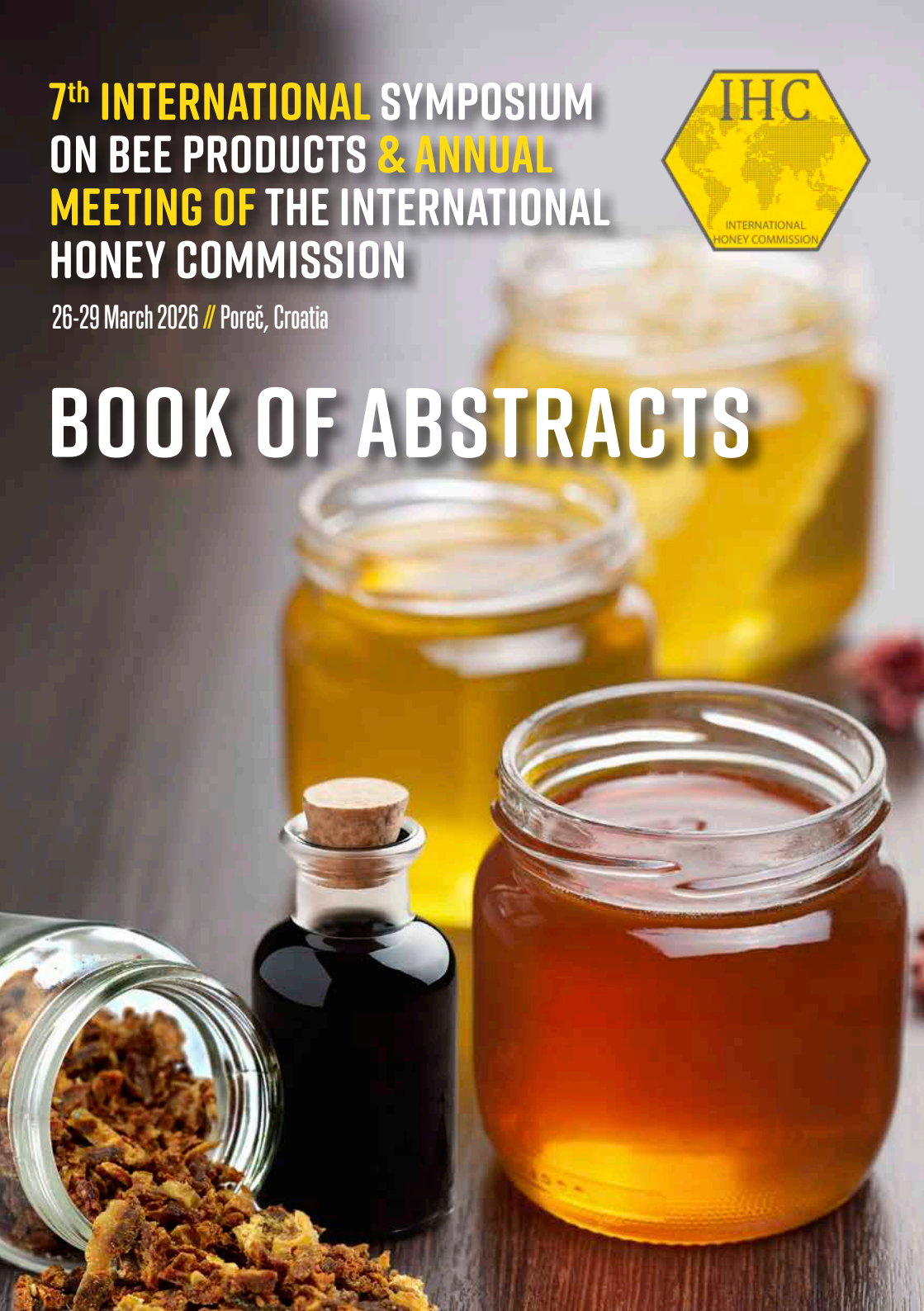
7th INTERNATIONAL SYMPOSIUM ON BEE PRODUCTS & ANNUAL MEETING OF THE INTERNATIONAL HONEY COMMISSION

26-29 March 2026 // Poreč, Croatia

BOOK OF ABSTRACTS



Published by:
University of Rijeka, Faculty of Medicine, Rijeka, Croatia

***Editor in Chief*:**
Lidija Svečnjak (University of Zagreb Faculty of Agriculture, Zagreb, Croatia)

Editorial Board:
Ivana Flanjak (Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia)
Irena Brčić Karačonji (Institute for Medical Research and Occupational Health, Zagreb, Croatia)
Lidija Svečnjak (University of Zagreb Faculty of Agriculture, Zagreb, Croatia)
Dražen Lušić (Faculty of Medicine, University of Rijeka, Rijeka, Croatia)

Organisers:
International Honey Commission (IHC)
University of Rijeka, Faculty of Medicine, Croatia
Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia
Teaching Institute for Public Health of Primorje-Gorski Kotar County, Rijeka, Croatia
Beekeeping Association "Nektar", Poreč, Croatia
Associations of Primorje-Gorski Kotar County – Beekeepers Federation of Primorje-Gorski Kotar, Omišalj, Croatia

In collaboration with:
University of Zagreb Faculty of Agriculture, Zagreb, Croatia
Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia

Institutional support:
City of Poreč, Croatia
Region of Istria, Croatia
Croatian Beekeeping Federation
European Beekeeping Association (EBA)
Ministry of Agriculture, Forestry and Fisheries of the Republic of Croatia
B-THENET
NextGeneration EU – CCBE-Propolis

Under the Auspices
The Speaker of the Croatian Parliament, Mr. Gordan Jandroković

***Designed by*:** GRAFIK, Rijeka, Croatia

***Press*:** GRAFIK, Rijeka, Croatia

ISBN: 978-953-8341-80-9

Web page: https://www.ihc-platform.net/

7th INTERNATIONAL SYMPOSIUM ON BEE PRODUCTS & ANNUAL MEETING OF THE INTERNATIONAL HONEY COMMISSION

Organising Committee:

Dražen Lušić, IHC President, Head of the Organising Committee,
Faculty of Medicine, University of Rijeka, Croatia
Jasna Bošnir, Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia
Irena Brčić Karačonji, Institute for Medical Research and Occupational Health, Zagreb, Croatia
Ivana Tlak Gajger, Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia
Blanka Bilić Rajs, Josip Juraj Strossmayer University of Osijek,
Faculty of Food Technology Osijek, Osijek, Croatia
Ivan Kovač, Beekeeping Association "Nektar", Poreč, Croatia
Nedjeljko Mrakovčić, Associations of Primorje-Gorski Kotar County –
Beekeepers Federation of Primorje-Gorski Kotar, Omišalj, Croatia

Scientific Committee:

Lidija Svečnjak, Head of the Scientific Committee, University of
Zagreb Faculty of Agriculture, Zagreb, Croatia
Ivana Flanjak, Josip Juraj Strossmayer University of Osijek,
Faculty of Food Technology Osijek, Osijek, Croatia
Ofélia Anjos, Polytechnic University of Castelo Branco, Castelo Branco, Portugal
Maria Dimou, Aristotle University of Thessaloniki, School of Agriculture, Thessaloniki, Greece
Panagiota Gotsiou, Mediterranean Agronomic Institute of Chania (CIHEAM), Chania, Greece
Andresa Aparecida Berretta, Apis Flora Indl Coml Ltda / ABEMEL, Ribeirão Preto, São Paulo, Brazil
Gian Luigi Marcazzan, Council for Agricultural Research and Economics -
Honey bee and Silkworm Research Unit (CREA-API), Bologna, Italy
Ismail Emir Akyıldız, Balparmak, Istanbul, Turkey
Aslı Elif Tanuğur Samancı, BEE&YOU, Istanbul, Turkey
Dušanka Milojković-Opsenica, University of Belgrade, Faculty of Chemistry, Beograd, Serbia
Juraj Majtan, Institute of Molecular Biology, Bratislava, Slovakia
Igor Jerković, University of Split, Faculty of Chemistry and Technology
/ Mediterranean Institute for Life Sciences, Split, Croatia
Mojca Korošec, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Content

Preface **5**
Foreword of the President of the Region of Istria **7**
Foreword of the Mayor of the City of Poreč **9**
Foreword of the President of the Beekeeping Association Nektar **11**

SYMPOSIUM PROGRAMME

Thursday, 26 March 2026 12
Friday, 27 March 2026 14
Saturday, 28 March 2026. 16
Sunday, 29 March 2026 19

PLENARY PRESENTATIONS

Why are European borders open to fraudulent honey? 21
New approaches in honey traceability—examples of chemical markers pathways. 22

ORAL PRESENTATIONS

Session: Authenticity, traceability, quality, and safety 23
Session: Characterisation of bee products 52
Session: Evidence-based studies on beneficial properties 66
Session: Sustainability in bee products production. 78
Session: Other products & by-products of apiculture 84
Session: Novel analytical approaches 91

POSTER PRESENTATIONS

Session: Authenticity, traceability, quality and safety 100
Session: Characterisation of bee products 114
Session: Evidence-based studies on beneficial properties 130
Session: Novel analytical approaches 136
Session: Sustainability in bee products production. 140
Session: Other products & by-products of apiculture 150

Preface



We are delighted to welcome you to the 7th International Symposium on Bee Products which is held in conjunction with the Annual Meeting of the International Honey Commission (IHC) taking place from March 26-29, 2026, at the Hotel Parentium Plava Laguna, located on the Adriatic coast in the historic town of Poreč, Croatia. As in previous years, the Symposium serves as a vital platform for international experts, researchers, scientists, professionals, and stakeholders in the field of apiculture and bee products to come together and share their latest findings, innovations, and ideas. As global interest in natural health products and sustainable apiculture continues to rise, the need for collaboration and evidence-based research becomes even more essential. The Scientific Programme and Book of abstracts cover a comprehensive range of topics related to bee products (honey, propolis, bee pollen/bee bread, beeswax, royal jelly, and bee venom), as well as various aspects of apiculture affecting the production of bee products. Participants have the opportunity to attend interesting plenary lectures, scientific sessions (oral and poster presentations), and interactive workshops and panel discussions, all designed to promote in-depth discussions and knowledge exchange.

Core themes include authenticity, traceability, quality and safety of bee products, characterisation of bee products, evidence-based studies on beneficial properties of bee products, sustainability in bee products production, other products and by-products of apiculture and novel analytical approaches. Additionally, sessions address regulatory developments and

consumer trends. A central feature of the event is the Annual Meeting of the International Honey Commission (IHC). This gathering of global experts plays a crucial role in setting and revising international standards for bee product quality, analytical methods, and labelling. Attendees are encouraged to actively participate in shaping the future guidelines, and cooperative research and strategies.

The venue, Hotel Parentium Plava Laguna, offers an ideal setting for this international event. Nestled in a pine forest by the sea, and equipped with state-of-the-art conference facilities, the hotel ensures a comfortable and inspiring environment for both scientific / academic exchange and informal networking. Attendees can enjoy the peaceful atmosphere, excellent service, and breath-taking views of the Adriatic coastline. Beyond the scientific program, participants have the opportunity to explore Poreč, a town rich in historical and cultural heritage, as well as natural beauty of its landscape. From its UNESCO-listed Euphrasian Basilica and Roman ruins to its charming streets and local gastronomy, Poreč promises a memorable cultural experience.

We warmly welcome scientists, researchers, professionals, beekeepers, students, and industry representatives from around the world joining us in Poreč. Whether you are deeply involved in research or newly interested in the world of bee products, this symposium offers a unique chance to connect, learn, and contribute. We are delighted to welcome you to an inspiring international exchange of knowledge dedicated to the research of bee products, and we are truly honoured to host you in Poreč, Croatia!

I wish you an engaging and productive symposium.

Professor Dražen Lušić, PhD, IHC President
Head of the Organizing Committee

Foreword of the President of the Region of Istria



Beekeeping in Istria represents much more than an agricultural activity – it is an expression of the harmony between people and nature, a part of our identity, and an important foundation for the sustainable development of the region. Over generations, Istrian beekeepers have passed down knowledge, experience, and respect for nature, creating products that carry the authenticity of the landscape from which they originate.

At a time when environmental preservation and food safety are among the key global challenges, the role of beekeeping is gaining additional importance. Bees are irreplaceable pollinators, and their contribution to the preservation of biodiversity and the stability of ecosystems is vital for agriculture and quality of life. Caring for bees therefore means caring for our future. The Istrian region, with its diverse and rich flora, enables the production of specific types of high-quality honey. Among them, ivy honey is particularly notable – a product of strong character and unique properties, produced late in the season and considered a rarity and a specialty within the broader European context.

Alongside it, Istrian beekeepers are also recognized for other types of honey bearing protected designations of origin, such as sage honey, black locust honey, chestnut honey, linden honey, honeydew honey, winter savory honey, and blossom honey, further confirming the diversity and quality of our beekeeping heritage. Obtaining the protected designation of origin for Istrian honeys is an important step in recognizing and safeguarding this value. It guarantees quality, origin, and traceability, protects producers, and contributes to strengthening competitiveness

in the demanding EU market. It also confirms that Istria rightfully holds its place among regions that uphold excellence and authenticity. Beekeeping also plays a significant role in the development of rural areas and in strengthening the local economy. It promotes sustainable forms of production, provides additional income, and contributes to preserving life in rural communities.

All of this makes it an important part of the overall development strategy of our region. Guided by these values, we will continue to support the development of beekeeping, protect natural resources, and assist the people who, through their work, preserve this noble activity in Istria. For by preserving beekeeping, we also preserve what is most valuable to us – our nature, our tradition, and our identity.

Boris Miletić
President of the Region of Istria

Foreword of the Mayor of the City of Poreč



It is with great pleasure that we received the news about the upcoming 7th International Symposium on Bee Products & Annual Meeting of the International Honey Commission, to be held in Poreč. Alongside the many other internationally oriented events hosted in Poreč, this Symposium is an opportunity for us to welcome one of the largest beekeeping scientific events in the world, while also highlighting the regional and local recognition of beekeeping in our area.

The beekeepers' association Nektar, which operates in Poreč, and its members continuously present projects and activities they are working on, combining their profession with education and the promotion of honey. One such initiative is the development of urban apiaries in cooperation with the City, which we were among the first to launch in Croatia. The honey produced in these apiaries is of exceptional quality, and we share it with our citizens – from the youngest to the seniors – as well as showcase it through the City's promotional materials.

The strong yields of our urban apiaries, in addition to producing top-quality honey, also show that our urban green areas provide excellent forage for bees. We plant melliferous plants, thereby protecting our environment and enhancing public spaces in a meaningful way. The Nektar association has also initiated the promotion of ivy honey and educational activities on the importance of honey and beekeeping for all generations.

We are always pleased to support beekeeping and agriculture, as they represent key segments of our local economy, as well as an important part of preserving the traditions and authenticity of our city.

I wish you successful participation in the Symposium, and hope that you will take back with you pleasant experiences from our city.

