyzed via LC-MS/MS by using the respective isotopologues as internal standards [5].

It could be shown that no pronounced differences were observed between the MRP content of a home-made and the corresponding canned food item. The widely held thesis, both in public and in scientific communities, that industrial production leads to a higher heat load of food and thus to a higher daily intake of MRPs cannot be sustained.

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The effect of enzymatic oxidation, so called tea fermentation, on the antioxidant activity of commertial bagged teas

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Antioxidants are substances that are able to eliminate and inhibit oxidation reactions caused by the reactivity of free radicals, primarily reactive forms of oxygen and reactive forms of nitrogen. Among the most important natural antioxidants are polyphenols, flavonoids, flavanols and vitamins C and E. These substances are also found in extracts from the leaves of the tea tree *Camelia sinensis L. Kuntze*. Depending on the degree of enzymatic oxidation, also called fermentation of tea leaves, teas are divided into unfermented green teas, lightly fermented white and yellow teas, partially fermented oolongs and fermented black teas. Green and black teas are the most commercially consumed. Commercial black and green teas available in Slovakia were tested (bagged teas: Earl Gray Teekane, Royal Earl Gray Sir Winston Tea, Earl Gray Classic Lipton, Yellow Label Lipton, Gold Tea Special Collection Lipton, Black Label Premium Teekanne, Earl Gray Dilmah, Earl Gray Lord Nelson, Green Tea Lord Nelson, Green Tea Teekanne, Geen Tea Sir Winston Tea, Green Tea Greenfield, Green Tea Mistral, Green Tea Tesco, Green Tea Pure Pickwick, Green Tea Basilur). The manufacturers' recommended method of preparing the drink was pouring hot water with a temperature of 100 °C in the case of black teas and 100 °C or 80 °C in the case of green teas. Extracts were prepared from all tested teas with 100 °C water and with 80 °C water, too. The extraction time was followed strictly according to the manufacturer's recommendation (1-5 min.). The content of total polyphenols was determined by reaction with Folin-Ciocalteu's reagent [1], the total antioxidant capacity was determined by the ABTS method [2] and the ability to inhibit the reactivity of free radicals was determined by the DPPH method [3]. The content of total polyphenols in black teas was higher in extracts prepared by 100 °C water than in extracts prepared with 80 °C water. The amounts of polyphenols in black teas were from 264.2 mg to 378.2 mg of gallic acid equivalents in 1 g of dry tea leaves (100 °C), or from 205.9 mg to 340.9 mg of gallic acid equivalents in 1 g of dry tea leaves (80 °C). Earl Gray Dilmah black tea contained the highest amount of polyphenols. Even in green teas, the content of total polyphenols was higher in extracts prepared with 100 °C water. It was from 223.1 mg to 334.4 mg of gallic acid equivalents, or in 80 °C extracts it was from 206.3 mg to 249.9 mg of gallic acid equivalents in 1 g of dry tea leaves. Green Tea Pure Pickwick contained the highest amount of polyphenols. The total antioxidant capacity (TAC) was determined by reaction with the cation radical ABTS and expressed in Trolox equivalents. On average, black teas had a higher total antioxidant capacity (at 100 °C it was from 1.433 to 2.403 mmol TE in 1 g of dry tea leaves, at 80 °C it was from 1.256 to 2.336 mmol TE in 1 g of dry tea leaves). Tea Earl Gray Dilmah had the highest TAC. Green teas prepared at 100 °C showed a TAC of 1.742 – 2.356 TE, resp. at 80 °C 1.424 – 2.207 TE in 1 g of dry tea leaves. Green Tea Pure Pickwick had the highest TAC. At 100 °C, the difference between black and green teas was on average 12.1% in favor of black teas. At 80 °C, green teas had an average of 13.5% lower TAC than black teas. The ability to inhibit reactive nitrogen species (RNS) was determined by reaction with DPPH. In the case of black teas, the inhibition was from 29.3% to 35.5% (100 °C), resp. from 25.3% to 32.1% (80 °C). Yellow Label Lipton tea had the best ability to inhibit RNS. In the case of green teas, RNS inhibition was from 27.6% to 34.6% (100 °C), the most significant inhibition was determined with Green Tea Greenfield. In the extracts prepared with 80 °C water, RNS inhibition was in the range of 25.8-30.3%. Green Tea Tesco had the highest activity. The results show that a higher amount of substances with antioxidant activity is obtained by preparing tea with 100 °C water, and the tested commercial bagged black teas are a better source of these substances than the tested commercial bagged green teas.

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Effect of variety on physicochemical properties of coated chestnuts along short storage

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The present work was performed under the RevestCAST project, which intends to optimise the industrial processes of edible coating application to preserve chestnut along the post-harvest. Chestnut is a perishable commodity that spoils quickly during storage due to weight loss and mould development [1,2]. In the harvest of 2022, the main objective of the work was to study the influence of chestnut variety on the physicochemical properties of coated fruits over three weeks of storage. Three varieties were studied, namely: Martaínha, Longal and Judia. The edible coatings applied were chitosan and chitosan with a rosemary extract. The coated and uncoated samples (control) were stored at room temperature to simulate the retail conditions because, generally, chestnuts are found in supermarkets without temperature control. The physicochemical properties determined were: the number of rotten fruits, external and interior colour, texture, water activity and moisture, ash, protein and reducing sugar contents. Longal was the variety that presented the lowest number of rotten fruits. Regarding the treatments, none of them showed to be efficient in reducing fruit rotting. Concerning the colour, the storage time, and, in some situations, the application of edible coatings may affect this parameter; however, no trend was observed. In terms of variety, Judia presented the highest number of significant differences between treatments and storage time for the interior colour. Regarding texture, the control was the treatment that revealed the highest number of significant differences. These results suggest that edible coating application can induce fewer fruit texture modifications along storage. All water activity values were higher than 0.6, allowing microbial growth. In all situations, the water activity decreased with storage, showing the occurrence of weight loss. In line with this, the moisture content also fell along the storage for the three varieties. The ash content varied between 1.53-2.58%, 1.45-2.19% and 1.58-2.52% for Martaínha, Longal and Judia, respectively, without any trend along storage time. Also, no significant differences were observed between treatments. Concerning the proteins, when considering 0 and 3 weeks, some significant differences were observed between varieties and treatments, showing Judia the highest values, mainly in control and with chitosan coating. With only two exceptions, no significant differences were observed between the 0 and 3 weeks for each treatment. Similar results were obtained for the reducing sugars. In the beginning, the Judia control (uncoated) and Judia with chitosan coating presented the highest means (0.90 and 0.76 g glucose/100 g d.w, respectively). Nevertheless, after three weeks, this behaviour was no more observed, not being detected significant differences between the treatments. With two exceptions, no significant differences were observed between the 0 and 3 weeks, suggesting that the formation of reducing sugars through starch and/or sucrose hydrolysis was not evident.

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A critical insight into the changes of aroma-active compounds during fruits and vegetables processing

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Flavor plays an important role in food choice, directly affecting consumer acceptance. To better evaluate the effect of processing technology on the characteristic aroma, the molecular sensory approaches, including odor activity values and detection frequency analysis, were first applied to the identification of aroma-active compounds in selected fruits and vegetables (raspberry, melon, and peas). The effect of high-temperature short-term sterilization, high-static-pressure processing, and fermentation on the sensory and aroma profile of melon juice, raspberry juice, and pepper, respectively, were investigated by the application of quantitative descriptive analysis, electronic nose and SPME-GC/MS. Different processing exhibited a varying effect on the release of aroma-active compounds in selected fruits. To reveal the variation mechanism of characteristic aromas in selected fruits during processing, the methods of the exogenous addition of isotope-labeled precursors were used to clarify the formation of off-flavor in heat-sterilized melon juice, while a combined method of targeted metabolomics, pathway enrichment, and correlation analysis was used to analyze the formation pathways of aroma-active compounds in baked peas, fermented pepper and jujube pulp. These findings not only enrich the basic theory of flavour chemistry in fruit and vegetable during processing but also provide a scientific basis for the development of the control of flavor quality in processed fruit and vegetables.

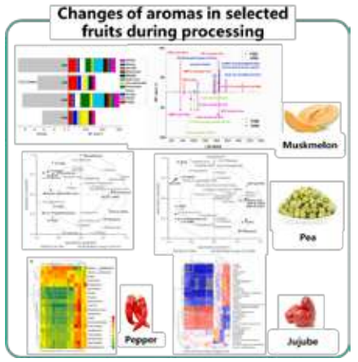

Fig.1 Graphical abstract

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α-Dicarbonyl compounds in honeys and honey-related products

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Dietary α-dicarbonyl compounds are one of the main groups of compounds that are formed during sugar caramelization and the Maillard reaction. Other sources in food may be fermentations and lipid oxidations. αDicarbonyl compounds are key intermediates in the formation of flavour-active, coloured, potentially toxic and redox-active products during non-enzymatic browning reactions [1]. Relatively high levels of α-dicarbonyl compounds, mostly (deoxy)glycosuloses and fragmentary ones such as methylglyoxal, are expected in foods that are rich in monosaccharides and reducing oligosaccharides, having lower water activity value, with the limited possibility of αdicarbonyl compounds to react with other food components and that are heat-treated or long-term stored. Among such foods are, for example, fruit juices (concentrates), sugar-sweetened soft drinks, syrups, and honeys [2]. Fresh honey is a supersaturated liquid with a very high amount of glucose and fructose and some minor oligo- and higher saccharides as well as free amino acids and enzymes. Honey is well-known not only as natural sweetener but also for its healing potential. In addition to their main role in honey ageing and browning, αdicarbonyl compounds may be partly involved in antibacterial effects of honey [3].