Mayor of the City of Poreč – Parenzo
Loris Peršurić

Foreword of the President of the Beekeeping Association Nektar, Poreč



Dear Ladies and Gentlemen, participants of the 7th International IHC Symposium 2026, on behalf of all members of the Nektar Beekeepers' Association, it is my great pleasure to extend a warm welcome to you! For many generations, the people of our region have nurtured and developed beekeeping, strongly supported by our natural environment, climate, and human dedication. In more recent times, with the growth of tourism, awareness of nature conservation has increased significantly – seen especially through the planting of melliferous plants in and around the city, as well as within tourist resorts.

The products of our beekeepers enrich both the gastronomic scene and the overall tourist offering of our region. We pay special attention to our project dedicated to the study of ivy honey, which has generously rewarded us with excellent results over the past several years. Caring for pollinators is both an imperative and a great responsibility for the beekeepers of our area. Our intention is to share our achievements with you, highlight the challenges we face, and, above all, ensure that we are fully at your disposal during your stay in Poreč.

On behalf of all beekeepers, and personally, I wish all participants a productive and inspiring Symposium. Welcome!

Ivan Kovač
President of the Beekeeping Association Nektar, Poreč

SYMPOSIUM PROGRAMME

Thursday, 26 March 2026

09:30–10:30 **Symposium opening ceremony**

10:30–11:00 **Plenary lecture 1**

Why are European borders open to fraudulent honey?
Andreas Thrasyvoulou
Aristotle University of Thessaloniki, Thessaloniki, Greece

11:00–11:30 **Plenary lecture 2**

New approaches in honey traceability—examples of chemical markers pathways
Igor Jerković
Faculty of Chemistry and Technology; Mediterranean Institute for Life Research, University of Split, Split, Croatia

11:30–12:00 **Coffee break**

11:30–12:00 **Poster session**

12:00–13:30 **AUTHENTICITY, TRACEABILITY, QUALITY, AND SAFETY—*PART 1***

Session Chairs: Dario Lasić & Dražen Lušić

Combining multi-dimensional DNA sequencing data with multi-platform physicochemical and isotopic $\delta^{13}C$ analyses to uncover adulterated honey
Luca Fontanesi, Anisa Ribani, Samuele Bovo, Valeria Taurisano, Giancarlo Quaglia

Multivariate methods for authenticating the geographical and botanical origin of honey: a response to the legislative requirements of Directive (EU) 2024/1438
Matej Pospiech, Zdeňka Javůrková, Jan Dobeš, Marie Bartlová, Bohuslava Tremlová

Melissopalynology characterisation of five Portuguese honeys with protected designation of origin
Paulo Russo-Almeida, Irailde Lima, Ofélia Anjos, Miguel Vilas-Boas

Environmental DNA metabarcoding explores geographical patterns in honey
Seonmi Kim, Soon Ok Woo, Hong Min Choi, Pureum Im, Suwon Park, Yun-Gon Son, Samgyul Lee, Munseon Lee

Unveiling the diversity of Spanish honey production: origin, quality, and authenticity
María Carmen Seijo, María Shantal Rodríguez-Flores, Sonia Harbane, Manuela Chouza-Carou, Ana Pascual-Maté, Amelia González-Porto, Olga Escuredo

Effects of processing and packaging on the physicochemical properties of honey
Taylan Samanci, Aslı Elif Tanuğur Samanci, Elif Yorulmaz Önder

13:30–14:30 **Lunch break**

14:30–16:00 **AUTHENTICITY, TRACEABILITY, QUALITY, AND SAFETY—*PART 2***

Session Chairs: Ivana Flanjak & Maria Dimou

Chemometric profiling of honey samples in Turkey (2016–2025): a decadal survey of physicochemical and carbohydrate signatures consistent with in-hive sugar feeding
Ufuk Alpat, İlknur Coşkun, Tuğçe Daştan, Özge Erdem, Sezer Acar, Özlem Yılmaz, Özlem Cengiz, Ayça Karakuş, Fatma Ezer, Fatma Gevrek, Emel Damarlı

Challenges and scientific gaps in determining the botanical origin of honeydew honey
Jelena Trifković & Dušanka Milojković-Opsenica

Production technology of Belarusian willow honey (*Salix* L.): from early spring nectar flow to botanical identification
Volha Haurylik

Metabolomic profiling of black locust honey from different geographical origins
Pureum Im, Soon Ok Woo, Sangmi Han, Hyo Young Kim, Hongmin Choi, Seonmi Kim, Samgyul Lee, Su Won Park, Munseon Lee, Yun Gon Son

The MISTRAL project: portable solutions for authenticity, traceability, and sustainability in honey production
Marco Ciulu, Muhammad Aqib Khurshid, Alessandra Bossi, Giovanna Felis, Elisa Salvetti, Francesco Pagliarini, Gianni Zoccatelli, Federica Mainente

DNA metabarcoding reveals the botanical diversity of Australian honey
Zahra Karimi, Sophie Dodd, Zoltan Kevei, Tatiana Stead, Keegan Blignaut, Maria Anastasiadi

14:30–16:00 **WORKSHOP**

WG Residues and Contaminants

16:00–16:30 **Coffee break**

16:00–16:30 **Poster session**

16:30–18:00 **PANEL DISCUSSION**

Is honey fraud a systemic weakness or a chosen policy?
Panellists: *Andreas Thrasyvoulou, Nik Lupše, Miguel Vilas-Boas, Dario Lasić, Dražen Lušić*

Friday, 27 March 2026

09:00–11:00 **AUTHENTICITY, TRACEABILITY, QUALITY, AND SAFETY—*PART 3***

Session Chairs: Lidija Svečnjak & Mojca Korošec

Pesticides in pollen collected by honey bees near orchards and vineyards
Christina Kast & Marion Fracheboud

The quality of beeswax blocks and comb foundations in Poland
Katarzyna Kusyk & Teresa Szczęsna

Assessment of the quality of beeswax available on the Polish market
Ewa Waś

Production variables, analytical control, and quality assurance of bee venom
Dario Lasić, Maja Budeč, Gordana Jurak, Danijel Brkić, Jasna Bošnir

Spectroscopic methods for botanical and geographical authentication of honey from Mediterranean area
Davide Martini, Silvia Magro, Massimo De Marchi, Giovanni Niero

The role of accompanying pollen in determining the geographical origin of monofloral honey across Europe: a focus on black locust honey (*Robinia pseudoacacia* L.).
Ghalia Kassem

11:00–11:30 **Coffee break**

11:00–11:30 **Poster session**

11:30–13:30 CHARACTERISATION OF BEE PRODUCTS

Session Chairs: Dušanka Milojković-Opsenica & Juraj Majtan

Kalokimithia (Ballota) honey: a rare honey type produced only in Crete, Greece
Panagiota Gotsiou, Sofia Loupasaki, Ilektra Remoundou, Spyridon Grigorakis, Fatima Zahra Dahmani, Fotini Papadopoulou, Vasilios Liolios, Dimitrios Kanelis, Maria-Anna Rodopoulou, Chrysoula Tananaki

The characterisation of the sensory profiles of British monofloral honeys
Gino Abdul-Jabbar, Rebecca Beveridge, Helen Rogers, Raffaele Dall'Olio, Gian-Luigi Marcazzan

Preliminary results of Slovenian sweet cherry honey characterisation
Aljaž Debelak, Urška Ratajc, Boris Potočnik, Simon Golob, Marinka Kregar, Jasna Bertoncelj, Mojca Korošec

Identification of culturable microbiota associated with the transformation of bee pollen into bee bread
Valentina Odorčić, Mirna Mrkonjić Fuka, Lidija Svečnjak

Insights into sugar composition and FTIR-ATR spectroscopy of stingless bee honey
Jéssica B. Ressutte, Diego Galvan, Cynthia F.P. da Luz, Ana Margarida Gonçalves, Kemilla S. Rebelo, José Augusto G. Sattler, Luis Passarinha, Eugenia Gallardo, Wilma A. Spinosa, Ofélia Anjos

Sensory profiling and acceptance of lesser-known Slovenian and Argentinean honey types among young consumers
Mojca Korošec, Jasna Bertoncelj, Anja Bolha, Eva Marija Čad, Lucía Elisabet Brelis, Veronica Busch, Blaž Ferjančič

Melissopalynology coupled with multivariate analysis to investigate the geographical origin of Italian honeys
Martina Denami, Lorenzo Braga, Maria Lucia Piana, Anna Maria Mercuri

11:30–13:30 WORKSHOP

WG Propolis

13:30–14:30 Lunch break

14:30–16:30 WORKSHOP

WG Sensory Analysis

16:00–16:30 **Poster session**

16:30–17:00 **Coffee break**

17:00–19:00 **IHC Meeting Assembly**

***Quo vadis* IHC? (Opening remarks)**
Dražen Lušić, IHC President

20:00–22:00 **Gala dinner**

Saturday, 28 March 2026

09:00–11:00 **EVIDENCE-BASED STUDIES ON BENEFICIAL PROPERTIES**

Session Chairs: Panagiota Gotsiou & Ivana Tlak Gajger

From hive to hospital: navigating the regulatory maze of medical-grade honey
Juraj Majtan, Jana Godocikova, Viktoriia Chirkova, Martin Safranek

Propolis: metamorphosis of medicine
James Fearnley

From chemical composition to biological function: propolis as a model phenolic system
Dušanka Milojković-Opsenica, Aleksandra Dramićanin, Darija Vukić Lušić

Functional validation of a buccal spray formulated with Brazilian green and red propolis: selective antimicrobial, antioxidant and anti-inflammatory activities
Andresa Aparecida Berretta, Luana Zamarrenho, Jéssica Aparecida de Lima, Monia Melo, Rachel Monteiro, Gabriel Guimarães Calefi, Anna Livia Oliveira Santos, Júlia Gomes Teixeira, Emilly Camargo Lopes, Mariana da Silva Honorio, Nathalia Baptista, Giovanna Borini, Juliana Issa Hori, Carlos Henrique Gomes Martins, José Mauricio Sforcin

Analysis of bioactive compounds and evaluation of the cyto- and genoprotective effects of poplar-type propolis and its major flavonoid component galangin
Mateo Jakac, Dražen Lušić, Irena Brčić Karačonji, Nediljko Landeka, Andreja Jurič, Nevenka Kopjar, Darija Vukić Lušić

Development of a natural wound and burn healing cream: a novel formulation with propolis and honey

Aslı Elif Tanuğur Samanci, Taylan Samanci, Elif Yorulmaz Önder, Esra Birinci

Improvement of haematological and biochemical parameters and white blood cell resistance to irinotecan treatment after one month of the common ivy (*Hedera helix* L.) honey consumption
Nevenka Kopjar, Mateo Jakac, Andreja Jurič, Irena Brčić Karačonji, Dražen Lušić

11:00–11:30 **Coffee break**

11:00–11:30 **Poster session**

11:30–13:30 **SUSTAINABILITY IN BEE PRODUCTS PRODUCTION AND OTHER PRODUCTS & BY-PRODUCTS OF APICULTURE**

Session Chairs: Andresa Aparecida Berretta & Ismail Emir Akyıldız

Contract beekeeping: a sustainable approach to quality and traceability
Taylan Samanci, Aslı Elif Tanuğur Samanci, Elif Yorulmaz Önder

Honey quality standards, use of antibiotics and development of AMR in *Paenibacillus larvae* strains—one health risk communication
Violeta Santrac, Giovani Formato, Nik Lupše, Ivana Tlak Gajger, Xesús Feás, Aslı Özkırım

Calorimetric evaluation of patties and energy content in adult honeybees (*Apis mellifera*) linked to diet-dependent changes in gut microbiota
Ana-Marija Kovač, Maja Ivana Smodiš Škerl, Bojan Papić, Darja Kušar, Damir Pavliček, Ivana Tlak Gajger

Sporopollenin microcapsules from bee pollen for targeted cancer therapy and sustained antioxidant release: a sustainable natural platform
Volkan Aylanc, Seymanur Ertosun, Lalehan Akyuz, Ceren Mutlu, Soraia I. Falcao, Nuno Vale, Cristina Freire, Miguel Vilas-Boas

Chemical and bioactive evaluation of propolis-based preservation strategies in artisanal cheeses
Aritson Tolentino, Seymanur Ertosun, Volkan Aylan, Paula Rodrigues, Miguel Vilas-Boas, Soraia Falcão

Characteristics and quality of Spanish artisanal meads
M. Teresa Sancho, Leire Cantero, Guillermo Puertas, Miguel A. Fernández-Muiño, Sandra M. Osés

Characterisation of artisanal Spanish meads by their sugars, glycerol, inositol and ethanol contents
Miguel A. Fernández Muiño, Guillermo Puertas, Leire Cantero, Sandra M. Osés, M. Teresa Sancho

13:30–14:30 Lunch break

14:30–16:00 NOVEL ANALYTICAL APPROACHES
Session Chairs: Aslı Elif Tanuğur Samancı & Miguel Vilas-Boas

Hyperspectral imaging and AI-driven chemometrics: a new frontier for rapid, non-destructive honey authenticity assessment
Fatjon Hoxha, Sony George, Dolores Pérez-Marín

Honey as a source of entomological environmental DNA: metabarcoding insights into plant-sucking insects across agricultural and forest landscapes
Valeria Taurisano, Anisa Ribani, Samuele Bovo, Giuseppina Schiavo, Valerio Joe Utzeri, Francesca Bertolini, Luca Fontanesi

Royal jelly vesicles as a new drug-delivery system for liquid metal nanoparticles
Mario Kurtjak, Ena Kramarić, Anna Placci, Valentina Marassi, Vanja Baričević, Lana Arsić, Dario Stipanić, Iva Lovrović, Anja Harej Hrkać, Mladenka Malenica

Assessment of extraction strategies for bioactive compounds recovery from propolis of the Montesinho Natural Park (Portugal)
Sandra Barbosa, Maria Fernanda Sousa, Nicolas Justus, Ana R. Circuncisão, Maria Carolina Ribeiro, Olívia Pereira, Cristina Almeida-Aguiar, Susana M. Cardoso

Application of front-face fluorescence spectroscopy combined with chemometrics for the characterisation and adulteration detection of honey
Dany El-Obeid, Ghenwa Abou Chrouch, Dany Yammouni, Jad Rizkallah

16:00–16:30 Coffee break

16:30–18:00 WORKSHOP

WG Palynology of Bee Products

18:00–19:00 Symposium closing ceremony

Sunday, 29 March 2026

10:00–16:30 Technical tour

Depart from Poreč in the morning for a scenic drive through the beautiful Istrian countryside. On the way to Hum, we will make a special stop in Poreč to visit the urban apiary, a project run jointly by the local beekeeping association "Nektar" and the City of Poreč. Here, you'll learn about urban beekeeping and see the hives up close. Continue the journey to Hum, officially the smallest town in the world. Upon arrival, enjoy a guided tour through the charming medieval streets, learning about Hum's history, local traditions, and unique legends.

After the guided walk, enjoy some free time for photos, independent exploration, or a visit to the Aura Museum, which showcases everyday life in the past, including traditional businesses, crafts, and tools used in small towns like Hum. The excursion continues with a short drive to a traditional Istrian konoba, where a relaxed lunch awaits. Enjoy a hearty homemade meal accompanied by local drinks while a live accordion player adds a lively and cheerful atmosphere. In the afternoon, after good food, music, and authentic Istrian hospitality, return to Poreč, arriving approximately between 16:00 and 17:00. This excursion combines history, culture, gastronomy, and unique local experiences in the heart of Istria.

Why are European borders open to fraudulent honey?

Andreas Thrasyvoulou

Aristotle University of Thessaloniki, Thessaloniki, Greece

E–MAIL: *thrasia@agro.auth.gr*

Honey fraud represents one of the most serious challenges facing apiculture, food authenticity, and consumer protection today. Despite extensive EU legislation, large quantities of adulterated and fake honey continue to enter the European market and are distributed as authentic products. This presentation examines the mechanisms that allow fraudulent honey to pass EU borders, focusing on triangular trade practices, weaknesses in border controls, and regulatory limitations. Using data from the European Commission's 2023 Coordinated Action "From the Hives", it demonstrates that most EU Member States have imported honey classified as non-compliant or "suspicious," yet these consignments were ultimately released into the market. The key problem lies in the legal meaning of "suspicious" honey. Analytical methods capable of detecting sophisticated adulteration are not yet fully validated at the EU level and therefore cannot be used to legally block consignments. As a result, approximately 80,000 tonnes of adulterated honey entered the EU during 2021–2022. Additionally, adulterated honey is incorrectly classified as a low-risk product under Regulation (EU) 2017/625, resulting in minimal physical border checks. The absence of a European Union Reference Laboratory for honey and the limited mandate of the Honey Platform further delays the development of enforceable analytical methods. The presentation concludes that without reclassifying adulterated honey as a higher-risk product and establishing legally validated analytical methods, European borders will remain vulnerable to honey fraud, with serious consequences for consumers, beekeepers, and market integrity.

New approaches in honey traceability– examples of chemical markers pathways

Igor Jerković

University of Split, Faculty of Chemistry and Technology; Mediterranean Institute for Life Research, Split, Croatia

E-MAIL: *igor@ktf-split.hr*

The reports of specific or nonspecific chemical markers of the honey botanical origin have been focused on their identification by preparative, chromatographic, and spectroscopic techniques from the collected honey samples. However, limited studies investigate the chemical markers pathways from the source to the final product. Therefore, the approach to the honey traceability involving the corresponding nectar or honeydew as the starting source or honeybee-sac has been developed. Few examples are presented. *Citrus unshiu* Marc. nectar (NE)/honey-sac (HoS)/honey (HO) pathways of the headspace, volatiles and semivolatiles determined by gas chromatography–mass spectrometry (GC-MS) indicated that HO headspace composition was significantly different in comparison to NE and HoS with respect to phenylacetaldehyde and linalool derivatives abundance as the consequence of the hive conditions and the bee enzyme activity. Comparison of *Mentha* spp. honey organic extractives [headspace solid-phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE) followed by GC-MS] with the corresponding bee-sac extractive indicated that methyl syringate and vomifoliol were transferred to the honey while terpendiol I was partially transformed to hotrienol in ripened honey. *Acer monspessulanum* L. honeydew (HD)-honeydew honey (HDH) pathway identified octanal and nonanal by HS-SPME/GC-MS as transferred markers. Ultra performance liquid chromatography (UHPLC-DAD-QqTOF-MS) in HD determined flavonoid glycosides, flavonoid galloyl glycosides, ellagic and protocatechuic acids while HDH was dominated by the corresponding flavonoid aglycons indicating the hydrolysis pathway. The chemical markers pathways research is meaningful for the honey traceability.

SESSION: AUTHENTICITY, TRACEABILITY, QUALITY, AND SAFETY

Combining multi-dimensional DNA sequencing data with multi-platform physicochemical and isotopic $\delta^{13}C$ analyses to uncover adulterated honey

Luca Fontanesi, Anisa Ribani, Samuele Bovo, Valeria Taurisano, Giancarlo Quaglia

University of Bologna, Department of Agricultural and Food Sciences, Animal and Food Genomics Group, Bologna, Italy
Giancarlo Quaglia Independent Chemist, Treviso, Italy

CORRESPONDING AUTHOR: luca.fontanesi@unibo.it

One of the most significant challenges confronting the global apicultural sector is the widespread availability of honey that fails to comply with regulatory standards, as demonstrated by the numerous reports of adulterated products. The most prevalent form of adulteration involves diluting honey with sugar syrups. This practice is particularly attractive to fraudulent operators due to the limitations of current analytical methods in reliably detecting their presence. Additional forms of fraud include false declarations of the geographical, botanical, and entomological origins of honey. Given the complexity of honey fraud and the breadth of information required for its detection, a multi-analytical approach is essential. In this study, we applied an integrated set of analytical methodologies to investigate authentic, suspected, and adulterated honey samples from various countries. The applied methods included: 1) next-generation DNA sequencing, comprising five plant and entomological metabarcoding assays and whole-genome shotgun sequencing, supported by advanced bioinformatic analyses to identify multiple environmental DNA signatures in honey; 2) melissopalynological analysis to characterise pollen composition; 3) assessment of various standard physicochemical parameters; 4) standardised isotopic techniques, namely elemental analyser (EA) for determining C-4 plant sugar in honey and liquid chromatography coupled

to isotope ratio mass spectrometry (LC-IRMS) method for determining the $\delta^{13}C$ ratios of mono-, di-, and trisaccharides in honey; 5) targeted liquid chromatography-high resolution MS (LC-HRMS) to identify sugar syrup markers. The combined results from these complementary methods enabled effective classification of honey samples as authentic or adulterated. This pilot study demonstrates that a multi-method strategy is essential for reliable detection of honey fraud. Ongoing efforts focus on developing a comprehensive reference database integrating multiple analytical datasets to further strengthen counterfeit honey identification.

Multivariate methods for authenticating the geographical and botanical origin of honey: a response to the legislative requirements of Directive (EU) 2024/1438

Matej Pospiech, Zdeňka Javůrková, Jan Dobeš, Marie Bartlová, Bohuslava Tremlová

University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Brno, Czech Republic

CORRESPONDING AUTHOR: mpospiech@vfu.cz

The new Directive (EU) 2024/1438 of the European Parliament and of the Council, amending Council Directive 2001/110/EC on honey, introduces stricter rules for origin labelling. For honey blends, it will be mandatory to indicate the countries of origin in descending order of their weight, together with the percentage share of each country, with a tolerance of only 5%. This legislative shift requires robust analytical tools capable of not only detecting adulteration but also precisely verifying geographical origin. This contribution summarises the results of multi-year research focused on the characterisation of Czech and Central European honeys (V4 countries) using advanced methods. The efficiency of melissopalynological analysis combined with Geographic Information Systems (GIS) and the CORINE Land Cover (CLC) database was evaluated for predicting the pollen profile based on land cover within the bees' flight range. Furthermore, the relationship between the mineral profile of honey and soil types (e.g., cambisol, fluvisol) in the hive locality was investigated, confirming a strong correlation between specific elements (e.g., B, Ca, Mg, Ni, and Mn) and soil characteristics. The results indicate that while physicochemical parameters alone allow for origin discrimination with 89% accuracy, combining them with melissopalynology 100% and mineral profiling (86.5%) increases the discriminatory power of the models.

The study confirmed specific characteristics of Czech honeys, such as the darker colour of rape honey (Brassica honey) compared to the European average. The study concludes that to meet the requirements of the new directive and ensure effective official control, it is essential to use multivariate statistical models integrating data on pollen spectrum, mineral composition, and physico-chemical properties, supported by geographical data on land cover.

Melissopalynology characterisation of five Portuguese honeys with protected designation of origin

Paulo Russo-Almeida[1], Irailde Lima[2], Ofélia Anjos[3], Miguel Vilas-Boas[4]

[1]Universidade Trás-os-Montes e Alto Douro (UTAD), Inov4Agro, Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Quinta de Prados, Vila Real, Portugal
[2]Universidade de Trás-os-Montes e Alto Douro, Departamento de Zootecnia, LabApis, Vila Real, Portugal
[3]Polytechnic Institute of Castelo Branco, CERNAS-IPCB-Research Centre for Natural Resources, Environment and Society, , Castelo Branco, Portugal
[4]Campus de Santa Apolónia, Instituto Politécnico de Bragança, CIMO, LA SusTEC, Bragança, Portugal

CORRESPONDING AUTHOR: prusso@utad.pt

In 1994, Portugal was assigned with nine honey Protected Designations of Origin (PDO) by the EU. Given the limited information available at that time and the interval since its approval, it is expectable to find some changes in the flora underlying the aforementioned honeys, as a result of modifications in the land use and/or climate changes. The "BeeLand" project aims to update the regulated specifications for some Portuguese PDO honeys. In this work we present the results of the qualitative and quantitative melissopalynology analysis of five PDO honeys (Mel do Parque de Montesinho, Mel da Serra da Lousã, Mel da Terra Quente, Mel de Barroso, and Mel do Ribatejo Norte). Qualitative melissopalynology analysis was performed using the acetolysis method while quantitative analysis followed the filtration method. Overall, 117 samples were evaluated for the study, distributed along the different PDOs. The results revealed distinct pollinic spectra among the PDO honeys studied. Mel do Parque de Montesinho was predominantly characterised by *Castanea sativa*, followed by *Rubus* spp. and *Fabaceae* (genera *Cytisus*, *Genista* and *Ulex*). Mel de Barroso also showed a strong dominance of *Castanea sativa*, with relevant contributions from *Erica* spp., *Rubus* spp., and

Fabaceae. In Mel da Terra Quente, the pollinic profile was more diverse, with *Fabaceae*, *Jasione montana*, *Castanea sativa*, *Rubus* spp., *Echium* spp., and *Lavandula pedunculata* contributing in similar proportions. Mel da Serra da Lousã was mainly associated with *Erica* spp. and *Castanea sativa*, while *Rubus* spp., *Fabaceae,* and *Eucalyptus* spp. were present at lower levels. Mel Ribatejo Norte was characterised by *Echium* spp. as the main pollen type, followed by *Castanea sativa*, *Trifolium* spp., *Eucalyptus* spp., *Stachys* spp., and *Lavandula* spp. According to the quantitative pollen analysis, all PDO honeys were classified as Class III. Overall, the results revealed discrepancies between the nectar sources defined in the current PDO specification documents and the floral resources effectively exploited by bees, highlighting the need for updating these specifications.

Acknowledgements: the authors are grateful to the program *Promove* from Fundação "*La Caixa*", in collaboration with BPI and the Foundation for Science and Technology (FCT, Portugal) within the scope of project BeeSustain, PD23-00019. Thanks also for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020, UIDP/00690/2020), SusTEC (LA/P/0007/2021) and UID/04033/2023: Centre for the Research and Technology of Agro-Environmental and Biological Sciences and LA/P/0126/2020 (https://doi.org/10.54499/LA/P/0126/2020). This work was further supported by the European Union's NextGenerationEU recovery funds under Project BeeLand (10/C05-i03/2021.PPRR-C05-i03-I-00008)

Environmental DNA metabarcoding explores geographical patterns in honey

Seonmi Kim, Soon Ok Woo, Hong Min Choi, Pureum Im, Suwon Park, Yun-Gon Son, Samgyul Lee, Munseon Lee

National Institute of Agricultural Science, Department of Agricultural Biology, Wanju-gun, Jeonbuk-do, Republic of Korea

CORRESPONDING AUTHOR: *ryheekim19@korea.kr*

Honey contains diverse biological DNA derived from plants, microorganisms, and surrounding environments, reflecting the ecological conditions of its production area. In this study, environmental DNA metabarcoding was applied to explore geographical patterns in domestic black locust honey collected from 13 regions in Korea. To characterise the biological composition of honey, 16S rRNA and ITS2 marker regions were analysed, resulting in the detection of a wide range of microbial and plant-derived DNA taxa. The identified microbial DNA included honeybee-associated, environmental, and plant-associated microorganisms, in addition to the primary nectar source *Robinia pseudoacacia*, multiple plant taxa such as *Actinia*, *Toxicodendron*, *Rubus,* and *Rosa* species were consistently detected across samples. Notably, the composition and relative abundance of plant-derived DNA profiles exhibited regional variation among the honey samples. These results suggest that environmental DNA metabarcoding could capture region-related patterns embedded in honey, providing insights into the ecological characteristics of honey production environments.

Unveiling the diversity of Spanish honey production: origin, quality, and authenticity

María Carmen Seijo[1], María Shantal Rodríguez-Flores[1], Sonia Harbane[1], Manuela Chouza-Carou[1], Ana Pascual-Maté[2], Amelia González- Porto[3], Olga Escuredo[1]

[1]Universidade de Vigo, Campus Auga, Instituto de Agroecoloxía e Alimentación, Facultade de Ciencias, Grupo de Investigación en Sistemas Agroambientais, Ourense, Spain
[2]Universidad Isabel I, Burgos, Spain
[3]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha, Centro de Investigación Apícola y Agroambiental – CIAPA, Marchamalo (Guadalajara), Spain

CORRESPONDING AUTHOR: *mcoello@uvigo.es*

The honey market faces significant challenges that endanger the long-term viability of beekeeping, an activity of major ecological importance. Adulteration, frauds, and confuse labels are practices that erode consumer trust. Although honey has been widely studied, key aspects related to geographical and botanical origin are unclear, making its definition essential to verify authenticity, support local honey and protect the future of beekeeping. This communication summarises the results obtained in a three-year project funded by the Spanish National Research Agency to improve knowledge in Spanish honey production. More than 1,000 honey samples were collected across the national territory in collaboration with the beekeeping sector. Different analyses were conducted, including physicochemical parameters, sensory evaluation, melissopalynological analysis, enzymatic activity, volatile compounds, polyphenol and flavonoid profiles, as well as functional properties such as antioxidant and antimicrobial capacity. More than 25 honey types were classified and described according to botanical and geographical origin. In addition to multifloral honey, northern and western regions are characterised by honey types such as *Castanea*, *Eucalyptus*, *Rubus*, *Erica*, *Calluna*, *Arbutus* and honey-

dew. Meanwhile in central, eastern and southern regions Lamiaceae plants (*Lavandula*, *Rosmarinus*, *Thymus*, *Satureja*, among others), Fabaceae (*Vicia*, *Dorycnium*, *Hedysarum*), *Echium*, *Helianthus*, together singular botanical sources (*Persea*, *Asparagus*, *Pimpinella*, *Coriandrum*...) are characteristics. Multivariate statistical analyses were applied, allowing the establishment of distinctive compositional and functional profiles for the different honey types. Significant variations were observed in moisture content, electrical conductivity, enzyme activity (diastase and invertase), polyphenol concentration, and biological activity. Occasionally, some samples do not meet EU legislation requirements, this is the case of electrical conductivity for some nectar honeys. These findings show that integrated analytical and statistical methods are valuable for honey authentication and quality control. Regulatory updates are also needed to clearly define quality characteristics of different honey types, helping to preserve authenticity and prevent fraud.