This work deals with the quantity and profile of α-dicarbonyl compounds in honeys available on the Czech and Slovak market and in some by-products and products from honey, such as royal jelly and mead. α-Dicarbonyl compounds were analyzed as their quinoxaline derivatives using HPLC-PDA and LC-MS methods. The differences in α-dicarbonyl compounds were studied in multifloral, some monofloral and honeydew (forest) honeys. The effect of heat treatment and storage was evaluated in honeys matured naturally as well as in several accelerated ways. The effects of creaming, fructose/glucose ratio, content of other saccharides, colour, reducing power and some other parameters on the amounts of α-dicarbonyl compounds were investigated. To describe the potential transformation of α-dicarbonyl compounds, the amounts and development of some Maillard attributes and more advanced products such as 5-hydroxymethylfurfural were monitored. The amount and composition of αdicarbonyl compounds depend on both the origin and treatment of honey and honey-derived products.

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POSTER PRESENTATIONS

T7

Food adulteration, authenticity and traceability

Legume authentication method based on free amino acid composition

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Legumes belong to the oldest and the most significant group of food. They are rich in proteins, fiber, carbohydrates, vitamins and minerals. Legumes do not contain gluten so they become more popular lately, especially in bakery industry. The aim of this work was to determine the authenticity of legumes: bean, green bean, faba bean, peas and grass pea. The authentication method was based on amino acid composition. Free amino acids were extracted from the legume samples and derivatized as described in [1]. The analysis was performed using GC-MS device with injection volume of 0.2 µL in splitless mode. Peak surface areas of identified amino acids were collected and data matrix was created for further data processing. Multivariate statistics were shown to be a very useful processing tool in the field of food authentication and fraud detection [2]. Linear discriminant analysis (LDA) was performed to classify the samples according to their botanical origin. LDA score plot is presented in Fig. 1. Green bean samples are marked in purple and are located in the first quadrant of the LDA score plot. Beans, marked in red, are located mostly in the fourth and partly in the first quadrant, but clearly separated from the green bean samples. Samples of faba bean are marked in yellow and located in the second quadrant, while peas (green) and grass pea (blue) are grouped together in the third quadrant of the LDA score plot. Even though peas and grass pea are in the same quadrant, it can be found that they form two separated clusters with an exception of one peas sample (28). Proposed method based on GC-MS analysis of free amino acids coupled to LDA as a multivariate statistic tool, shows a great potential in legume authentication.

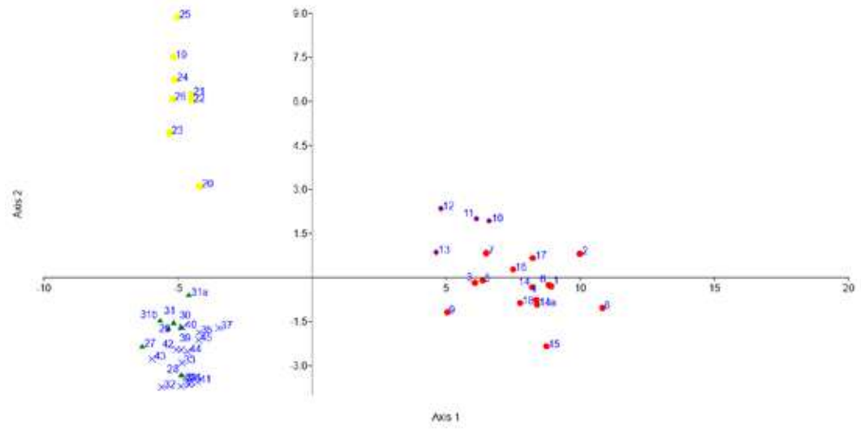

Fig.1. LDA score plot of legume samples separation according to their botanical origin

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Food fraud in Europe - a 5 year overview

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Food fraud (also agri-food fraud) is one of important challenges related to food safety in the food-supply chain. Food fraud has negative effects on both consumers and food business operators. There is no clearly defined definition of food fraud in the European Union (EU) legislation [1] compared to food standards [2]. However, cases that include 1) violation of EU legislation, 2) consumer deception, 3) perpetrators economic advantage and 4) intentional non-compliance, are considered fraud [3]. Every year different food fraud cases are discovered around the world and news of different scandals reach the consumers. With improvements in international cooperation and great scientific effort in different method development significant progress has been made.

The aim of our study was to review the significant food fraud events that occurred in Europe in a five year period (from 2017 to 2021), with an emphasis on the significance of food fraud cases among different hazard categories and the food products that were more commonly affected by food fraud. The examined events were reported by the Alert and Cooperation Network (ACN) and Rapid Alert System for Food and Feed (RASFF). ACN and RASFF, which is a part of ACN, are important for the exchange of agri-food related information between EU Member States. Food fraud cases were very diverse and in some cases even involved internationally coordinated actions of cross-border investigations and operations.

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POSTER PRESENTATIONS

T8

Novel methods for food chemistry

MALDI-TOF-MS for protein profiling and classification of food. Application to quinoa grains

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Quinoa is a grain originary from the Andean region and can adapt to very different agroecological conditions. It is considered a superfood with a high content of lipids, vitamins, minerals and, especially, proteins, hence its consumption is rapidly increasing [1-3]. This raising interest in quinoa has propelled the demand and cost, being quinoa foodstuff highly susceptible to adulteration with cheaper cereals [4]. In this study, we describe a novel approach for protein profiling, peak detection and classification of commercial quinoa grain varieties based on the combination of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and chemometrics [2]. First, we developed a MALDI-TOF-MS method to obtain characteristic mass spectra profiles of soluble protein extracts from different quinoa varieties from Peru and Bolivia. Then, MALDIquant was used to accurately detect the peaks, with characteristic m/z and intensity values, which constitute the MALDI-TOF-MS protein fingerprints. Finally, unsupervised and supervised multivariate data analysis methods (i.e. principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), respectively) were applied to efficiently classify and differentiate the quinoa varieties, while tentatively identifying the most important proteins for the discrimination [1,2]. The proposed methodology could find application in quality control and food fraud prevention programs. Furthermore, it could be also applied to protein profiling of other food products, presenting complex mass spectra profiles with highly overlapped peaks.

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Spectrofluorimetric characterization of ozonated olive oils

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Ozonated olive oils are commonly used for the treatment of skin diseases (1). The reaction between ozone (O_3) and olive oil's fatty acids produces a series of compounds (aldehydes, peroxides and ozonides) with healing properties. Among them, the bactericidal activity stands out (2). The analytical techniques commonly used to detect and quantify the above-mentioned compounds are nonspecific, economically expensive, and require a large investment of time (3). Spectrofluorimetric techniques have been widely used to characterize olive oils with different purposes (analyse the quality of the oils, detect adulterations, etc…) in a specific, fast and economic way (4). However, no articles have been found in the bibliography in which these techniques are used for the characterization of ozonated oils. The development of this technique for the detection and quantification of peroxidised compounds in ozonated olive oils would be a great novelty for the scientific community. Part of the beneficial properties of olive oil for health reside in the presence of these phenolic compounds (5). Until now it is not known how the ozonation process affects phenolic compounds and to what extent they disappear as the amount of ozone applied increases. Our results have shown (see Fig. 1) that spectrofluorimetric techniques could be very useful for detecting and quantifying the oxidized compounds responsible for the curative properties of ozonated olive oil. As it is shown in Fig.1, as the duration of the ozonisation time increases, the intensity of the oxidised compounds increases as well (ex ʎ 300-400, em ʎ 300-400) and the staining corresponding to the polyphenols decreases (ex ʎ 260-310; em ʎ 310- 370). In addition, this novel technique would allow quantifying the intensity and subsequently the concentration of these compounds which, as can be seen, goes from 148 i.u. in the untreated oil, up to 643 i.u. in the treated olive oil (ozonated for 24h).

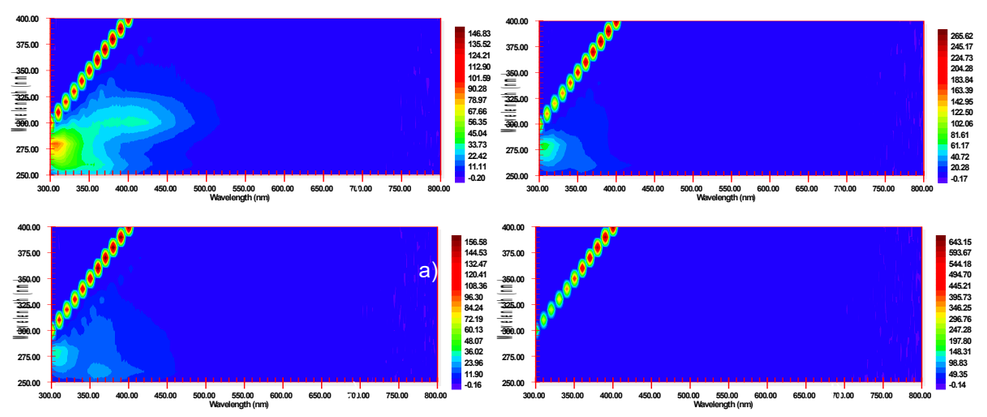

Fig.1. Excitation emission matrix of a) untreated olive oil, b) 12h ozonated olive oil, c) 16h ozonated olive oil and d) 24h ozonated olive oil.

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Determination of antioxidant properties of turmeric spice extracts prepared by ultrasound assisted (UAE) and classical solvent extraction (CSE)

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Turmeric spice is obtained by grinding the rhizome of *Curcuma longa* (Fig. 1b) – plant from ginger family (Zingiberaceae). India is the world's leading producer of this spice, with a share of 80%. Turmeric is used in cooking as a spice, a coloring agent, a medicinal agent in folk medicine, and in recent years as a supplement in official medicine. Turmeric is a source of over 100 compounds, the main one being curcumin. Curcumin is responsible for numerous medicinal effects: anti-cardiovascular, anti-inflammatory, antibacterial, antifungal, antiviral, antidiabetic, skin protective, radioprotective, antigastrointesinal properties, antioxidant, immunomodulating, anticarcinogenic, and Alzheimer's [1].

In this paper, the antioxidant properties of ground turmeric spice, purchased in a local market, were examined. Extracts (Fig.1a, 1c) were prepared using 80% acetone and warm water (50 ℃), by ultrasound assisted (UAE) and classical solvent extraction (CSE) techniques. Antioxidant properties were determined using CUPRAC, FRAP, TAC and DPPH$^{\cdot}$ assays, previously described by Kilibarda et al. [2].

(a) (b) (c)

Fig.1. Turmeric: a – acetone extracts; b – rhizome of *Curcuma longa*; c – water extracts

The obtained results (Tab.1) indicated a statistically significantly higher antioxidant activity of acetone extracts compared to water extracts, in all assays. The extraction technique (UAE and CSE) did not statistically significantly affect the antioxidant properties of extracts in all assays, except for DPPH$^{\cdot}$. In DPPH$^{\cdot}$, the highest antioxidant activity was observed in UAE acetone extract. Contrary, UAE water extract exhibited the lowest antioxidant activity.

In general, turmeric is considered as a spice with high antioxidant activity and is recommended in the treatment of various illness [3].

Table 1. Antioxidant properties of turmeric

Extraction technique	Solvent	CUPRAC (mg/g AAE) mean ± SD	FRAP (mg/g AAE) mean ± SD	TAC (mg/g AAE) mean ± SD	DPPH$^{\cdot}$ (% Inh) mean ± SD
CSE*	80% Acetone	3.27 ± 0.27 ^{a}	2.57 ± 0.05 ^{a}	14.83 ± 1.02 ^{a}	71.23 ± 0.52 ^{b}
UAE**		3.29 ± 0.27 ^{a}	2.42 ± 0.19 ^{a}	15.84 ± 0.80 ^{a}	76.10 ± 0.05 ^{a}
CSE*	Warm water (50°C)	2.00 ± 0.05 ^{b}	1.46 ± 0.05 ^{b}	5.04 ± 0.25 ^{b}	13.69 ± 1.10 ^{c}
UAE**		1.94 ± 0.02 ^{b}	1.33 ± 0.11 ^{b}	6.19 ± 0.34 ^{b}	4.68 ± 0.29 ^{d}

*CSE – classical solvent extraction; **UAE – ultrasound assisted extraction. Different letters in the some column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

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Green extraction of phenolic compounds and carotenoids from pulp and peel of mango criollo by ultrasound assisted extraction with deep eutectic solvents

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Introduction. New green solvents and innovative extraction technologies (ultrasound, high-pressure processing, microwave, pulsed electric fields, supercritical fluid extraction, etc.) and combination of both are employed to reduce the use of petroleum-based solvents to obtain safer extracts in line to promote green and sustainable process. Deep eutectic solvents (DES) have been studied as promising green alternatives to replace conventional petroleum derived solvents in the extraction of bioactive compounds from agrifood by-products due to their unique physicochemical properties, such as chemical and thermal stability, no flammability, low toxicity, high electrical conductivity, and a good solubilizing capacity for determined compounds (1). Mango (*Mangifera indica*) criollo is found in the northeast region of Argentina. These under commercialized fruits can be exploited as a source of bioactive compounds (2). **Objective**. To evaluate the capacity of different DES combined with ultrasound-assisted extraction (UAE) for the extraction of phenolic and carotenoid compounds from peel and pulp of mango criollo.

Material & Methods. Peel and pulp products from mango criollo harvested in Corrientes (Argentina) were extracted with four DES (ratio1:60 for pulp and 1:30 (w:w) for peel). DES were prepared by mixing the desired molar proportions of HBA and HBD (table 1) under constant agitation at 70±2 °C (3). Ultrasound equipment (500 Watts-20 kHz) with a probe tip of 19 mm and fixed at 60% of amplitude during 5 min was used. A clean-up phase was done with C-18 sep-pack cartridges. Total phenolic compounds (TPC), polyphenolic and carotenoid compounds by HPLC-DAD and antioxidant activity (AA) ($DPPH^{\cdot}$, $ABTS^{\cdot+}$) were determined. Results were compared with conventional extraction (CE) with MeOH/H_2O (80:20) for polyphenolic compounds and diethyl-ether/petroleum ether (1:1) for carotenoids (4 & 5). **Results**. DES-4 values in pulp for total carotenes by HPLC (38.27±6.62 µg/g dw), mainly ß-carotene, corresponded with a 90% of carotene content recovery respect to CE. DES-4 values in pulp for TPC (12.10±1.08 mg GAE/g dw), $DPPH^{\cdot}$ (49.44±3.44 µmol TEAC/g dw) and $ABTS^{\cdot+}$ (64.05±6.16 µmol TEAC/g dw) were 4, 8 and 6 times higher than those found in CE. In peel, DES-4 was also effective for TPC and AA meanwhile DES-2 provided the best results for total carotenoids, mainly ß-carotene and lutein. DES-2 values in peel for total carotenoids (92.03±4.7 µg/g dw) corresponded with 71% of carotenoid content recovery respect to CE. DES-4 value in peel for TPC (23.06±1.22 mg GAE/g dw), $DPPH^{\cdot}$ (241.12±2.01 µmol TEAC/g dw) and $ABTS^{\cdot+}$ (537.99±2.36 µmol TEAC/g dw) were 4, 10 and 22 times higher than those found in CE. **Conclusions**. DES-4 provided good yields for the extraction of both carotenoids and phenolic compounds and antioxidant activity in pulps, but in peel DES-4 provided better results only for TPC and AA and DES-2 for total carotenoid content.

Table 1. Deep eutectic solvents (DES)

	HBA	HBD	Other	Molar ratio	Viscosity-cps
DES-1	β-alanine	DL-Malic acid	H_2O	1:1:3	750 ± 30
DES-2	β-alanine	Citric acid	H_2O	1:1:3	2350 ± 25
DES-3	Choline chloride	Ethylene glycol	----	1:2	150 ± 15
DES-4	Choline chloride	Glycerol	-----	1:2	450 ± 20

HBA, hydrogen bond acceptors; HBD, hydrogen bond donors.

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Citrus sinensis: Comparative Untargeted Metabolic Profiling Using Liquid Chromatography-Mass Spectrometry and Multivariate Data Analysis to Uncover Authenticity

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Citrus trees bear some of the most popular fruits are grown globally for food [1], medicinal [2] and other industrial applications [3] with a total annual production of nearly 85 million tons [4]. Asia is the original home of *C. sinensis* [5], which is now found all across the Pacific and warm regions of the globe [6]. Ailments include constipation, cramps, colic, diarrhea, bronchitis, cough, cold, obesity, menstruation disorders, angina, hypertension, anxiety, depression, and stress have all been treated with *C. sinensis* traditionally [7,8,9]. Flavonoids, steroids, hydroxyamides, alkanes and fatty acids, coumarins, peptides, carbohydrates, carbamates and alkylamines, carotenoids, volatile compounds, and nutritional components like potassium, magnesium, calcium, and sodium have all been found in the fruits, peel, leaves, juice, and roots of *C. sinensis* [8,9,10]. Therefore, phytochemical evidence is highly demanded to support the potential nutraceutical value of orange fruit.