Acknowledgements: this project is funded by the Spanish National Research Agency "Valorization and authentication of Spanish apicultural products based on their composition" (Project PID2022-141679OR).

Effects of processing and packaging on the physicochemical properties of honey

Taylan Samanci[1], Aslı Elif Tanuğur Samanci[2], Elif Yorulmaz Önder[3]

[1]Sustainable Agriculture and Ecology Association, Ankara, Türkiye
[2]SBS Bilimsel Bio Çözümler San. ve Tic. A.Ş., İstanbul, Türkiye
[3]BEE&YOU R&D Center, İstanbul, Türkiye

CORRESPONDING AUTHOR: taylan.samanci@sbs-turkey.com

Honey is a natural product composed of sugars, enzymes, organic acids, vitamins, and bioactive compounds, making it a valuable food with both nutritional and medicinal properties. However, processing and packaging techniques significantly influence its physicochemical properties, including enzyme activity, hydroxymethylfurfural (HMF) levels, moisture content, pH, electrical conductivity, colour, total phenolic content, antioxidant activity, and sugar composition. These changes affect honey's quality, authenticity, and consumer perception. This study evaluates the effects of heat treatment, filtration, and packaging on these physicochemical parameters. In this study, a scientifically designed experimental approach was applied to examine the physicochemical changes occurring in honey during various processing steps. A total of 14 honey samples, consisting of seven monofloral and seven multifloral varieties, were analysed. Key assessments included enzyme activity (invertase and diastase), HMF formation, moisture content, pH, electrical conductivity, and colour changes. The findings provided a comprehensive understanding of how processing conditions influenced multiple quality attributes of honey, emphasising the need for optimised processing and packaging strategies to maintain its natural composition and functional properties. Minimising heat exposure and optimising packaging are crucial to preserving honey's overall quality, including its enzymatic activity, chemical stability, and sensory characteristics. These findings highlight the importance of implementing better regulatory standards and quality control measures to ensure consumer trust and product authenticity.

Chemometric profiling of honey samples in Turkey (2016–2025): a decadal survey of physicochemical and carbohydrate signatures consistent with in-hive sugar feeding

Ufuk Alpat[1], İlknur Coşkun[1,2], Tuğçe Daştan[1], Özge Erdem[1], Sezer Acar[1], Özlem Yılmaz[1], Özlem Cengiz[1], Ayça Karakuş[1], Fatma Ezer[1], Fatma Gevrek[1], Emel Damarlı[1]

[1]Balparmak R&D Center, İstanbul, Turkey
[2]Yıldız Technical University, Food Engineering Department, İstanbul, Turkey

CORRESPONDING AUTHOR: *ufuk.alpat@balparmak.com.tr*

This study investigates patterns in honey quality and sugar fingerprints for anomaly detection potentially consistent with non-compliant practices. Honey samples (n = 30,734) collected in Turkey between 2016 and 2025 were analysed for physicochemical parameters (moisture, electrical conductivity, free acidity, diastase activity, HMF, and starch/pollen ratio) and carbohydrate profile (fructose, glucose, sucrose, maltose, erlose, trehalose, isomaltose, turanose, maltotriose, and melezitose). The starch/pollen ratio was determined microscopically according to Louveaux et al. and included as an exploratory indicator of exogenous starch granules potentially associated with starch-containing supplementary feeds or enzyme carrier materials. The botanical origins were blackthorn, cedar, chestnut, citrus, cotton, lavender, linden, oak, pine, sunflower, thistle, thyme, clover, chaste tree, and multifloral honeys. Without colony feeding records, the study uses a data-driven forensic approach, comparing the observed patterns with those reported for authentic floral honeys and for honeys produced when colonies are intensively sugar-fed. Although erlose can form from sucrose through enzyme-mediated reactions and is not unique to any single cause, we observe that elevated sucrose together with atypically high erlose tends to occur as a characteristic pattern. We therefore treat this combined

pattern as a key marker profile, rather than relying on erlose or sucrose alone. Exploratory and confirmatory analyses (correlation, regression/ANCOVA models, distribution and percentile analyses, and analyses performed separately by botanical origin and year) are used to identify subpopulations showing a "sugar-feeding–consistent" signature, namely concurrent elevation of sucrose and related oligosaccharides together with lower free acidity, electrical conductivity, and diastase activity. We interpret these co-occurring changes as reflecting reduced nectar foraging (lower contributions of nectar-derived organic acids and minerals) and altered enzymatic processing in the hive, which generates a different fingerprint from authentic honey profiles. Overall, the work proposes a scalable chemometric screening framework for large honey datasets to prioritise samples for confirmatory authentication testing, while explicitly acknowledging that compositional evidence alone cannot prove feeding history on a per-sample basis.

Challenges and scientific gaps in determining the botanical origin of honeydew honey

Jelena Trifković & Dušanka Milojković-Opsenica

University of Belgrade, Faculty of Chemistry, Belgrade, Serbia

CORRESPONDING AUTHOR: *jvelicko@chem.bg.ac.rs*

Determination of honey authenticity, as one of the most important confirmations of food quality, is essential for the protection of both consumers and producers. Honey authenticity can be considered from two perspectives: authenticity with regard to production and authenticity with regard to labelling, i.e., botanical and geographical origin. Proper labelling in the promotion of honey as a functional food, i.e., a value-added food product, is an important task. The literature describes various chemical compounds, such as sugars, amino acids, minerals, phenolic compounds, and volatile compounds, which essentially represent the "chemical fingerprint" of different types of honey and can be used as markers of their origin. Consequently, the development and application of analytical methods for determining honey authenticity is a current issue and has been the subject of numerous studies in recent decades. Reliable analytical techniques, together with clearly defined criteria and standards in the field of honey quality control, are necessary and represent the only secure tools against adulteration. Although honeydew honey has a distinct nutritional value and is attributed with higher antibacterial and antioxidant properties compared to blossom honey, it is less studied and less familiar to consumers. In order to prevent adulteration and fraud, standardised methods for the routine quality control of honeydew honeys with regard to their botanical and geographical authentication are required. The scientific gap in reporting the botanical origin of honeydew honey should be addressed in more detail, even though no objective method currently exists for the characterisation and differentiation of honeydew honeys. Nevertheless, the botanical origin of honeydew is important not only for its biological activity, but also for honey repeatability, traceability, and marketing.

Production technology of Belarusian willow honey (*Salix L.)*: from early spring nectar flow to botanical identification

Volha Haurylik

Institute of Meat and Dairy Industry, Minsk, Republic of Belarus

CORRESPONDING AUTHOR: *gavrilik.o@gmail.com*

Willow honey is produced in early spring from nectar collected predominantly from plants of the genus *Salix* and represents a rare monofloral honey in Belarus. Although traditionally valued for its delicate sensory properties, the lack of standardised technological and sensory descriptions complicates its objective identification among early-spring honeys. This study integrates technological, biological, analytical, and sensory approaches to establish a reference profile for Belarusian willow honey. Three honey samples were obtained from strong overwintered colonies located in the Pinsk and Grodno districts (Belarus) during the short willow nectar flow (31 March–3 April 2025). Production conditions, including weather dynamics and colony status, were documented together with structured sensory evaluation. The results revealed a stable sensory profile closely linked to early-spring production technology. Willow honey was characterised by a golden-amber colour, smooth buttery texture, and a medium tendency toward crystallisation. The aromatic profile showed medium intensity with dominant light woody and herbal notes. These characteristics were shaped by the extremely short nectar flow, minimal botanical competition, and the activity of overwintered foragers. Early harvesting under low-temperature conditions limited oxidative and thermal changes, preserving sensory freshness and visual clarity. Pollen analysis confirmed monofloral origin, with willow pollen accounting from 67.4 to 79.5% of pollen grains. No pesticide residues or veterinary medicinal products were detected, reflecting nectar collection prior to

agricultural treatments. The integration of technological observations with structured sensory and analytical analysis provides a scientific basis for botanical authentication, standardisation, and recognition of Belarusian willow honey as a distinct early-spring monofloral product.

Metabolomic profiling of black locust honey from different geographical origins

Pureum Im, Soon Ok Woo, Sangmi Han, Hyo Young Kim, Hongmin Choi, Seonmi Kim, Samgyul Lee, Su Won Park, Munseon Lee, Yun Gon Son

Rural Development Administration, National Institute of Agricultural Sciences, Department of Apiculture, Republic of Korea

CORRESPONDING AUTHOR: *wkgur0727@korea.kr*

Concerns regarding the mixing of honey from different geographical origins and the reliability of origin labelling have increased the need for scientific characterisation of honey. In this study, non-targeted metabolomic profiling was applied to investigate differences in the chemical characteristics of black locust honey (*Robinia pseudoacacia* L.) according to geographical origin. A total of 14 black locust honey samples collected from different regions in Korea and four samples from China were analysed using UPLC-Q-TOF MS-based metabolomics. Multivariate statistical analysis using partial least squares discriminant analysis (PLS-DA) revealed a statistically significant difference between honey samples from different origins ($p < 0.05$), despite the limited explanatory power of the model ($R^2X = 0.195$, $R^2Y = 0.407$). Variable importance in projection (VIP) analysis (VIP > 0.7, $p < 0.05$) identified 16 metabolites as major contributors to the discrimination between origins. The metabolites contributing to origin differentiation mainly included amino acids and amino acid derivatives, sphingolipids such as phytosphingosine and dihydrosphingosine, and fatty acid–derived amides including oleamide and palmitamide. Differences in the relative abundance of these metabolites between honey samples from different origins were visualised using heatmap and box plot analyses. In addition, comparison of metabolomic profiles among black locust honey samples collected from different regions within Korea indicated regional variation in metabolite composition,

suggesting that local production environments may influence honey metabolomic characteristics. Overall, this study demonstrates that metabolomic profiling can reveal origin-dependent chemical differences in black locust honey and provides baseline data for future studies aimed at honey origin discrimination using expanded sample sets.

The MISTRAL project: portable solutions for authenticity, traceability, and sustainability in honey production

Marco Ciulu, Muhammad Aqib Khurshid, Alessandra Bossi, Giovanna Felis, Elisa Salvetti, Francesco Pagliarini, Gianni Zoccatelli, Federica Mainente

University of Verona, Dept. Biotechnology, Verona, Italy

CORRESPONDING AUTHOR: marco.ciulu@univr.it

Beekeeping is increasingly threatened by multiple stressors, including climate change, intensive pesticide use, expanding anthropogenic pressures on rural ecosystems, and rising production costs. These factors disproportionately affect small-scale apiculture and contribute to declining honey yields. Market instability is further exacerbated by the circulation of counterfeit and adulterated honey in the Italian and European markets, which reduces the competitiveness of genuine local products and accelerates the abandonment of beekeeping practices. The MISTRAL research project (University of Verona) aims to address these challenges by developing a multidisciplinary approach based on portable analytical solutions aimed at (i) defining a screening procedure to authenticate and trace the origin of honeys in the market, (ii) implementing field-deployable methods for detecting neonicotinoid pesticides, hazardous to both bees and humans, and (iii) establishing innovative approaches for profiling microbial communities naturally present in honey to derive biologically relevant indicators of bee health. Portable FT-NIR instrumentation was employed to develop authentication and traceability models for Italian honeys. This approach enables the acquisition, within seconds and without any type of sample pre-treatment, of a spectral "fingerprint" containing the information required to detect adulterants and verify geographical origin. More specifically, the resulting models allow estimation of adulterant content with an error below 15%, and enable discrimination between Italian honeys and those of other origins with an

average accuracy of approximately 80%. In parallel, pesticide-detection sensors were developed using molecularly imprinted polymers (MIPs) based on surface plasmon resonance plastic optical fiber (SPR-POF), which selectively bind target molecules and generate measurable signals even at low concentrations. For instance, the detection of the pesticide acetamiprid was possible at concentrations below 319 nM, which is the threshold set by EFSA. Finally, the microbiological analysis relied on an experimental protocol designed to obtain a phylogenetic fingerprint, i.e., a microbial signature generated following DNA extraction from honey. A comparative analysis of the microbial profiles of different honey varieties is currently underway.

DNA metabarcoding reveals the botanical diversity of Australian honey

Zahra Karimi[1], Sophie Dodd[1], Zoltan Kevei[1], Tatiana Stead[1], Keegan Blignaut[2], Maria Anastasiadi[1]

[1]Cranfield University, Faculty of Engineering and Applied Sciences, Centre for Soil, Agrifood and Biosciences, Cranfield, Bedfordshire, UK
[2]Duxton Bees Proprietary Limited, Pallamana, Australia

CORRESPONDING AUTHOR: m.anastasiadi@cranfield.ac.uk

Honey is a natural product whose composition reflects the floral diversity of surrounding landscapes, making it a valuable indicator of foraging ecology, authenticity, and product quality. Australia produces a wide range of honeys with distinctive profiles due to its rich and diverse native flora. In this study, DNA metabarcoding was used to investigate the botanical diversity of Australian honey. A total of 34 honey samples were analysed, including 14 samples sourced directly from Australian beekeepers (Duxton Bees and Hampson Honey) and 20 commercially available products from the Australian market. DNA metabarcoding revealed that beekeeper-sourced honeys were predominantly composed of plant species from the family *Myrtaceae*, with a particularly strong representation of *Eucalyptus* spp., highlighting the significant contribution of native flora to Australian honey. In contrast, commercial honeys exhibited a broader and more heterogeneous floral composition, dominated by *Fabaceae*, *Papaveraceae*, *Rosaceae*, and *Fagaceae*, consistent with multi-regional or blended origins. Multivariate analyses, including principal coordinates analysis (PCoA) and PERMANOVA, confirmed significant differences in floral profile between beekeeper-sourced and commercial honeys. Overall, this study demonstrates the effectiveness of DNA metabarcoding in characterising the botanical origins, quality, and authenticity of Australian honey.