The present study aimed to utilize multi-targeted metabolic profiling technique *viz*., nLC–MS to assess for metabolites heterogeneity (Fig. 1) among the different parts of *C. sinensis* (albedo, flavedo, and juice) presenting the first comparative insights into metabolite profiles derived from various parts of Valencia orange fruits collected from different countries. Nano-liquid chromatography coupled to a high-resolution electrospray-ionization quadrupole-time-of- flight mass spectrometer (nLC-ESI-qTOF-MS) led to the detection of 66 metabolites belonging to different classes including phenolic acids, coumarins, flavonoid glycosides, limonoids, terpenes, and fatty acids identified based on their MS^n spectra. Eleven metabolites were detected for the first time in *Citrus sinensis:* citroside A, sinapic acid pentoside, di-hexosyl-diosmetin, apigenin-*C*-hexosyl-*O*-pentoside, chrysoeriol-*C*-hexoside, perilloside A, hydroxy-sphingenine, xanthomicrol, coumaryl alcohol-*O*-hexoside, gingerol and ionone epoxide. Comparing different fruit parts, a number of flavonoids with proposed bioactivities, i.e. naringenin-*O*-hexoside, hesperetin-*O*-deoxyhexoside, sakuranetin-*O*-hexosyl-*O*-deoxyhexoside, and demethylnobiletin, were completely absent from the juices, but present most prominent in the peel. Hence, the peels have considerable prospective for the development and utilization in the future. The comprehensive nLC-ESI-MS/MS metabolic profiling followed by multivariate data analyses (Figure 1) suggested that the differences between orange parts were much more obvious than the geographical source, with flavedo peel from Uruguay being most distant from others attributed to the abundant levels of hydroxylinoleic acid, nobiletin, dihydroxy-trimethoxyflavone, demethylnobiletin and tangeretin. Generally, albedo was richer in mono-methoxylated flavonoids, while flavedo was richer in poly-methoxylated flavonoids and hydroxylated fatty acids. Differences among orange samples were primarily due to the diversity of flavonoids and coumarins, which can affect the product quality of orange parts.

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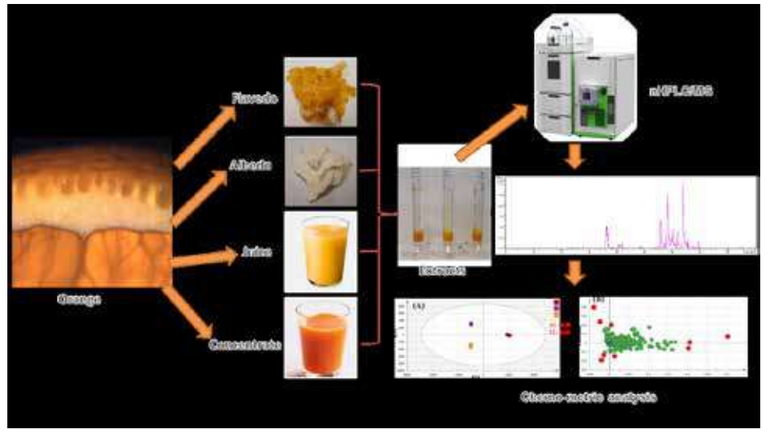

Figure 1. Overview of sample preparation and chemical profiling of different orange compartment.

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Optimization of ultra-sound assisted extraction and conventional solvent extraction of TPC and DPPH from sea buckthorn pomace and hempseed hull

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Globally, around 1,3 billion of tons of food waste is created every year. Vegetal waste (of fruit and vegetable origins) contributes to nearly 60% of this amount, exceeding all other types of food wastes (FAO, 2011). The reason behind such huge amount of vegetal waste is due to growth of population and a shift to more vegetarian based diets followed by high demand of fruit and vegetable production, poor handling, and error during production as well as retailers and consumers behavior towards wastage (Malenica et al., 2022). Vegetal waste contributes significantly to environmental pollution due to current disposal methods such as landfills and disposing of waste in waterbodies (Wadhwa & Bakshi, 2013). In addition, vegetal waste due to its high moisture is prone to quick microbiological contamination, which further affects environmental pollution (Coman et al., 2019). However, vegetal waste is a rich source of bioactive compounds which is the reason why numerous innovative waste management strategies have been implemented such as extraction of bioactive compounds and their utilization in food, pharmaceutical and cosmetic industries (Sagar et al., 2018). Polyphenolic compounds are plant secondary metabolites which exhibit numerous health promoting activities with emphasis on their high anti-inflammatory activity and anti-oxidant activities (Fratianni et al., 2019). Present study involved evaluation of total phenolic content followed by antioxidant analyses and evaluation of DPPH, using ultrasound-assisted extraction and conventional solvent extraction. The first aim of the study was optimization of ultrasound-assisted extraction parameters to gain the maximum yield of TPC and DPPH. For the optimization of parameters in ultrasound-assisted extraction, the response surface method with Box–Behnken design was used. The influence of time and ultrasound amplitude was assessed. The analyses were carried out with spectrophotometric method. The optimal extraction conditions for hempseed hull were extraction time of 17, 5 min with 35% amplitude and for sea buckthorn pomace it was 30 min with 20% amplitude (70% ethanol, *w*/*w*). The second aim of the study was optimization of conventional solvent extraction, where the influence of different solvent was evaluated as well as the mass of sample per 50ml of solvent. Both hempseed hull and sea buckthorn pomace showed optimal extraction conditions with 2,5g per 50ml of solvent while the maximum yield of TPC for hempseed hull was achieved with 70% of Ethanol and for sea buckthorn pomace 96% of Ethanol. Some more interesting results will be presented.

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Synthesis and characterization of ReS_2-CB[n]-0D nanodots Development of an analytical method for Ponceau-4R determination

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Due to their small size, the nanodots of transition metal dichalcogenides have unique properties such as photoluminescence, which allows their application in the development of analytical methodologies for the determination of certain analytes. The interaction of the analyte with the nanodots may lead to an inhibition of the intensity of the native fluorescence of the nanomaterial, if the analyte absorbs part of the emitted photons. This inhibition allows the indirect detection of non-fluorescent analytes, such as synthetic colourants that are used in drinks and sweets.[1]

In this work, rhenium disulfide nanodots (ReS_2-0D) have been synthesized in the presence of Cucurbi[n]uril, ReS_2-0D-CB[n], in order to evaluate the effect of this macrocyclic receptor in the interaction with Ponceau-4R. The synthesis was performed following two different strategies: "top-down" and "bottom-up". The first one is based on liquid exfoliation in a green solvent such as cyrene assisted by ultrasound and the "bottom-up" on an hydrothermal reaction employing cysteine and ammonium perrhenate as precursors. In addition, the nanodots were synthesized in the absence of the receptor, ReS_2-0D, for comparison. The obtained ReS_2-0D and ReS_2-CB[n]-0D nanodots were characterized by spectroscopic techniques (UV-Visible absorption and fluorescence) and atomic force microcopy to study the nanodots structure and size. The interaction of ReS_2-0D and ReS_2-CB[n]-0D nanodots with the analyte was studied by calculating the Stern-Volmer constants. After selecting the most suitable conditions, the developed method was applied for the fluorescent determination of Ponceau-4R.

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Application of a flow injection system with carbon paste/copper Schiff base composite electrode on the ascorbic acid determination

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An amperometric sensor with carbon paste/copper Schiff base composite electrode was applied to the ascorbic acid determination. A simple flow injection system was used for bringing all solutions to the flow through the amperometric cell. A tetradentate Schiff base copper(II) complex with ethylenediamine-bridge and two phenyl groups as substituents were used for carbon paste electrode modification (Figure 1) [1]. The modified sensor showed increased sensitivity toward ascorbic acid oxidation by 60% compared to the unmodified carbon paste electrode and a decrease of oxidation potential by 200 mV.as reagent the sensor gave a linear response for up to 75 µmol/L of ascorbic acid. Optimal FIA conditions were a working potential of 0.6 V *vs*. Ag/AgCl, sample loop volume of 50 µl, reaction loop volume of 100 µl, and 1 ml/min flow rate. Briton Robinson buffer was used for sample preparation (0.04 mol/L, pH 5). Under optimized flow parameters, working potential, gasket thickness, sample and reaction coil volumes, and flow rate, the developed flow system gave a linear response from 5 to 75 µmol/L of ascorbic acid. The calculated limit of detection was 4.5 µmol/L, calculated as 3σ/s which corresponds to the absolute value of 40 ng for the 50 µL sample loop. The precision for six consecutive injections of 5 and 75 µmol/L ascorbic acid solutions was determined as relative standard deviation and it was 5.8% and 1.5%, respectively.

The flow injection system enabled up to 120 analyses per hour. An optimized flow system was applied to the determination of ascorbic acid in effervescent tablets (ET1 and ET2), drinks (MD) and supplements (MS1 and MS2), and tea sample, after dilution of samples only. The obtained FIA results were statistically compared with titration, and, at a 95% confidence level, there is no significant difference between the means found for both methods, since the calculated t value (1.455) is lower than the critical t value (2.571) (Table 1).