Pesticides in pollen collected by honey bees near orchards and vineyards

Christina Kast & Marion Fracheboud

Agroscope, Swiss Bee Research Centre, Bern, Switzerland

CORRESPONDING AUTHOR: christina.kast@agroscope.admin.ch

Honey bees can be exposed to a variety of pollutants, including pesticides from agriculture. The aim of this study was to investigate the types of pesticides found in bee pollen from apiaries in agricultural environments with orchards (three study sites), vineyards (two study sites) or both (one study site). Four hives were sampled per study site. We investigated the seasonal timing of bee pollen contamination by collecting samples one day per week from April to August 2024 using pollen traps installed in front of the hive entrances. Bee pollen from the four colonies was pooled for each date and study site, resulting in a total of 123 samples. Analytical methods based on ultra-high-performance liquid chromatography coupled with mass spectroscopy enabled the identification of 30 pesticides (out of 50 analysed), including 14 fungicides or their transformation products, 10 insecticides, three acaricides or their transformation products, two herbicides, and one synergist. The highest prevalences were observed for the fungicides cyprodinil (52%), trifloxystrobin (43%), difenoconazole (39%), and mandipropamid (37%), and for the insecticide acetamiprid (33%). Maximal concentration levels for acetamiprid (79 µg/kg), cyprodinil (24,543 µg/kg) and trifloystrobin (441 µg/kg) were measured at study sites near orchards during the flowering period of the fruit trees. By dividing the bee pollen samples into several colour fractions, these pesticides could be assigned to apple, pear or cherry pollen using melissopalynology. High mandipropamid levels in bee pollen (maximum concentration of 563 µg/kg) were measured in June at study sites near vineyards. At one of the study sites, this fungicide could be attributed to pollen from grapevines (*Vitis sp*.). Our study showed that honey bees

near fruit or wine-growing areas are exposed to high pesticide levels. The mortality rate in bee colonies observed at the flight entrances was not above normal levels. However, high pesticide levels in pollen can be problematic for the use of pollen as a dietary supplement.

The quality of beeswax blocks and comb foundations in Poland

Katarzyna Kusyk & Teresa Szczęsna

The National Institute of Horticultural Research, Apiculture Division, Bee Products Quality Testing Laboratory, Skierniewice, Poland

CORRESPONDING AUTHOR: *katarzyna.kusyk@inhort.pl*

The aim of this study was to conduct monitoring tests on the quality of beeswax blocks and comb foundations in Poland. Samples for testing were provided by beekeeping organisations and individual beekeepers from across the country in 2025. A total of 170 samples were tested, including 81 samples of crude beeswax and 89 samples of comb foundations. Tests were carried out to detect adulteration of beeswax blocks and comb foundations with paraffin and stearin by FTIR-ATR spectroscopy. Samples that did not show the presence of these adulterants (at a detection limit of 3%) were tested to characterise their physicochemical properties (melting point, acid value, saponification value, and iodine value) and long-chain hydrocarbon composition using GC-MS. The crude beeswax and comb foundations samples did not contain any added stearin (with a detection limit for these substances set at 3%). The tested beeswax blocks samples also did not contain paraffin of foreign origin (≥ 5%), and the number of comb foundations samples adulterated with these substances in relation to the total number of samples tested was only 8%. The melting point of unadulterated samples ranged from 63.3 to 64.9 °C (average 63.8 °C), the acid value from 18.3 to 21.6 mg KOH/g beeswax (average 19.8 mg KOH/g of beeswax), saponification value from 72.5 to 105.3 mg KOH/g beeswax (average 90.5 mg KOH/g beeswax, and iodine value from 7.2 to 13.7 g I_2/100 g of beeswax (average 11.5 g I_2/100 g of beeswax). The hydrocarbon composition determined by GC-MS for these samples is comparable to the results obtained at Bee Products Quality Testing Laboratory in previous studies. The results obtained in 2025 indicated a better quality of crude beeswax and comb foundations traded on the domestic market than in 2022.

Assessment of the quality of beeswax available on the Polish market

Ewa Waś

HoneyLab Teper & Waś, Puławy, Poland

CORRESPONDING AUTHOR: *ewa.was@honeylab.pl*

The poor quality of crude beeswax and comb foundation is one of the problems currently facing the beekeeping industry in many European countries. There are various reasons for the difficult situation on the beeswax market, the most important of which are: insufficient domestic supply, lack of legal regulations concerning the quality of this product and the reference method used to detect its adulteration, and, as a consequence, the lack of an effective quality control. The aim of the study was to assess the quality of beeswax available on the Polish market. For the detection of beeswax adulteration, we have implemented one of the most advanced methods of infrared spectroscopy Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR). Over five years of research, we have analysed 2,335 of samples. Adulterants were detected in 31.3% samples out of total analysed. Paraffin was the most commonly used beeswax adulterant (in 97.1%), but adulteration with stearin and other foreign substances also occurred sporadically. Most of the samples (84.5%) in which paraffin was identified contained between 3–5% of this adulterant. In other cases, the degree of adulteration with paraffin was higher and ranged from: more than 5 and up to 10% (6.2%); more than 10 and up to 20% (3.0%); more than 20 up to 30% (2.0%); more than 30 up to 50% (0.3%), and more than 50% (4.1%). Our results represent the largest dataset on beeswax adulteration in Poland. Although the actual scale of the problem is difficult to estimate, our results show that beeswax adulteration occurs quite frequently and, in some samples, the degree of adulteration is rather high.

Production variables, analytical control, and quality assurance of bee venom

Dario Lasić, Maja Budeč, Gordana Jurak, Danijel Brkić, Jasna Bošnir

Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia

CORRESPONDING AUTHOR: *dario.lasic@stampar.hr*

Bee venom is a high-value natural product with expanding use in pharmaceutical research, apitherapy, and cosmetic applications. As interest in its commercialisation grows, regulatory expectations and the implementation of Good Beekeeping Practices (GBP) have become essential for ensuring consistent quality, safety, and traceability. This overview focuses on quality issues, together with regulatory considerations and the role of GBP in standardised bee venom production, supported by analytical quality assessment. The composition of bee venom, including key bioactive components such as melittin, apamin, and phospholipase A_2, is influenced by multiple production-related factors. These include honey bee subspecies, colony health and nutrition, environmental conditions, seasonal variation, and venom harvesting protocols. Regulatory guidance increasingly emphasises control of these variables to reduce batch-to-batch variability and to ensure reproducible biological activity. This overview aims to outline recommended GBP for bee venom production, including hygienic handling of collection equipment, controlled electrical stimulation parameters, and appropriate harvesting intervals to minimise colony stress. Documentation, traceability, and operator training will be discussed as critical elements aligned with quality management systems. Analytical methods play a central role in regulatory compliance and quality assurance. The presentation will review validated analytical techniques used to confirm identity, purity, and potency of bee venom, as well as to detect contaminants. These data support compliance with emerging pharmacopeial and EU regulatory frameworks for natural bi-

oactive substances. Emphasis will be placed on integrating analytical quality control with GBP to support sustainable production, protect bee welfare, and ensure safe downstream applications. The adoption of clear regulatory guidance and standardised practices is essential for the responsible development of bee venom as a regulated natural product.

Acknowledgments: this study was partly carried out within the "Food Safety and Quality Center" (K.K.01.1.1.02.0004), project funded by the European Regional Development Fund.

Spectroscopic methods for botanical and geographical authentication of honey from Mediterranean area

Davide Martini, Silvia Magro, Massimo De Marchi, Giovanni Niero

University of Padova, Dept. of Agronomy, Food, Natural Resources, Animals and Environment, Legnaro, Italy

CORRESPONDING AUTHOR: davidemartini0397@gmail.com

Identifying the geographical and botanical origin of honey is critical to ensure authenticity. A wide range of spectroscopic techniques has been applied for honey authentication, including ultraviolet-visible (UV-VIS) spectroscopy, Fourier-transform infrared spectroscopy, near-infrared (NIR) spectroscopy, Raman spectroscopy, nuclear magnetic resonance, isotope ratio mass spectrometry (IRMS), and gas chromatography-mass spectrometry (GC-MS). Some of them require expensive instrumentation, complex sample preparation, and specialised expertise (e.g., GC-MS or IRMS). In this study, VIS, NIR spectroscopy, and energy-dispersive X-ray fluorescence (ED-XRF) were applied as methods potentially providing a rapid, non-destructive, and more accessible alternative for honey authentication. This study evaluated the effectiveness of diverse spectroscopic techniques in predicting the geographical and botanical origin of honeys of the Mediterranean area (Italy n = 20, Spain n = 6, France n = 8, Greece n = 11, and Croatia n = 9). Fifty-six honey samples were sourced from commercial retailers, including black locust (n = 14), chestnut (n = 15), linden (n = 13), and lavender (n = 12). Spectra were acquired using two NIR devices (benchtop and portable), one VIS colorimeter, and a benchtop ED-XRF instrument. Prediction models were developed using partial least square discriminant analysis (PLS-DA). Spectral data were divided into a calibration set (including 70% of the samples) and a validation set (including 30% of the samples). Model performance was assessed using sensitivity, specificity, positive and negative predictive values, and balanced accuracy for both the calibration

and validation sets. In external validation, the VIS colorimeter achieved the highest performance for botanical origin (accuracy = 1). Conversely, the geographical origin showed limited predictability, with accuracy values ≤ 0.57 across all the spectroscopy techniques. Findings of the present study highlight the considerable potential of spectrometry-based approaches for discriminating botanical origins, while emphasising the need for further methodological refinement to improve the robustness of geographical origin classification.

The role of accompanying pollen in determining the geographical origin of monofloral honey across Europe: a focus on black locust honey (*Robinia pseudoacacia* L.).

Ghalia Kassem

Eurofins Food Integrity Control Services GmbH, Riterhude, Germany

CORRESPONDING AUTHOR: *ghalia.kassem@ftdach.eurofins.com*

Monofloral honeys reveal the floral source, while accompanying pollen adds a geographic signature. This study investigates how accompanying pollen profiles, pollen diversity, and pollen proportions in black locust (*Robinia pseudoacacia* L.) honeys from European countries can refine geographic origin determination. Samples of different monofloral honeys were analysed by pollen analysis (microscopic identification) and statistical analysis. Results have shown that accompanying pollen profiles reveal distinct regional signatures in black locust honey, reflecting the surrounding flora of each production area. This leads to the conclusion that accompanying pollen represents a valuable complementary tool for authenticating and tracing the origin of European monofloral honeys, with implications for quality control, origin labelling, and honey biodiversity research.

Kalokimithia (Ballota) honey: a rare honey type produced only in Crete, Greece

Panagiota Gotsiou[1], Sofia Loupasaki[1], Ilektra Remoundou[1], Spyridon Grigorakis[1], Fatima Zahra Dahmani[1], Fotini Papadopoulou[2], Vasilios Liolios[2], Dimitrios Kanelis[2], Maria-Anna Rodopoulou[2], Chrysoula Tananaki[2]

[1]CIHEAM-Mediterranean Agronomic Institute of Chania, Laboratory of Analytical Chemistry and Chemistry of Natural Products, Greece
[2]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Apiculture & Sericulture, Greece

CORRESPONDING AUTHOR: yiota@maich.gr

Kalokimithia (or Ballota) honey is a rare type of nectar honey produced only in the Chania region of the island of Crete (Greece) and nowhere else in the world, due to the limited distribution of the plant 'Kalokimithia' [*Ballota pseudodictamnus* (L.) Benth. subsp. *pseudodictamnus*, syn. of *Pseudodictamnus mediterraneus* Salmaki & Siadati *subsp. mediterraneus*]. The plant has so far been recorded only in western Crete, the island of Antikythira (Greece), and specific areas of Cyrenaica (northern Libya). The significant production of Kalokimithiá honey in Chania region creates favourable future prospects for its systematic commercial exploitation. In response, the Region of Crete has funded a two-year project to investigate and characterise this honey. Kalokimithia honey is characterised by low moisture content (mean = 15.5%) and low electrical conductivity (0.158–0.471 mS/cm). Diastase activity and free acidity were determined at medium levels (mean = 18 DN and 20.5 meq/kg, respectively), while its low pH (mean = 3.7) is consistent with a slightly acidic aftertaste. It is a very light-coloured honey (mean = 23 mm Pfund, Lmean* = 37.7) comparable to acacia and citrus honey. The fructose/glucose ratio is close to unity, indicating a relatively rapid crystallisation tendency. Its characteristic feature is the relatively high sucrose content (mean = 3.1%), with

17% of the samples exceeding the 5% limit set by the Directive 2001/110/EC. Melissopalynological analysis revealed *Ballota* pollen grains in the characteristic honeys ranging from 37 to 98% (mean = 65.3%), while 40% of the samples were found to have less than 45%, indicating that the Ballota pollen could be under-represented in the unifloral honey. In more than 70% of the *Ballota* honey samples, thyme pollen grains were found (1–13%), which is attributed to the successive and partially simultaneous flowering of the two plants. Sensory evaluation showed a very light-coloured honey with clarity and high viscosity, medium aroma with floral, fruity odour, and strong sweetness and aftertaste.

The characterisation of the sensory profiles of British monofloral honeys

Gino Abdul-Jabbar[1], Rebecca Beveridge[1], Helen Rogers[1], Raffaele Dall'Olio[2], Gian-Luigi Marcazzan[3]

[1]The Honey Guild of the United Kingdom, UK
[2]Bee Sources, Italy
[3]CREA – Research Centre for Agriculture and the Environment & The Italian Register of Experts in Sensory Analysis of Honey, Italy

CORRESPONDING AUTHOR: *gino.jabbar@honeyguild.co.uk*

The sensory profiles of monofloral honeys provide insight into the organoleptic properties of specific botanical sources. In addition, this may be used to probe further aspects of honey such as quality and an objective measure of terroir. Here we present for the first-time extensive work carried out on the sensory profiles of British monofloral honeys. The sensory profiles were determined using the method of honey sensory analysis with a panel consisting of accredited members of the *Italian Register of Experts in the Sensory Analysis of Honey*. The method consisted of a blind tasting employed over three distinct phases of sensory evaluation. First, tasters individually assessed olfactory, aroma and texture of honey samples labelled solely by a string of digits. Second, tasters were provided with sealed transparent tasting glasses labelled by a string of digits to individually evaluate the aspect and colour. It was not possible to detect olfactory, aroma and texture during this stage. Thirdly, the honeys were tasted for a second time as a panel. Each honey was tasted and discussed, with majority verdicts, points of variation, and a panel sensory description of each honey labelled solely by a string of digits determined. In total 15 UK monofloral profiles were characterised: borage *Borago officinalis,* Himalayan balsam *Impatiens glandulifera,* field bean *Vicia faba*, rapeseed *Brassica napus,* apple *Malus*, lime *Tilia,* hawthorn *Crataegus monogyna,* blackberry *Rubus,* blueberry *Vaccinium,* sunflower *Helianthus annuus,*

sea lavender *Limonium*, tree of heaven *Ailanthus altissima*, Ivy *Hedera helix*, bell heather *Erica cinerea*, and ling heather *Calluna vulgaris*. In the discussions that follow we explore how differences in our results from those documented in continental Europe may result from the effect of the UK's maritime northern European climate and geography which may support a better understanding of European honey terroir and support efforts towards geographic authenticity.

Preliminary results of Slovenian sweet cherry honey characterisation

Aljaž Debelak[1], Urška Ratajc[1], Boris Potočnik[1], Simon Golob[1], Marinka Kregar[2], Jasna Bertoncelj[3], Mojca Korošec[3]

[1]Slovenian Beekeeper's Association, Lukovica, Slovenia
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia
[3]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

CORRESPONDING AUTHOR: aljaz.debelak@czs.si

Beekeepers have begun using new beekeeping techniques to exploit previously untapped sources of bee pastures. As a result, the production of lesser-known honey types has been steadily increasing. In recent years, beekeepers from the Coastal-Karst region and southeastern Slovenia have begun producing sweet cherry (*Prunus avium*) honey with growing consistency. The objective of this study was to determine the characteristics of this lesser-known monofloral honey and to assist beekeepers in its proper labelling. A total of 11 sweet cherry honey samples were collected between 2023 and 2025. Physicochemical parameters, including water content, electrical conductivity, and the fructose-to-glucose ratio, were analysed using standard analytical methods (refractometry, conductometry, and HPLC). The samples were analysed for pollen content using standard melissopalynological methodology. The quantitative sensory profile was determined by a six-member trained panel of honey assessors. The water content of the samples analysed ranged from 14.6% to 17.0%. Electrical conductivity varied between 0.60 and 1.12 mS/cm, with an average value of 0.85 mS/cm. Five (45%) samples exhibited an electrical conductivity exceeding 0.80 mS/cm. The fructose-to-glucose ratio ranged from 1.07 to 1.47, with a mean value of 1.24. The average proportion of *Prunus* pollen was 24%, ranging from 5% to 58%, based on total pollen count. The sample containing only 5% *Prunus* pollen contained a very high content of *Myosotis* pollen (87%). On

average, the most abundant pollen types were *Acer*, *Prunus*, *Myosotis, Castanea sativa*, and *Salix*, listed in descending order of representation. When excluding pollen from over-represented and non-nectariferous plants, the proportion of *Prunus* pollen ranged from 15% to 68%. In the liquid state, the honey samples exhibited a colour ranging from medium amber to dark amber. Natural crystallization may occur rapidly; in the crystallized state, the colour ranged from light amber to light amber with a grey-green hue. The taste was described as medium sweet to sweet, weakly to moderately sour and, in some cases, slightly bitter. The odour and aroma were of medium persistence. Predominant aroma included warm (almond, marzipan, cherry stone), floral (cherry blossom), and fruity (cherry fruit, cherry compote, processed fruits) notes.

Identification of culturable microbiota associated with the transformation of bee pollen into bee bread

Valentina Odorčić[1], Mirna Mrkonjić Fuka[1], Lidija Svečnjak[2]

[1]University of Zagreb Faculty of Agriculture, Department of Microbiology, Zagreb, Croatia
[2]University of Zagreb Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia

CORRESPONDING AUTHOR: vodorcic@agr.hr

Honey bees collect pollen and mix it with their salivary gland secretions and nectar, thus producing bee pollen (BP). They further transfer BP into the hive and store it in honeycomb cells, where, under the influence of moisture, enzymes, yeasts, and lactic acid bacteria, it ferments into a bee bread (BB). However, the microbiota involved in BP-to-BB transformation process remains insufficiently investigated. Therefore, the aim of this study was to identify the culturable microbiota present during the BP to BB transformation. BP and BB samples were collected from three colonies of Carniolan honey bees (*Apis mellifera carnica*) at the experimental apiary of the University of Zagreb Faculty of Agriculture. Sampling was conducted on different days (day 0, 1, 3, 5, 7, 10, 13, 17) during the transformation process (different fermentation stages) to monitor changes in the microbiota. Fresh multifloral BP (n = 3) and corresponding BB (n = 12) samples were used to isolate bacteria and fungi on selective and non-selective nutrient media. All isolates (n = 375) were identified to the genus or species level by MALDI-TOF MS or Sanger sequencing. In total, 24 different genera were identified, comprising bacterial (n = 294) and fungal (n = 81) isolates. The most prevalent bacterial species was *Apilactobacillus kunkeei* (n = 213), which predominated in all analysed samples. Among the fungal isolates, the yeast *Metschnikowia* spp. (n = 38) predominated and were present throughout the fermentation process. Both *Apilactobacillus* spp. and *Metschnikowia* spp. are commonly found

in habitats such as flowers, fruits, and the digestive tracts of insects. Moreover, *Apilactobacillus kunkeei* is a fructophilic lactic acid bacterium (FLAB) efficient in lactic fermentation, while *Metschnikowia* spp. play a key role in the unique nutritional profile and stability of BB by competing with spoilage organisms and producing beneficial compounds. Therefore, these predominant species likely play the most significant role in the transformation pathway of BP into BB.

Acknowledgements: this research was funded by the Paying Agency for Agriculture, Fisheries and Rural Development of the Republic of Croatia (APPRRR, class: 320-09/23-07/0011) within the frame of the project entitled "Chemical, molecular and antimicrobial profiling of bee pollen, bee bread and propolis (Bee-PROfiling)" (2024-2025).

Insights into sugar composition and FTIR-ATR spectroscopy of stingless bee honey

Jéssica B. Ressutte[1], Diego Galvan[2], Cynthia F.P. da Luz[3], Ana Margarida Gonçalves[4], Kemilla S. Rebelo[5], José Augusto G. Sattler[6], Luis Passarinha[7,8], Eugenia Gallardo[4], Wilma A. Spinosa[1], Ofélia Anjos[9,10]

[1]State University of Londrina, Department of Food Science and Technology, Londrina, Brazil
[2]Federal University of Santa Catarina, Department of Chemistry, Florianópolis, Brazil
[3]Environmental Research Institute, Biodiversity Conservation Center, Palynology Laboratory PALINO-IPA, São Paulo, Brazil
[4]Universidade da Beira Interior, Faculdade de Ciências da Saúde, Departamento de Ciências Médicas, RISE-Health, Covilhã, Portugal
[5]Federal University of Amazonas, Faculty of Agricultural Sciences, Department of Agricultural and Soil Engineering, Manaus, Brazil
[6]Ritter dos Reis University Center, School of Health Sciences, Faculty of Nutrition, Porto Alegre, Brazil
[7]NOVA University Lisbon, NOVA School of Science and Technology, Chemistry Department, UCIBIO-Applied Molecular Biosciences Unit, Caparica, Portugal
[8]Universidade NOVA de Lisboa, NOVA School of Science and Technology, Associate Laboratory i4HB-Institute for Health and Bioeconomy, Caparica, Portugal
[9]Polytechnic University of Castelo Branco, Research Centre for Natural Resources Environment and Society, CERNAS-IPCB, Castelo Branco, Portugal
[10]Centre of Plant Biotechnology of Beira Interior, Spectroscopy and Chromatography Laboratory, CBP-BI, Castelo Branco, Portugal

CORRESPONDING AUTHOR: ofelia@ipcb.pt

This study investigated several samples of honey from five genera of stingless bee from Brazil, focusing on sugar profiling and FTIR-ATR spectroscopy. HPLC-RID sugar analysis revealed substantial variation in the levels of key carbohydrates: glucose, fructose, maltose, raffinose, and trehalulose. The honey produced by genera *Tetragona*, *Tetragonisca*, and *Frieseomelitta* exhibited particularly high levels of trehalulose and

maltose, whereas the produced by *Melipona* and *Scaptotrigona* samples showed higher levels of monosaccharides. There were strong correlations, including a negative relationship between trehalulose + maltose and the major monosaccharides. This highlights sugar composition as a central discriminating factor between bee genera. FTIR-ATR spectroscopy, particularly in the 1800–750 cm^{-1} region, has proven to be a valuable tool for identifying spectral fingerprints associated with carbohydrate functional groups. Differences in band intensities across genera reflect variations in sugar composition and other matrix constituents. Multivariate analysis of the FTIR spectral data revealed three regions to be the most influential for class separation: 1610–1557 cm^{-1}, 1126–960 cm^{-1}, and 1170–915 cm^{-1}. These regions primarily correspond to carbohydrate vibrations, which reinforces the central role of sugar chemistry in differentiating samples. When combined with chemometric models (PLS-DA and LDA), both sugar profiling and FTIR-ATR analysis enabled the robust discrimination of honey produced by *Melipona*, *Scaptotrigona* and *Tetragonisca* species. As conclusion, the study demonstrates that sugars and FTIR-ATR spectral data are the most decisive analytical dimensions for understanding entomological influences (determined through palynological analysis) and ensuring the authenticity and traceability of stingless bee honey.

Acknowledgements: thanks for financial support by national funds FCT/MCTES through the projects UID/00681/2025 (DOI: 10.54499/UID/00681/2025) of Research Centre for Natural Resources, Environment and Society (CERNAS), Applied Molecular Biosciences Unit UCIBIO (UIDB/04378/2020 and UIDP/04378/2020) and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020), and the project BioLivingLABS, Collective Actions – Transfer of scientific and technological knowledge, COMPETE2030-2024-6, Project Number 17807, co-financed by the European Regional Development Fund (ERDF) throughCOMPETE2030 (Thematic program for Innovation and Digital Transition).

Sensory profiling and acceptance of lesser-known Slovenian and Argentinean honey types among young consumers

Mojca Korošec[1], Jasna Bertoncelj[1], Anja Bolha[1], Eva Marija Čad[1], Lucía Elisabet Brelis[2], Veronica Busch[2], Blaž Ferjančič[1]

[1]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
[2]Universidad Nacional de Entre Ríos, Instituto de Ciencia y Tecnología de Alimentos de Entre Ríos, Entre Rios, Argentina

CORRESPONDING AUTHOR: *mojca.korosec@bf.uni-lj.si*

In light of climate change, bee forage resources are shifting, altering the types of honey available. As taste strongly influences food acceptance—and since repeated exposure is often required for familiarity-this study evaluated the acceptance of four lesser—known honey types among young consumers. Two samples each of common ivy (*Hedera helix*), buckwheat (*Fagopyrum esculentum*), and sweet cherry (*Prunus avium*) honey from Slovenia, and two of coronilla (*Scutia buxifolia*) from Argentina, were evaluated by 35 young consumers (avg. age 23.3 ± 1.3 years). Acceptance was measured using a 9-point hedonic scale, while odour and flavour were described using the Check-All-That-Apply (CATA) method. Botanical origin, water content, and electrical conductivity were pre-verified by a professional panel. Hedonic scores differed significantly, with coronilla honey receiving the lowest ratings (2.8–2.9) and sweet cherry honey the highest (5.3–5.9). Coronilla honey was primarily described by "animal" and "unpleasant" odours and "faecal" flavour. Sweet cherry honey was characterised by "floral" flavour and "sweet" taste, though 36.8% found its odour "unpleasant". Common ivy honey (5.0) was perceived as "mildly floral," while buckwheat honey (4.8) exhibited "unpleasant" odour but "caramel" flavour. CATA analysis revealed that "unpleasant," "faecal," and "animal" flavours negatively correlated with acceptance, while "floral," "sweet," and "cotton candy" notes showed positive corre-

lations. Results indicate that while young consumers distinguish between these sensory profiles, overall acceptance is low. Given the increasing prevalence of these honey types, appropriate presentation is essential to ensure consumers recognise their biological and nutritional value.

Melissopalynology coupled with multivariate analysis to investigate the geographical origin of Italian honeys

Martina Denami[1], Lorenzo Braga[1], Maria Lucia Piana[2], Anna Maria Mercuri[1]

[1]University of Modena and Reggio Emilia, Department of Life Sciences, Laboratory of Palynology and Paleobotany, Modena, Italy
[2]Piana Ricerca e Consulenza s.r.l., Castel San Pietro Terme, Italy

CORRESPONDING AUTHOR: martina.denami@studenti.unimore.it

Honey traceability is increasingly required by consumers and food control institutions as indispensable element of food authenticity and quality. The growing demand for clear and reliable honey labelling has highlighted the importance of improving analytical approaches capable of differentiating honeys according to their geographical origins, even when derived from the same botanical source. Melissopalynology is the method traditionally applied to determine the botanical origin of honey using the relative abundance of nectariferous pollen grains. In addition, the analysis of the entire pollen spectrum can support the determination of geographical origin, as it reflects the floristic composition of the production areas. However, this evaluation is often based on qualitative assessment and expert judgement rather than on multivariate statistical evidence. The study aimed to evaluate whether pollen spectra, analysed through an exploratory multivariate approach, highlight patterns consistent with the geographical origin of honey samples from two Italian regions, Valle d'Aosta (northern Italy) and Calabria (southern Italy). A total of 267 honey samples were melissopalynologically analysed, including 180 samples from Valle d'Aosta, produced in 2025 and 87 samples from Calabria, produced between 2023 and 2024. To improve melissopalynological routine analysis, pollen data were explored using multivariate statistics and ordination methods. The results highlighted differences

between honey samples from the two regions. Samples from Calabria were associated with pollen taxa such as *Hedysarum coronarium*, *Echium*, *Olea*, and *Fraxinus*, typical of Mediterranean and sub-Mediterranean vegetation. Conversely, honeys from Valle d'Aosta were characterised by pollen types including *Rubus*, *Tilia*, *Prunus*, Ericaceae (possibly *Rhododendron*), and *Myosotis*, representative of temperate montane and subalpine floras. These results indicate that these techniques can support melissopalynological analyses in the regional characterisation of honeys. The pollen taxa contributing the most to the differentiation of samples correspond to those traditionally used in botanical and geographical interpretation, highlighting the potential of multivariate analyses and ordination techniques as complementary tools to improve routine melissopalynological analysis.

From hive to hospital: navigating the regulatory maze of medical-grade honey

Juraj Majtan, Jana Godocikova, Viktoriia Chirkova, Martin Safranek

Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia

CORRESPONDING AUTHOR: *juraj.majtan@savba.sk*

Medical-grade honey (MGH) is increasingly utilised in clinical practice, particularly for wound care applications. However, transforming natural honey into MGH faces substantial regulatory and quality challenges. The lack of precise definitions, quality standards, and biological potential criteria for MGH complicates its registration as a medical device and CE-marking compliance. Updated EU medical device regulations have created additional hurdles for existing and new MGH-containing wound care products, requiring manufacturers to meet stringent documentation and testing requirements that are currently not well-defined for honey-based products. The quality control challenges begin with honey authenticity. The 2021 European Commission's "From the Hives" action revealed that 46% of imported honey was adulterated with sugar syrups, making the selection of appropriate raw materials for MGH production extremely difficult. Current international honey standards in Codex Alimentarius focus primarily on physicochemical parameters such as hydroxymethylfurfural (HMF) and diastase number, which show limited reliability, particularly for thermally treated or gamma-sterilised samples. Critically, none of these standards are associated with biologically active compounds or therapeutic activities required for medical device registration. Recent research demonstrated that over 40% of commercially available honeys exhibited antibacterial activity comparable to artificial sugar solutions, highlighting that conventional quality parameters fail to predict therapeutic efficacy. This disconnects between food-grade

standards and medical requirements creates significant obstacles in the regulatory pathway for MGH development. This study aims to address these challenges by defining key quality standards for MGH, including defensin-1 content, polyphenolic substances, hydrogen peroxide levels, and glucose oxidase activity. The biological activities, including antibacterial, antioxidant, and anti-inflammatory will be comprehensively presented in order to establish minimum quantitative requirements suitable for medical device registration.