Fig.1. The structure of copper(II) Schiff base complex

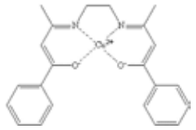

Table 1. The concentration of AA in various samples obtained by FIA and titrimetry (n=3)

Sample	FIA-AMP (mg/g)	Titration (mg/g)	Declared value (mg/g)
ET1	19.2±1	18.8±2	20
ET2	17.5±1	20.0±2	20
MD	1.65±0.25	1.95±0.3	2.1
MS1	628±40	667±20	658
MS2	286±5	297±10	205
Tea	8.7±0.2	10.7±0.2	10

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Raman spectroscopy as a tool for chemical characterisation of 12 Serbian fruits

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Nowadays, Raman spectroscopy is widely utilized for analyzing various plant materials which provides data about the chemical composition and structural characteristics (“fingerprint”). It is also used for semi-quantitative and quantitative analyses [1,2]. This paper reports the evaluation of phytochemical composition of fruits that commonly grow in Serbia using Raman microspectroscopy, and the differentiation between the samples according to their chemical composition by applying Principal Component Analysis (PCA) of the obtained Raman spectra. Twelve fruits belonging to the families Rosaceae (red raspberry - Rubus idaeus L, blackberry -Rubus ceasius L., strawberry - Fragaria vesca L., rosehip - Rosa canina L., plum - Prunus domestica L., blackthorn - Prunus spinosa L., sour cherry - Prunus cerasus L., apple - Malus pumila Miller, common hawthorn - Crategus monogyna Jacq., medlar - Crataegus germanica L.), Grossulariaceae (red currant - Ribes rubra L.) and Ericaceae (blueberry - Vaccinium myrtillus L.) were studied. Fruits were selected with respect to their significance in every-day diet and use in food industry. For fruits, Raman scattering was excited by wavelength of 785 nm using XploRA Raman spectrometer. PCA was performed using PAST software [3].

Food processing is essential to preserve and extend the shelf life of food products. Most of the studied fruits are intended for fresh consumption, whereas all of them can be processed. Apples, cherries and plums are processed, by juicing, making syrups, fermentation or canning [4]. Berries could be used in products like fruit pulp, jams, juices, and nectars [5]. The blackthorn fruits are mostly used in jellies, syrups, vinegar, and conserves as well as for liquor making. Rosehip is used in the making of traditional probiotic drinks, soups, various beverages, whereas the fresh or dried fruits of hawthorn are used to make preserves, teas, and food supplements [6]. Medlar fruits are used to make jams, marmalades, jelly, candy, sauces, and wines [7].

The differences between the fruit samples related to the composition and structural diversity of the detected compounds were observed in the spectra, which confirmed the complexity of the studied fruits. The obtained spectra included characteristic bands that may be associated to the most important compounds found in fruits, carbohydrates, carotenoids and phenolic compounds [1,2]. The band characteristic for the (C=C) structural feature of phenolics (around 1600 cm-1) was medium intensity in the Raman spectra of some berries (blackberry, strawberry, red currant), sour cherry and medlar, whereas a very week intensity band was observed in the raspberry and apple spectra. The band associated to (C=C) of flavonoids was weak and medium intensity in the spectra of hawthorn and blueberry, respectively. The most abundant macronutrients in the majority of the studied fruits are carbohydrates, and the bands related to this class of compounds appeared with different intensity in the 400-1460 cm-1 region. The carotenoids “fingerprint” was dominant in the rosehip and hawthorn spectra. The score plot of the first and second PCs described 66.18% of the data variance. It can be seen that the data along the PC1 axis can be divided into eight classes of objects: cherry, plum, thorn, blueberry, blackberry and apple differed from other fruit samples in carbohydrate content (according to higher intensity loadings at 393 and 1066 cm-1), while hawthorn, rosehip, currant, strawberry and raspberry differed from the previously mentioned fruits in carotene content (loadings at 998, 1150 and 1516 cm-1). PC2 described mainly the similarities in carotenoid content between hawthorn, rosehip, sour cherry and plum, suggesting a similar carotenoid profile of these fruits. The obtained results confirmed the differences in the chemical profile of the studied fruits. Raman spectroscopy as a non-destructive, simple, rapid, low-cost and eco-friendly method combined with PCA, could be used as a valuable tool for providing an insight into chemical profiles of the most consumed Serbian fruits.

Acknowledgments: This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200116) and CNPq.

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Targeted UHPLC-MS/MS and DNA-based methods: a complementary approach for the botanical authentication of ginkgo plant food supplements

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Medicinal plants are widely used in plant food supplements (PFS), which are legally considered as foods under Directive 2002/46/EC. Among medicinal plants, *Ginkgo biloba* is one of the most consumed and broadly included as an ingredient in PFS with claimed activity for enhancing memory and cognition. The main pharmacologically active compounds reported in *G. biloba* are flavonol glycosides and terpene tri-lactones (bilobalide and ginkgolides), these last being ginkgo-specific and believed to cross the blood-brain barrier due to their lipophilic character, acting on the central nervous system [1]. The large demand of *G. biloba* leaf in the global market and the wide consumption of ginkgo products makes them potential targets for economically motivated adulteration by the substitution with other plant species of lower cost, such as *Styphnolobium japonicum* rich in flavonol glycosides, or by directly adding synthetic phytochemicals, mainly low cost flavonol aglycones such as quercetin. Therefore, this work aimed at comparing DNA and chemical-based approaches to authenticate the botanical origin of ginkgo-containing PFS. In the first approach, a previously validated specific real-time PCR with a hydrolysis probe targeting *G. biloba* DNA [2] was used while the second approach consisted of the development and validation of an ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) method targeting the main compounds of *G. biloba* and flavonol aglycones. Both systems contributed to identify possible authenticity issues in commercial samples. The combination of results suggested that 4 out of 19 samples were adulterated. Two samples showed evidence of the addition of quercetin from other source than ginkgo, while for other two samples results suggest a reduced amount of ginkgo material. Both DNA and chemical approaches proved to be efficient, accurate and robust tools, providing complementary results that are useful for the control of PFS.

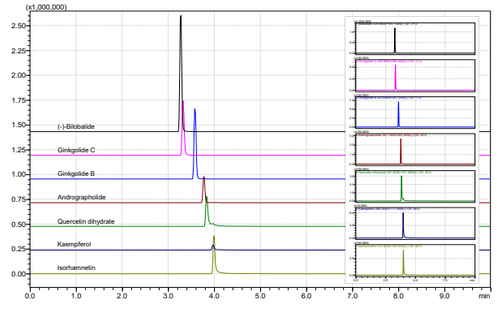

Fig.1. Overlaid individual chromatograms of the compounds analysed in the negative ionisation mode.

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Mos_2 Quantum Dots- Based Optical Sensing Platform For The Analysis Of Synthetic Colorants. Application To Quinoline Yellow Determination.

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We present the development of a novel fluorescence sensor for the analysis of synthetic colorants. It is based on MoS_2 quantum dots obtained by a hydrothermal method and incorporated as fluorophore into the matrix of PVC membranes, which are deposited on quartz substrates by spin-coating. It was proven, as in these conditions, MoS_2 quantum dots maintain the fluorescent properties that they present in solution. Experiments carried out in solution displayed a maximum emission when they were excited under 310 nm. This initial fluorescence decreases linearly in presence of various synthetic colorants namely quinoline yellow, tartrazine, sunset yellow, allura red, ponceau 4R and carmoisine. The two possible mechanisms that can explain this quenching effect, colorants absorbing photons emitted by quantum dots and/or competing with the nanomaterial for photons coming from the excitation source, were evaluated. The most pronounced effect was observed with quinoline yellow, as a result of a mixed mechanism. The optimized methodology developed for the determination of quinoline yellow showed a linear concentration range between 5.4 and 55.0 µg with a limit of detection of 1.6 µg. The sensor was applied to the determination of quinoline yellow in a food colour paste obtaining results in good agreement with those obtained by HPLC-UV-vis measurements.

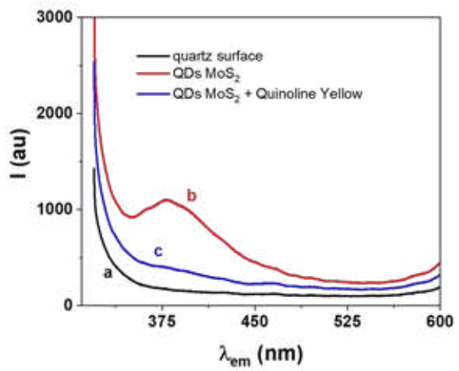

Fig.1. Emission spectra at λex 310 nm of a) quartz surface, b) MoS_2-QDS @PVC/quartz sensor, c) MoS_2-QDS @PVC/quartz sensor + 150 µL of 275 µg/mL QY.

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2D-ReS_2 & diamond nanoparticles-based sensor for the simultaneous determination of sunset yellow and tartrazine in a multiple-pulse amperometry FIA system

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In this work, we present the simultaneous analysis of sunset yellow (SY) and tartrazine (TR) by a flow injection with multiple pulse amperometric detection (FIA-MPA)-based methodology. As transducer, we have developed a novel electrochemical sensor based on the synergistic effect of ReS_2 nanosheets and diamond nanoparticles (DNPs). We have selected ReS_2 nanosheets among several transition dichalcogenides (TiS_2, WS_2) for the sensor development since it leads to a better response towards both colorants. Scanning probe microscopy characterization shows that the surface sensor is composed by scattered and stacked ReS_2 flakes and large aggregates of DNPs (Figure 1A). With the designed system, the gap between the oxidation potential values of sunset yellow and tartrazine is wide enough to allow the simultaneous determination of both dyes (Figure 1B). Under the optimum potential pulse conditions (0.8 V and 1.2 V) during 250 ms, a flow rate of 3 mL/min and a volume injection of 250 µL, detection limits of 3.51×10^{-7} M and 2.39×10^{-7} M for sunset yellow and tartrazine, respectively, were obtained. The method exhibits good accuracy and precision with Er minor than 13% and RSD lower than 8% and a sampling frequency of 66 samples per hour. Pineapple jelly samples were analysed by the standard addition method, obtaining 53.7 mg/Kg and 29.0 mg/Kg of sunset yellow and tartrazine, respectively. From the analysis of fortified samples, recoveries of 94% and 105% were obtained.