Acknowledgements: this work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences VEGA 2/0011/26.

Propolis: Metamorphosis of medicine

James Fearnley

Apiceutical Research Centre, International Propolis Research Group, Whitby, North Yorkshire, UK

CORRESPONDING AUTHOR: *James.fearnley@beearc.com*

Attitudes towards orthodox medicine are changing. Increasing numbers of consumers are questioning both the premise and the efficacy of targeted synthetic pharmaceutical medication. The predicted exponential growth of alternatives (personalised, functional, natural and complementary medicine) illustrates how consumers are looking for gentler, less invasive therapeutic forms with fewer side effects. The growth of research and consumer interest in the medicinal properties of propolis, and bee products in general, is part of this movement. However, current research continues to focus on key actives and anti-activity (anti-bacterial, anti-inflammatory, anti-viral etc.). Yet research over recent years points increasingly to the unique relationship between local flora, i.e. local chemistry in determining the unique biological properties of propolis. With propolis now seen as fundamental to the immune system of the honeybees and with local ecology providing the honeybee with unique biological immunity, the notion of single active chemicals with standardised cause and effect is fading. Given that human beings have been using propolis medicinally for millennia, i.e., the source of unique immune support for the honeybee, it seems reasonable to expect a similar correlation for human beings. The discovery of an anti-trypanosome chemical in propolis from an area where sleeping sickness was present in the human population highlights the potentially vital importance of this interrelationship between the ecology of the plant, the honeybee and the human being. Medicine is undergoing a form of metamorphosis away from specific targeted and standardised to local, ecological and contextual. "One mans medicine is another man's poison" *Propolis: Metamorphosis of Medicine* explores how propolis is articulating this movement.

From chemical composition to biological function: propolis as a model phenolic system

Dušanka Milojković-Opsenica[1], Aleksandra Dramićanin[1], Darija Vukić Lušić[2]

[1]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia
[2]University of Rijeka, Faculty of Medicine, Rijeka, Croatia

CORRESPONDING AUTHOR: dusankam@chem.bg.ac.rs

Propolis is a chemically complex natural product whose physicochemical properties and associated biological activity are largely governed by its botanical origin and the qualitative and quantitative distribution of phenolic acids, flavonoids, and related secondary metabolites. Pronounced compositional variability renders propolis a valuable model system for investigating structure-activity relationships within multicomponent natural matrices. Rather than resulting from the action of individual constituents alone, its biological effects are more accurately interpreted through the integrated phenolic profile and interactions among structurally diverse compounds. Although isolated flavonoids, such as galangin, exhibit intrinsic bioactivity, whole propolis extracts consistently demonstrate enhanced protective effects, indicating synergistic interactions determined by molecular composition. These effects are primarily associated with redox-related mechanisms, including antioxidant and radical-scavenging activity, metal-chelating properties, and modulation of cellular processes involved in maintaining genomic stability. Accordingly, comprehensive physicochemical characterisation—combining qualitative profiling with quantitative evaluation of key phenolic components—is an essential prerequisite for establishing meaningful correlations between chemical composition and biological function. Overall, propolis emerges as a chemically rich and structurally diverse natural product with significant potential for mitigating oxidative and genotoxic stress, while simulta-

neously serving as a model system for studying composition-function relationships in complex bioactive materials.

Acknowledgements: this research was funded by the project European Union – NextGeneration EU – CCBE-Propolis – uniri-iz-25-164.

Functional validation of a buccal spray formulated with Brazilian green and red propolis: selective antimicrobial, antioxidant and anti-inflammatory activities

Andresa Aparecida Berretta[1], Luana Zamarrenho[1], Jéssica Aparecida de Lima[1], Monia Melo[1], Rachel Monteiro[2], Gabriel Guimarães Calefi[3], Anna Livia Oliveira Santos[3], Júlia Gomes Teixeira[3], Emilly Camargo Lopes[4], Mariana da Silva Honorio[4], Nathalia Baptista[1], Giovanna Borini[1], Juliana Issa Hori[1], Carlos Henrique Gomes Martins[3], José Mauricio Sforcin[4]

[1]Research, Development & Innovation Laboratory, Apis Flora Indl. Coml. Ltda (Apis Flora), Ribeirão Preto, SP, Brazil
[2]Microbiology Laboratory, Apis Flora Indl. Coml. Ltda (Apis Flora), Ribeirão Preto, SP, Brazil
[3]Federal University of Uberlândia (UFU), Institute of Biomedical Sciences (ICBM), Laboratory of Antimicrobial Testing (LEA), Uberlândia, MG, Brazil
[4]Campus Botucatu, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, SP, Brazil

CORRESPONDING AUTHOR: andresa.berretta@apisflora.com.br

The oral cavity hosts a complex microbiota that plays a key role in oral health. Disruption of microbial balance may lead to dysbiosis and oral infections. Therefore, evidence-based oral care strategies that selectively inhibit pathogenic microorganisms while preserving beneficial bacteria are highly desirable. Bee products, particularly Brazilian green and red propolis, have been widely investigated due to their biological activities and health benefits. This study aimed to functionally validate a buccal spray formulated with Brazilian green and red propolis, combined with pomegranate, jambu, and licorice extracts (Propomax® Clinical-Irritation Relief), by evaluating its antimicrobial, antioxidant, and anti-inflammatory activities. The biological effects of individual ingredients and their binary combinations were also assessed to better understand their contribution

to the overall formulation. A total of 22 samples were analysed, including the complete buccal spray, six isolated ingredients, and 15 combinations of two components, all prepared in the same proportions used in the final product. Antioxidant activity was evaluated using DPPH and FRAP assays, anti-inflammatory effects were assessed by measuring TNF-α secretion in LPS-stimulated macrophages, and antimicrobial activity was determined using agar diffusion and minimal inhibitory concentration (MIC) assays. The buccal spray exhibited consistent antioxidant, antimicrobial, and anti-inflammatory effects, with antioxidant capacity comparable to pomegranate extract. Green propolis showed the strongest antimicrobial activity among individual ingredients, while combinations containing red propolis and pomegranate exhibited strain-dependent effects. Notably, the buccal spray demonstrated superior antimicrobial efficacy in MIC assays compared to the combination of green and red propolis alone. Advantageously, the formulation showed broad-spectrum antimicrobial activity against pathogenic microorganisms while not inhibiting *Lactobacillus paracasei*, indicating a selective microbiome-modulating effect. Additionally, the buccal spray significantly reduced TNF-α production in LPS-stimulated macrophages. These findings support the functional properties of a buccal spray formulated with Brazilian green and red propolis as a complementary approach for promoting oral health while preserving microbial balance.

Analysis of bioactive compounds and evaluation of the cyto- and genoprotective effects of poplar-type propolis and its major flavonoid component galangin

Mateo Jakac[1,2], Dražen Lušić[2,3,4], Irena Brčić Karačonji[3,5], Nediljko Landeka[1], Andreja Jurič[5], Nevenka Kopjar[5], Darija Vukić Lušić[2,4]

[1]Teaching Institute of Public Health of Istria County, Pula, Croatia
[2]University of Rijeka, Faculty of Medicine, Croatia
[3]University of Rijeka, Faculty of Health Studies, Croatia
[4]Teaching Institute of Public Health of Primorje-Gorski Kotar County, Rijeka, Croatia
[5]Institute for Medical Research and Occupational Health, Zagreb, Croatia

CORRESPONDING AUTHOR: *mateo.jakac@gmail.com*

The growing global interest in complementary and alternative medicine over the past decade has drawn significant attention to propolis on the Croatian market as well. It is increasingly used as a dietary supplement to support immune function in humans and, more recently, in animals. Propolis is characterised by an exceptionally complex phenolic composition, which may result in both pro-oxidant and antioxidant effects depending on its concentration and chemical profile. The aim of this study was to determine the phenolic content and antioxidant capacity of poplar-type propolis (N = 104), collected from Istria and Primorje-Gorski Kotar County. We also investigated the cyto- and genoprotective effects of its ethanolic extract on healthy human peripheral blood lymphocytes, following exposure to cytotoxic drug irinotecan *in vitro*. All effects were studied in parallel in lymphocytes treated with galangin, a major flavonoid component of poplar-type propolis. Lymphocytes were exposed for 3 hours to three concentrations of propolis extract corresponding to its average daily dose, as well as to five- and ten-fold higher concentrations. Galangin was used as a reference compound under identical experimental conditions. Cyto- and genoprotective effects were assessed using the cytokinesis-block micronucleus cytome (CBMN Cyt) assay. Content of

bioactive compounds in propolis extracts ranged 0.8–131.1 mg GAE/g raw propolis for total phenolics, 0.4–40.4 mg GalE/g for flavones and flavonols, and 1.2–29.2 mg NE/g for flavanones and dihydroflavonones. Antioxidant activity (DPPH assay) ranged 0.4–185.6 TE/g. At the selected concentrations, propolis and galangin were highly biocompatible with lymphocytes and effectively reduced cytogenetic damage induced by irinotecan, with propolis providing greater cyto-/genoprotection than galangin alone at equivalent concentrations. Overall, the findings indicate that poplar-type propolis, due to its distinctive chemical composition, may have potential applications as a valuable natural health product. The results also encourage further investigation into the biological effects of propolis under alternative exposure scenarios. Nevertheless, further studies are necessary before firm conclusions can be established.

Acknowledgements: this research was funded by the European Union—Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. No. 533-03-23-0006), European Union – NextGeneration EU – CCBE-Propolis – uniri-iz-25-164, the Croatian Academy of Sciences and Arts, the University of Rijeka (grant uniri-iskusni-biomed-23-247 and Prometheus Fund), Hrvatska elektroprivreda d.d., and supported by the European Regional Development Fund (project RecIMI, KK.01.1.1.02.0007).

Development of a natural wound and burn healing cream: a novel formulation with propolis and honey

Aslı Elif Tanuğur Samanci[1], Taylan Samanci[2], Elif Yorulmaz Önder[3], Esra Birinci[3]

[1]SBS Bilimsel Bio Çözümler San. ve Tic. A.Ş., İstanbul, Türkiye
[2]Sustainable Agriculture and Ecology Association, Ankara, Türkiye
[3]BEE&YOU R&D Center, İstanbul, Türkiye

CORRESPONDING AUTHOR: asli@sbs-turkey.com

This study aimed to develop a novel wound and burn healing cream formulated with propolis and honey. The study sought to create an effective, natural, and affordable alternative to conventional synthetic treatments, leveraging the unique bioactive properties of bee products. The research included *in vitro* and *in vivo* studies to evaluate the cream's efficacy in wound healing. The formulation process involved optimising ingredient concentrations to maximise antibacterial, anti-inflammatory, antioxidant, and regenerative effects. *In vitro* tests assessed cellular proliferation, migration, and collagen synthesis. The cream formulation was expected to accelerate wound closure, reduce inflammation, and prevent scar formation. The combination of propolis and honey provided antimicrobial and tissue-regenerative properties. Preliminary studies suggested that the product might outperform existing wound care treatments by reducing healing time and minimising side effects. This study presented an innovative approach to wound and burn care, introducing a cost-effective, all-natural topical formulation. The cream had the potential to improve patient outcomes while reducing healthcare costs associated with prolonged wound healing. The successful development of this product contributed to the advancement of apitherapy and phytotherapy in dermatological applications.

Improvement of haematological and biochemical parameters and white blood cell resistance to irinotecan treatment after one month of the common ivy (*Hedera helix* L.) honey consumption

Nevenka Kopjar[1], Mateo Jakac[2,4], Andreja Jurič[1], Irena Brčić Karačonji[1,3], Dražen Lušić[3,4,5]

[1]Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
[2]Teaching Institute of Public Health of Istria County, Department of Epidemiology, Pula, Croatia
[3]University of Rijeka, Faculty of Health Studies, Department of Basic Medical Sciences, Rijeka, Croatia
[4]University of Rijeka, Faculty of Medicine, Department of Health Ecology, Rijeka, Croatia
[5]Teaching Institute of Public Health of Primorje-Gorski Kotar County, Rijeka, Croatia

CORRESPONDING AUTHOR: nkopjar@imi.hr

Common ivy honey is a rare, seasonal product derived from the autumn bloom of *Hedera helix L., whose* distinctive chemical composition leads to its rapid crystallisation. As it has been relatively rare honey and, so far not studied much, scientific evidence regarding its cytoprotective properties is lacking. Here, we present results from a pilot interventional case study in which a healthy male volunteer consumed 36 g of common ivy honey daily for 30 days. The honey was chemically and palynologically characterised prior to the intervention. The study compared haematological and biochemical parameters in peripheral blood, as well as DNA damage and cytogenetic markers, before and after the consumption period. Genome stability in white blood cells was assessed using the alkaline comet assay and the cytokinesis-block micronucleus assay following 24-h *in vitro* exposure to irinotecan, a well-established cytotoxic agent, tested at a concentration that matched the dose of 350 mg/m^2 of

drug administered as monotherapy to colorectal cancer patients. The results indicate that regular intake of the common ivy honey positively affected several haematological and biochemical indices and enhanced white blood cell resistance to irinotecan-induced genotoxicity. While acknowledging its limitations, this controlled case study underscores the potential of ivy honey and contributes early evidence supporting its future use in development of natural products designed to improve human well-being.

Acknowledgements: the study was funded by institutional funding provided by the Ministry of Science, Education and the Youth of the Republic of Croatia (no. 106-F25-00045), European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and European Union – NextGeneration EU – CCBE-Propolis – uniri-iz-25-164.

Contract beekeeping: a sustainable approach to quality and traceability

Taylan Samanci[1], Aslı Elif Tanuğur Samanci[2], Elif Yorulmaz Önder[3]

[1]Sustainable Agriculture and Ecology Association, Ankara, Türkiye
[2]SBS Bilimsel Bio Çözümler San. ve Tic. A.Ş., İstanbul, Türkiye
[3]BEE&YOU R&D Center, İstanbul, Türkiye

CORRESPONDING AUTHOR: taylan.samanci@sbs-turkey.com

Contract beekeeping is an innovative and sustainable model designed to enhance the traceability, quality, and economic stability of beekeeping operations. This approach is designed to ensure that bee products are produced under controlled conditions, allowing for a transparent supply chain from hive to consumer. Through this model, beekeepers receive purchase agreements under pre-agreed pricing structures, enabling them to focus on production and quality rather than market fluctuations. One of the key benefits of contract beekeeping is its contribution to sustainable apiculture and environmental protection. Beekeepers are provided with necessary training and equipment, ensuring that they adhere to best practices in hive management, disease prevention, and production that complies with strict residue and food safety standards. Additionally, detailed specifications define acceptable hive inputs (feeding, treatments, and materials), ensuring the natural integrity of the products. By implementing this system, high-quality bee products such as propolis, royal jelly, pollen, bee bread, and honey are consistently produced, benefiting both beekeepers and consumers. Türkiye, with its rich biodiversity and favourable climatic conditions, stands as a global leader in beekeeping. The country ranks among the top honey producers worldwide, with over 9.2 million hives and a significant portion of the population engaged in beekeeping. However, challenges such as environmental degradation, pesticide exposure, and unsustainable practices threaten the industry. The contract beekeeping model mitigates these risks by promoting

eco-friendly and ethical beekeeping methods while securing a fair-trade environment for beekeepers. This model also plays a crucial role in increasing the production of value-added bee products, which hold significant economic and medicinal importance. Scientific research highlights the superior antioxidant, antibacterial, and antifungal properties of propolis and other bee products when harvested and processed correctly. Ensuring robust traceability and quality control through contract beekeeping not only benefits local economies but also strengthens global market competitiveness. In conclusion, contract beekeeping is a sustainable, fair, and efficient model that enhances the quality, safety, and economic viability of beekeeping. By fostering strong partnerships between beekeepers and buyers, this system supports biodiversity, promotes ethical production, and provides consumers with premium, traceable bee products.