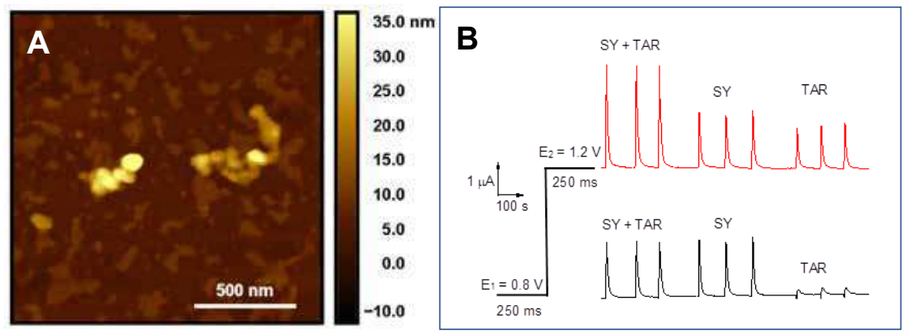

Fig.1. (A) AFM image of the Si/ReS_2/DNPs sample. (B) Potential pulse scheme and FIA-MPA response of SY, TAR and SY+TAR with GCE/ReS_2/DNPs sensor. Carrier solution of acetic acid/acetate buffer 0.1 M pH 5 at 3.0 mL/min.

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POSTER PRESENTATIONS

T9

Food contaminants

The risk of garden crops contamination grown in soils with an increased content of heavy metals

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Soil contamination by heavy metals (HMs) represents a threat to the environment. High concentrations of non-essential HMs in the soil become hazardous for food safety and human health. The most hazardous elements include Cd, Cr, Hg, and Pb [1]. In the territory of Slovakia, there are several areas with significant soil contamination. The Tekov region is one of these places. The areas of Nitra and Žiar nad Hronom, which are part of the Tekov region, are characterized by a slightly to strongly disturbed environment [2].

The contents of Ni, Pb, and Cd were determined in soils and cultivated agricultural crops (carrots, potatoes, and beans) from the plots of three locations in the Tekov region. Samples of soil and plant materials were collected from four sampling points on selected plots of the given locations in compliance with the relevant methodologies. The contents of heavy metals in both soil and plant materials were determined after sample treatment and preparation for analysis. The mobile forms of HMs (MF-HMs) were determined in soil extract by NH_4NO_3. The total contents of HMs (TC-HMs) were determined in soil extract by *aqua regia* after microwave digestion.

The contents of Ni, Pb, and Cd in the plant material were determined after their mineralization using a closed microwave digestion system (Mars X-Press 5) without the use of hydrogen peroxide by Flame AAS method (Ni) and Graphite Furnace AAS method (Cd, Pb). The same analytical methods were used to determine the soil's contents of Ni, Pb, and Cd.

According to Law 220/2004 [3], the risk element contents detected in soil samples were compared to the limit and critical values [4]. The contents of heavy metals determined in plant samples were evaluated according to the maximum allowed amounts given by Regulation (EC) [4,5], and Regulation SR [7].

The contents of mobile forms of HMs determined in soil samples were from 0.26 (Dolná Ves, carrot, potato) to 0.34 (Vráble, bean) mg Ni/kg, from 0.12 (Dolná Ves, bean) to 0.18 (Vráble, bean) mg Cd/kg, and from 0.14 (Dolná Ves, potato) to 0.34 (Vráble, bean) mg Pb/kg. The total contents of HMs in soil were from 23.1 (Dolná Ves and Kremnička, potato) to 52.9 (Vráble, potato) mg Ni/kg, from 1.13 (Kremnička, potato) to 2.19 (Vráble, carrot) mg Cd/ kg, and from 34.3 (Vráble, potato) to 67.9 (Dolná Ves, carrot) mg Pb/kg. The results showed that the soils from all sampling sites are contaminated with cadmium (both forms of Cd), and lead (mobile forms of Pb). In two cases was detected an increased content of Ni (total content of Ni).

Table 1 shows the Ni, Pb, and Cd levels detected in the plant samples. Based on the obtained results, it can be stated that the hygiene standards for Ni in potatoes, Cd in beans, and Pb in carrots have been exceeded.

Table 1. The content of heavy metals in plant materials (mg/kg fresh weight)

heavy metal		**Ni**			**Cd**			**Pb**	
cultivated crop	**carrot**	**potato**	**bean**	**carrot**	**potato**	**bean**	**carrot**	**potato**	**bean**
locality									
Vráble	0.54	0.63	3.37	0.02	0.01	0.04	0.27	BDL	BDL
Dolná Ves	0.53	0.55	2.39	0.08	0.03	0.13	0.56	BDL	0.09
Kremnička	0.50	0.64	2.04	0.02	0.02	0.10	0.48	BDL	0.05
Limit value	**2.5** [6]	***0.5*** [6]	***6.0*** [6]	***0.1*** [4]	***0.1*** [4]	***0.02*** [4]	***0.1*** [5]	***0.1*** [6]	***0.2*** [5]

Note: BDL – below detection limit

Acknowledgments: This publication was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund.

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Uptake of heavy metals by selected species of the genus *Allium*

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Toxic pollution is a critical environmental problem that seriously threatens human health and agricultural production. Consumption of vegetables contaminated with heavy metals can lead to various diseases in consumers. Heavy metals can accumulate in the kidneys and liver, which can disrupt many biochemical processes and lead to cardiovascular, neurological, kidney, and bone diseases [1].
The study aimed to evaluate the content of risky heavy metals (Cd and Pb) in the soil and subsequently in selected species of the genus *Allium*, specifically onion (Spirith, Nanas F1, Sedona F1), garlic (Bjetín, Eden Rose, Garpel), and leek (Aligator, Columbus, Elephant). We determined the total content of heavy metals (*aqua regia*), and mobile forms of heavy metals (1 M NH_4NO_3) in soils and compared the obtained results with the limit values given by Act no. 220/2004 (valid in the Slovak Republic) [2]. The obtained results indicate that the total contents of monitored heavy metals did not exceed the limit value. In the case of the content of mobile forms of monitored risk elements, the permitted legislative limits were exceeded (Pb: 0.31 - 0.59 mg.kg^{-1}; Cd: 0.16 – 0.18 mg.kg^{-1}). For lead, the limit value exceeded 3.1 to 5.9 times. In the case of Cd, the limit value was exceeded by 1.6 to 1.8 times.
Total heavy metal content in plant samples was determined using the Graphite Furnace AAS method. The limit of detection was set at 30.0 ng.kg^{-1}.The determined contents of heavy metals in onions were within the range 0.004 – 0.04 mg Cd.kg^{-1} FW fresh weight), ND – 0.10 mg Pb.kg^{-1} FW, in garlic within the range ND – 0.03 mg Cd.kg^{-1} FW, ND – 0.41 mg Pb.kg^{-1} FW, and in leek within the range 0.002 – 0.04 mg Cd.kg^{-1} FW and ND mg Pb.kg^{-1} FW. We found that Cd accumulated the most in the monitored leek varieties and lead accumulated the most in the monitored onion varieties. Each plant has a different reaction to different heavy metals. Some plants are sensitive, while other plants have a high tolerance to heavy metals [3]. The content of risk elements contained in the plant is a direct function of the presence of these minerals in the soil and depends on the amount of sunlight, air, and water, but also the pH of the soil and the mobility of the element [4]. Maximum level of cadmium was exceeded in onion cultivar Nanas F1, and leek cultivar Elephant. Maximum level of lead was exceeded in garlic cultivar Garpel. Despite exceeding the highest permissible amount of lead and cadmium according to the current legislation, this exceeded amount may not represent a potential danger to human health. World Health Organization (WHO) indicates tolerable weekly intake of Cadmium to 0.175 mg/person/week and lead to 1.750 mg/person/week [5]. Based on the calculation, the permissible weekly intake of cadmium and lead per person would not be exceeded.

Table 1. Cd and Pb content in analysed species

Species	Cultivar	Cd (mg.kg^{-1} FW)	Pb (mg.kg^{-1} FW)
Onion (*Allium cepa* L.)	Spirith	0.02±0.005	0.10±0.005
	Nanas F1	0.04±0.008	0.10±0.009
	Sedona F1	0.004±0.001	ND
Garlic (*Allium sativum* L.)	Bjetín	0.03±0.009	ND
	Eden Rose	ND	ND
	Garpel	ND	0.41±0.215
Leek (*Allium porrum* L.)	Aligator	0.03±0.006	ND
	Columbus	0.002±0.001	ND
	Elephant	0.04±0.007	ND
Maximum level [6, 7]		0.03 (0.05)	0.10

Acknowledgments: This research was funded by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund

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Inhibition of acrylamide in biscuits by fibre supplementation

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Acrylamide (AA) is classified as a probable carcinogen by the International Agency for Research on Cancer [1] and described as a neurotoxin. EFSA considers AA as a public health concern and AA levels across the EU have been subject to a benchmarking system since 2018 to force effective reduction of its content in food. Nevertheless, AA is ubiquitous in the human diet, since it can be found at different levels in a great variety of foods, such as fried and roasted potato products, bread, biscuits, and breakfast cereals, coffee and cocoa [2]. AA is formed when the amino acid asparagine reacts with reducing sugars, such as glucose and fructose, being the main chemical processes that occur known as the Maillard Reaction, the same reaction that 'browns' food and gives distinctive flavour. Asparaginase is frequently added to reduce acrylamide formation, but new alternatives are welcome by the food industry.

The aim of this work is to understand the impact of biscuits supplementation with fibre on AA formation and bioaccessibility on gastrointestinal tract after *in vitro* digestion (INFOGEST method). Fibre supplementation was done with apple pomace lyophilised and washed to remove free sugars that partially replaced wheat flour. Positive control biscuits were prepared without fibre enrichment and without asparaginase addition, while negative control biscuits were prepared using asparaginase, as usually done by the industry. AA content in baked biscuits was assessed by gas chromatography (GC) coupled to mass spectrometry (MS) after solid-liquid extraction and derivatization with xanthydrol [2].

Results show that the biscuits supplemented with apple pomace (containing around 20% fibre) presented less quantity of AA (132.3±8.3 µg/kg) than positive control biscuits without asparaginase and containing 5% fibre (215.3±11.3 µg/kg). The reduction of AA content due to fibre addition is notorious, but alone did not reach the reduction obtained in negative control biscuits prepared with asparaginase (65.02±11.3 µg/kg). However, both strategies can be combined to reduce the amount of asparaginase needed, and simultaneously increasing the fibre content and health benefits, since "high-fiber" food, being nutritionally improved in relation to the traditional recipe. Moreover, fibre acts as binder of AA during digestion and decreased significantly AA bioaccessibility on gastrointestinal tract after *in vitro* digestion (*p*-values<0.0025).

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Effect of dry-heat treatment on acrylamide and HMF formation in maize flour

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Thermal treatments have numerous applications as valuable tools for extending the shelf-life of flour by inactivating enzymes and reducing moisture content [1]. Besides the biological effects, the dry-heat process has a significant impact on the techno-functional, nutritional properties as well as bioactive compounds of flour [2]. However, during different thermal treatments, many potentially harmful compounds could be formed through the Maillard reaction. Recently, two heat-induced contaminants have gained much interest: acrylamide and HMF [3]. HMF forms as a result of the dehydration of hexose sugars or the Maillard reaction during heating [4], while acrylamide in foods is formed via the Maillard reaction from free asparagine in the presence of carbonyl compounds such as reducing sugars during thermal processes. The present study aimed to evaluate the effects of the thermal treatment at different temperatures: 100, 125, 135, 150, and 165°C, on the HMF and acrylamide formulation, as well as the antioxidant capacity of maize flours. The experimental material consisted of three maize hybrids with different colours and kernel types (white-standard, yellow and blue-popping). Maize samples were ground on a lab mill to a fine powder (<500µm) and flour samples were evenly spread thinly on a glass plate and thermally treated for 1 h in a ventilation oven Memmert UF55. In order to evaluate the effect of dry-heat treatment on the HMF and acrylamide formulations and antioxidant activity of maize flours all the results were compared with those of non-treated flours as a control. As we expected, no HMF was detected in untreated flour and the content of HMF increased with the rising of the applied temperature. The content of HMF ranged from 2.93 to 207.60 µg/kg, 2.03 to 113.62 µg/kg and 2.60 to 185.26 µg/kg in white, yellow and blue maize flours, respectively. Our results demonstrated a maximum HMF level at 165°C in the white maize sample (207.60 µg/kg), which was higher by approximately 10% and 45% than the level measured in the blue and yellow maize samples, respectively. The acrylamide ranged from 41.00 to 840.66 µg/kg, 31.40 to 666.70 µg/kg and 56.75 to 953.96 µg/kg in white, yellow and blue maize flour samples, respectively. It was observed that the dry-heat treatment of all the investigated products at higher temperatures produced slightly more acrylamide concentrations. No acrylamide was detected in untreated flour, as well as in maize flours heated at 100°C. The antioxidant capacity ranged from 10.05 to 13.32, 15.89 to 18.15 and 20.51 to 24.03 mmol Trolox Eq/kg in white, yellow and blue maize flour, respectively. Dry-heat treatment had a significant effect on the maize flour colour parameters and the results of the parameters L*, a*, and b* indicated that the flour showed a darkening and browning effect as the dry-heat treatment temperature increased. Browning also indicates the formation of melanoidins as the end-product of the Maillard reaction, which confirms the maximum value of antioxidant capacity at a temperature of 165°C. All results showed that dry heat treatment increases antioxidant capacity with increasing temperature, but also increases HMF and acrylamide. However, due to the different kernel structures of the used maize genotypes and the inter-relations between chemical compounds within the food matrix, the overall impact of dry-heat treatments was not completely elucidated.

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Relation of free asparagine content in small-grain cereals and the generation of acrylamide in the cookies

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Small-grain cereals plays an important role in food production. A wide range of cereal-based food products such as different bakery products, snack foods, cakes and cookies, breakfast cereals, porridges, beverages etc. can be processed from cereal grains, flour, bran or starch. However, the transformation of the raw small-grain cereals into food products is mostly accompanied by a thermal treatment that can result in the formation of processing contaminants such as acrylamide. Widely consumed thermally processed cereal-based food can have a great contribution to acrylamide dietary intake thus bearing a high public health risk and require attention and application of strategies for its reduction. As it has been well established that the amount of asparagine in the presence of reducing sugars during Maillard reaction is proportional to the formation of acrylamide in most of the food products [1], one key strategy is to select an ingredient with lower levels of free asparagine. The asparagine content in cereals is affected by the genetic basis, growing conditions, time of harvest and storage conditions [2]. The aim of this study was to establish the interrelationship between the initial content of free asparagine in different cereal flours and acrylamide in cereal cookies. For making whole-grain flour, eight genotypes of small-grain cereals were used: one genotype each of bread wheat, durum wheat, soft wheat, hard wheat, triticale, rye, hulless oat and hulless barley. Rye and durum wheat whole-grain flours, with free asparagine content of 603.2 and 530.3 mg/kg, respectively, followed hulless oat flour (859.8 mg/kg). The average content of free asparagine in whole-grain flour of hard wheat and triticale is 465 mg/kg. The third group, with the content of free asparagine varying about 277.6 mg/kg, consisted of whole-grain flour of hulless barley, soft and bread wheat. Wholegrain cereal flours were used to prepare cookies. The cookies were prepared according to the method described previously [3]. The cookies were baked at 180°C for 7, 10 and 13 min. Compared to the initial content in flours, the relative asparagine reduction in cereal cookies baked for 7 min (on average 69±16%) was lower than in cookies baked for 13 min (on average 87±8%). The complete reduction of asparagine was observed in hulless oat cookies. At 180°C, acrylamide was detected at all baking times, reaching a final content from 162.6 to 861.7 µg/kg after 13 min of baking in soft wheat-based cookies and hulless oat-based cookies, respectively. The content of acrylamide in cookies baked for 7 min ranged from 44.8 µg/kg 167.6 µg/kg. A gradual increase in acrylamide content was observed with the extension of the baking time in most samples. However, the acrylamide content was decreased after 10 min of baking in cookie made from the bread wheat and hard wheat. Our results confirm that free asparagine in cereal flour is one of the main factors in the formation of acrylamide in the cookie. Although the correlation data indicated that acrylamide in cereal cookies could not exactly correspond to the free asparagine in flour, it is evident that the cookies prepared from flour with the highest content of free asparagine had the highest content of acrylamide.

Acknowledgments: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200040).

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Sample preparation for isolation of microplastic particles from mussel samples

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Microplastics (MPs) are persistent and widespread environmental contaminants, raising concerns about their effects in biota. Both laboratory toxicity assays and biomonitoring require easy and effective digestion procedures to extract microplastics from biological samples. Both MP extraction and chemical characterization by spectroscopy require a very efficient digestion of biological material and removal of inorganic matter present in the seafood samples. Furthermore, it is very important to prepare representative sample of.

In this research, mussel samples were analysed on MPs content. The extraction of MPs was performed by multistep digestion with combined chemical and enzymatic digestion. The first step was chemical digestion using alkaline conditions (KOH 10%) at 60ºC during 24 hrs, second step was enzymatic digestion with pepsin and pancreatin and last step included H_2O_2 digestion 16 h at room temperature. The final step was final filtration of isolated MPs onto μFTIR filter and their further characterization. Micro FTIR (μFTIR, iN10 Nicolet, Thermo Fisher scientific) which couples a microscope with an infrared spectrophotometer; the instrument is equipped with an ultra-fast motorized stage and with a liquid nitrogen cooled mercury cadmium telluride detector (MCT detector).

In order to compare effects on sample preparation, two types of samples were analysed: frozen mussel samples and lyophilized samples. Mussel samples were lyophilized to obtain a homogenous powder. 0.5 g of lyophilized samples (which is equal to mass of one individual) or one frozen organism were subjected by digestion protocol.

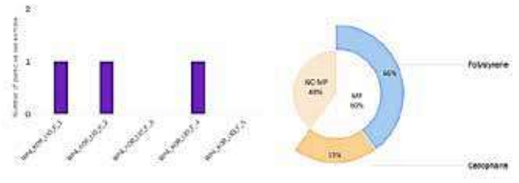

Fig.1. Number of particles per sample and type of MP particles

MPs content was 0 or 1 particle per organism or per 0.5 g of lyophilized sample, 40 % of samples did not contain MP. Polystyrene (PS), was the major polymer type found in samples. The polymer composition of microplastics in samples included polystyrene (66%) and cellophane (33%) (Figure 1). Our results suggested that microplastic pollution is widespread in commercial mussels.

Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965173.

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Chemical characterization and quantification of microplastics particles from mussel samples based on Micro-FTIR spectroscopy

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Seafood has been widely recognized as an integral part of a balanced and healthy diet, thanks to its high nutritional value and associated multiple health benefits. Seafood contamination with microplastics (MPs) have recently been highlighted as an emerging concern for global food security.[1]

According to definition, polymer particles with a diameter of less than 5 mm are referred to as microplastics particles (MPs)[2]. Most bivalves, as well as mussels, are filter feeders and can accumulate particles in their bodies. By consuming seafood, MP particles can be included in the human food chain. Most of the studies analyzed MPs content in environmental samples, instead of point-of-sales samples.

The aim of this study was to determine the abundance of microplastics in mussels collected from Croatia seafood market intended for human consumption. 5 clean laboratory criteria (100% cotton lab coat, clean glassware, filtered solutions, rinsed the outside of animals before dissection, and limited air exposure of samples) were fulfilled by our study. Digestion and manipulation of the sample was conducted in a clean hood. Procedural controls were included for each measurement. After extensive removal of biological material by alkaline, enzymatic and oxidation digestion, MPs were characterized by micro-Fourier-transform infrared spectroscopy (Micro-FTIR). It is a proven method for the identification of all types of polymers and allows a reliable differentiation between substances of natural and synthetic origin. Our approach included one individual per sample for micro-FTIR analysis, no sub-grouping of the sample for chemical characterization (100% of particles isolated from each sample have been analyzed by micro-FTIR) and manual confirmation of all recorded spectra with more than 60% match to the polymer material in the spectral library (Thermo-Fisher; using the OMNIC Picta^{TM} software). Soft tissue from inside the shell was not extensively washed to allow estimation of MPs intake during consumption.

Almost half of total samples (sample size=27) did not contain microplastic particles (52%). The highest number of particles per individual was 4 (Fig. 1). In total, we found 23 MPs particles in 27 individuals or 0.85 MPs per individual, range 0-4, and less than 0.3 MPs/g of soft tissue. In this research, major types of isolated MPs were polystyrene (PS), poly(ethylene terephthalate) (PET), polypropylene (PP) and polyethylene (PE), shown in Table 1.

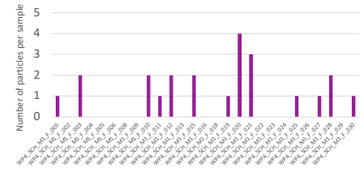

Fig.1. Number of particles found in Mussels from Croatia

Table 1. Major types of MPs found in mussels and their match

Type of MPs	Match with libraries, average
Poly(styrene), PS	73,5%
Poly(ethylene terephthalate), PET	84,33%
Polypropylene, PP	89,7
Polyethylene, PE	86,4

PE and PS are the most popular plastic materials used in consumer products and they have shorter service lives than other types of plastics.

Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965173.

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[2] A. I. Catarino, V. Macchia, W. G. Sanderson, R. C. Thompson, T. B. Henry, Environmental Pollution, 237, (2018), 675-684

Chemical characterization and quantification of microplastics particles from mussel samples based on Micro-FTIR spectroscopy

Tamara Mutić[1], Boban Anđelković[1], Dragana Stanić-Vučinić[1,2], Miloš Ilić[1], Tanja Ćirković Veličković[1,2,3,4,5*]

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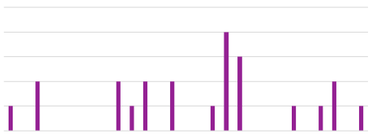
Seafood has been widely recognized as an integral part of a balanced and healthy diet, thanks to its high nutritional value and associated multiple health benefits. Despite to many seafood significant positive effects on human health, there are possibilities to find MPs particles in their tissues.[1]

According to definition, polymer particles with a diameter of less than 5 mm are referred to as microplastics particles (MPs)[2]. Most bivalves, as well as mussels, are filter feeders and can accumulate particles in their bodies. By consuming seafood, MP particles can be included in the human food chain. The impact of MPs on human health has been scarcely researched.

The aim of this study was to determine the abundance of microplastics in mussels collected from Croatia seafood market intended for human consumption. A standard protocol for both quantification and extraction of MP still does not exist. After alkaline, enzymatic and oxidation digestion, MPs were characterized by micro-Fourier-transform infrared spectroscopy (Micro-FTIR). It is a proven method for the identification of all types of polymers and allows a reliable differentiation between substances of natural and synthetic origin.

Almost half of total samples did not contain microplastic particles (52%). The maximum particles per individual was 4 (Fig. 1). In total, we found 23 MPs particles in 27 individuals or 0.85 MPs per individual. Expressing the whole content from the shell (soft wet tissue and water) it is 0.14 MPs / g. In this research, major types of isolated MPs were polystyrene (PS), poly(ethylene terephthalate) (PET), polypropylene (PP) and polyethylene (PE), shown in Table 1.



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Investigation of acrylamide and HMF formation in biscuits produced by different ingredients

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Acrylamide content is regulated for various cereal-based foods, and in particular for biscuits a limitation to 150 **µg**/kg it is required. Since biscuits are mainly consumed by younger populations, strict control of acrylamide and hydroxymethylfurfural (HMF) content is mandatory. Many studies have highlighted the influence of the raw material composition as well as the processing techniques on the formation of acrylamide and HMF.

This study aims to investigate the influence of various ingredients on the total amount of acrylamide and HMF formation in biscuits with or without cocoa. A method based on UV-VIS spectroscopy was used for the evaluation of total acrylamide content, in comparison with standard GC-MS measurement. The results show that the type of baking leavening agents (group of predominant inorganic salts) used influences the appearance of acrylamide during the baking process. It can thus be noticed the use of ammonium bicarbonate or a mixture of ammonium bicarbonate with sodium bicarbonate leads to a significant increasing in the concentration of acrylamide in the final product.

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Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine (ACRYRED)- COST ACTION 21149

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Since the discovery of acrylamide in food in 2002, research and technological development efforts have been made to reduce the levels of acrylamide by both academic and industrial groups. If asparagine levels in grains can be reduced through better varieties and farming practices, downstream acrylamide formation in cereal-based products could be reduced significantly. Here, we present the activities of ACRYRED project, a COST Action CA21149 (Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine), which started in October 2022 and is supported by COST (European Cooperation in Science and Technology).

ACRYRED's main aim is to understand the potential for mitigating acrylamide formation in foods produced from grains by establishing a multi-disciplinary network bringing together plant breeders, the agricultural grain farming community, global grain traders, European food processors, toxicologists, academic researchers from relevant disciplines, public regulators, and consumer and health interest groups.

Our specific objectives are: i) to facilitate the exchange of knowledge to encourage joint R&D planning between all scientific disciplines involved in reducing acrylamide levels in cereal-based food to be laid down in a strategic research planning for the next 5 years; ii) to stimulate research to identify supply chain management models to promote low free asparagine cereals (wheat, barley, maize, oats, rye) and low acrylamide cereal products; iii) to coordinate on plant breeding efforts to achieve a reduction in free asparagine levels in cereal cultivars; iv) to agree on a methodology and a process to describe average free asparagine content for individual cereal crop cultivars; v) to exchange knowledge and insights about the relationship between acrylamide formation during processing and desirable quality aspects such as aroma, taste, and colour; and vi) to link research on consumer preferences and willingness to purchase products to the topic of acrylamide and products with lower acrylamide levels.

We invite researchers, young scientists, plant breeders and stakeholders to join our working groups: WG1 – Interdisciplinary Exchange and Integration of Knowledge on Asparagine and Acrylamide, WG2 – Agronomy and Plant Breeding, WG3 – Chemistry & Processing, WG4 – Cereal Supply Chain Economy, and WG5 – Risk-benefit of MR Products and its Mitigation.

Acknowledgments: The authors would like to acknowledge the support by COST (European Cooperation in Science and Technology; www.cost.eu), in the framework of COST Action CA21149 (Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine (ACRYRED); https://acryred.eu/).

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