Honey quality standards, use of antibiotics and development of AMR in *Paenibacillus larvae* strains– one health risk communication

Violeta Santrac[1], Giovani Formato[2], Nik Lupše[3], Ivana Tlak Gajger[4], Xesús Feás[5], Aslı Özkırım[6]

[1]Public Institution Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Banja Luka, Republic of Srpska, Bosnia and Herzegovina
[2]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Italy
[3]Slovenian Beekeepers' Association, Slovenia
[4] University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia
[5]Academy of Veterinary Sciences of Galicia, Spain
[6]Faculty of Science, Bee and Bee Products Research Center, Turkiye

CORRESPONDING AUTHOR: violeta.santrac@virs-vb.com

This overview aims to point out misuse and/or overuse of antibiotics in beekeeping practices that pose significant risks to bee health, food safety, environmental integrity, and human health. Despite being prohibited in several regions, including the European Union, the metaphylactic application of antibiotics to treat infected colonies remains common worldwide. This practice exposes bees, hive products, and consumers to multiple risks: (1) antibiotic residues in honey and related products; (2) recurrence or persistence of infections in subsequent years; and (3) the emergence and spread of antimicrobial-resistant (AMR) strains of *Paenibacillus larvae* and other honey bee pathogens. Creating antimicrobial stewardship may have relevance within apiculture preventing honey from becoming a reservoir of resistance genes, particularly those *P. larvae*. Although international frameworks such as the Codex Alimentarius "Guidelines on Integrated Monitoring and Surveillance of Foodborne AMR" exist, global harmonisation is still lacking. Current microbiological assessments of honey do not adequately address *P. larvae* status, and approaches relying on mesophilic bacterial counts fail to reliably detect this pathogen. Moreover, translational studies exploring the fate of *P. larvae* spores in

the human gastrointestinal tract are absent, leaving unanswered questions about potential pathogenicity in vulnerable individuals may contribute as reason to doing more research in this area. These gaps highlight the urgent need for targeted surveillance and strengthened AMR stewardship in apiculture. Aligning antimicrobial stewardship principles with honey bee health is a necessary step. Coordinated international research is needed to establish the global antimicrobial resistance status of *P. larvae* and to improve additional standards that support quality and safety in honey market, contributing added value to consumers and the apicultural sector. Beekeeping aligns with the One Health framework and that can be recognised through established honey quality standards.

Calorimetric evaluation of patties and energy content in adult honeybees *(Apis mellifera)* linked to diet-dependent changes in gut microbiota

Ana-Marija Kovač[1], Maja Ivana Smodiš Škerl[2], Bojan Papić[3], Darja Kušar[3], Damir Pavličeк[4], Ivana Tlak Gajger[1]

[1] University of Zagreb, Faculty of Veterinary Medicine, Croatia
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia
[3] University of Ljubljana, Veterinary Faculty, Slovenia
[4]Croatian Veterinary Institute, Veterinary Institute Križevci, Croatia

CORRESPONDING AUTHOR: itlak@vef.unizg.hr

Supplementary feeding of honeybees (*Apis mellifera*) during dearth supports colony development. However, data on the effects of different feeding regimes on honeybee energy content and gut microbiota are limited. This study investigated diet-dependent shifts in energy reserves and gut microbiota during autumn supplementary feeding. In August 2024, 36 colonies were assigned to four feeding groups (nine colonies each): sugar syrup (I), commercial protein patty (II), pollen patty (III) and 2% spirulina-enriched sugar patty (IV). Colonies were sampled at the start (day 0) and end (day 43) of the trial. Dried bees and experimental patties were analysed by bomb calorimetry for gross energy (GE), while patties also underwent chemical analyses to estimate crude protein (CP) content. Pooled honeybee gut samples (n = 160) were analysed using full-length PacBio 16S rRNA sequencing and processed with QIIME2 for microbiota profiling. The energy density of patties was similar in groups II (15.63 ± 0.14 MJ/kg), III (15.64 ± 0.02 MJ/kg) and IV (15.72 ± 0.14 MJ/kg), while group I was highest at 16.45 ± 0.02 MJ/kg. CP content was 1.07% in the protein patty, 2.12% in the pollen patty and 3.42% the spirulina-enriched patty. The average GE of honeybees increased from 10.25 ± 0.29 to 10.75 ± 0.25 MJ/kg, with group I showing the highest value (10.83 ± 0.19 MJ/kg). Firmicutes and Proteobacteria accounted for 93% of gut microbiota,

with alpha diversity decreasing over time and beta diversity exhibiting feeding-specific clustering influenced by *Lactobacillus helsingborgensis* and *Snodgrassella alvi*. These results indicate that supplementary feeding affects both honeybee energy reserves and gut microbiota composition. Sugar syrup enhances short-term energy availability, while spirulina and pollen supplementation modulate gut microbiota, potentially supporting colony development and immunity.

Sporopollenin microcapsules from bee pollen for targeted cancer therapy and sustained antioxidant release: a sustainable natural platform

Volkan Aylanc[1,2,3], Seymanur Ertosun[1,2], Lalehan Akyuz[4], Ceren Mutlu[1], Soraia I. Falcao[1], Nuno Vale[3], Cristina Freire[2], Miguel Vilas-Boas[1]

[1]Campus de Santa Apolónia, CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal
[2]Universidade do Porto, Faculdade de Ciências, Departamento de Química e Bioquímica, LAQV-REQUIMTE, Porto, Portugal
[3]Center for Health Technology and Services Research (CINTESIS), PerMed Research Group, Porto, Portugal
[4]Aksaray University, Department of Molecular Biology and Genetics, Aksaray, Turkey

CORRESPONDING AUTHOR: mvboas@ipb.pt

Micro- and nanostructures are successfully used in various fields due to their engineering capabilities and functionality. Such structures can be obtained from organic and inorganic materials, but their complex production processes require time. In contrast, sporopollenin microcapsules (SMCs) derived from pollen grains have recently attracted attention for their durability and natural microcapsule form. This study investigated the use of SMCs from bee pollen pellets for the drug delivery of chemotherapeutics and the food application of propolis. *Helianthemum* sp. (Hel) type SMCs were loaded via a vacuum-assisted protocol with tamoxifen (TAM), 5-fluorouracil (5-FU), bee venom (BV), or combinations. Loaded-SMCs were coated with Eudragit (Eud) for pH-triggered intestinal/colonic release. The *in vitro* release profile was characterised in sequential gastrointestinal and colonic fluids. Formulations were tested on breast and colorectal cancer cells, as well as healthy fibroblasts. Quantum chemical calculations were performed to investigate drug-sporopollenin interactions. *Olea* sp. (Ol) type SMCs were loaded with propolis extracts and tested for their release behaviour and anti-radical effect on food preservation.

Hel-SMCs achieved high loading, with Eud coating markedly reducing premature gastric release. Co-delivery formulations produced strong synergy: co-encapsulated BV enabled cancer cell kill comparable to full chemotherapeutic doses at roughly half the drug concentration. Empty Hel-SMCs showed excellent biocompatibility with healthy cells. Molecular docking showed stronger TAM-SMC binding than 5-FU, explaining TAM's slower release. On the other hand, the loading capacity of propolis extract onto OI-SMCs was calculated as 12%. OI-SMCs continuously released propolis extract for up to 3 days, reaching 84%. SMCs with Eud coating provide a promising, sustainable platform for intestine/colon-targeted. Co-delivery with BV potentiates chemotherapy, enabling dose reduction and potentially lowering systemic toxicity. In parallel, SMCs enabled the sustained release of antioxidant propolis extract for food applications.

Acknowledgments: the authors acknowledge (FCT, Portugal) for financial support to CIMO (UIDB/00690/2020 and UIDP/00690/2020). SusTEC (LA/P/0007/2021), Volkan Aylanc (2021.07764.BD) and Seymanur Ertosun (2021.08361.BD). Thanks also to Promove program, financed by Fundação La Caixa, BPI, and FCT, through the project BEE4CHEESE (PL24-00071).

Chemical and bioactive evaluation of propolis-based preservation strategies in artisanal cheeses

Aritson Tolentino, Seymanur Ertosun, Volkan Aylan, Paula Rodrigues, Miguel Vilas-Boas, Soraia Falcão

Campus de Santa Apolónia, Instituto Politécnico de Bragança, CIMO, LA SusTEC, Bragança, Portugal

CORRESPONDING AUTHOR: *sfalcão@ipb.pt*

Honey, bee pollen, and propolis are valuable bee products, but in Portugal, honey has a greater market impact, highlighting the need to diversify beekeeping production and add value to the other bee products. The BEE4CHEESE project addresses this gap by exploring propolis and bee pollen as natural preservatives for artisanal cheeses, thereby enhancing product value while supporting traditional cheese-making in inland regions of Portugal. Propolis is widely used in pharmaceutical and food applications due to its bioactive properties. This study evaluated the chemical potential of propolis from northern Portugal for cheese preservation. The sample was characterised for physicochemical properties, phenolic composition, and antioxidant activity. Hydroethanolic extracts were analysed by LC-DAD-ESI-MS^n, with total phenolic and flavonoid contents measured spectrophotometrically, and antioxidant activity assessed via DPPH assay. Four types of cheeses (goat, sheep, cow-sheep mixture, and cow-sheep mixture with walnuts) were microbiologically monitored during maturation to identify target microorganisms for propolis inhibition. Raw propolis showed low moisture, moderate ash, and 14% beeswax, while the hydroethanolic extraction achieved a high yield of 71%. The phenolic composition revealed a typical profile of temperate poplar-type propolis, including phenolic acids and ester derivatives and flavonoids and their methylated or esterified derivatives (pinocembrin, pinobanksin and pinobanksin-3-***O***-acetate). The extract exhibited high TPC (364 mg/g) and TFC (79 mg/g), as well as strong antioxidant activity (EC_{50} =

0.013 mg/mL). Microbiological analysis showed a significant increase in *Staphylococcus aureus* and mould counts during cheese maturation. *S. aureus* was frequently detected in mixed cow-sheep cheeses with walnuts, whereas moulds were more prevalent in sheep cheeses. Overall, these results support the potential use of propolis as a natural, value-added preservative in the food sector, while contributing to the diversification and valorisation of beekeeping products.

Acknowledgments: this work was supported by national funds through FCT/MCTES (PIDDAC): CIMO UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); This work received financial support from Promove program, financed by Fundação La Caixa, BPI, and FCT, through the project BEE4CHEESE (PL24-00071).

Characteristics and quality of Spanish artisanal meads

M. Teresa Sancho, Leire Cantero, Guillermo Puertas, Miguel A. Fernández-Muiño, Sandra M. Osés

Universidad de Burgos (University of Burgos), Faculty of Sciences, Department of Biotechnology and Food Science, Burgos (Castilla-León), Spain

CORRESPONDING AUTHOR: mtsancho@ubu.es

Mead is likely the most ancient fermented beverage, made with honey, water, and yeasts that is increasingly gaining in popularity among consumers. Making mead help beekeepers add value to bee products while improving knowledge, marketing and commercialisation of honey derivatives. The purpose of this research was to characterise and determine the quality of eight artisanal meads made in different regions of Spain with honeys from different botanical origins and with different honey to water ratios (dry meads and sweet meads). Applying official, harmonised, and/or widely published methods for wine and honey, the following parameters were determined: colour "lightness" (L*), "red/green coordinate" (a*) and "yellow/blue coordinate" (b*), browning index (absorbance at 420 nm), pH, acidity (volatile, fixed and total), sulfur dioxide (free, bound and total), total phenolics, total flavones-flavonols, total flavanols, trolox equivalent antioxidant capacity (TEAC), and oxygen radical absorbance capacity (ORAC). The averages and ranges (in brackets) for each parameter were: L* 71.4 (66.2–73.7), a* -2.1 (from -3.3 to -1.2), b* 13.7 (6.9–28.9), browning index (A420) 0.427 (0.129–1.741), pH 3.2 (2.8–3.7); volatile acidity (g acetic acid/L) 0.5 (0.14–1.05), fixed acidity (g tartaric acid/L) 3.43 (2.39–5.74), total acidity (g tartaric acid/L) 4.05 (2.79–6.49), free SO2 (mg/L) 0.6 (nondetectable–0.2), bound SO2 (mg/L) 18.6 (9.6–49.6), total SO2 (mg/L) 19.2 (9.6–49.6), total phenolics (mg gallic acid/L) 189.1 (97.0–528.7), total flavones-flavonols (mg quercetin/L) 0.92 (0.38–1.78), total flavanols (mg catechin/L) 3.29 (1.19–8.23), TEAC (mmol trolox/L) 0.20 (0.13–0.42) and ORAC (mmol trolox/L) 14.1 (6.32–22.52).

Results fulfilled the limits for wines [C.D. Regulation (EU) 2019/934]. Sweet meads exhibited higher values of pH, volatile acidity, and total SO2 than dry meads. It was the botanical origin of the honey with which the mead was made, together with the honey to water ratio of the starting must that were the key factors as regards composition and antioxidant properties of mead.

Characterisation of artisanal Spanish meads by their sugars, glycerol, inositol, and ethanol contents

Miguel A. Fernández Muiño, Guillermo Puertas, Leire Cantero, Sandra M. Osés, M. Teresa Sancho

Universidad de Burgos (University of Burgos), Faculty of Sciences, Department of Biotechnology and Food Science, Burgos (Castilla-León), Spain

CORRESPONDING AUTHOR: mafernan@ubu.es

In this study, eight samples of Spanish artisanal meads from different botanical origins were analysed in order to contribute to their characterisation. Six samples were dry meads, and two samples were sweet meads, obtained with higher honey to water ratios than those of dry meads. The parameters studied included sugars, glycerol and inositol, determined by gas chromatography-mass spectrometry (GC-MS) after the Pourtallier derivatisation procedure, as well as the alcohol content and meads' Brix degrees. In the GC-MS analysis, nine compounds were detected and quantified including glycerol (3.88–10.36 mg/mL), inositol (0.56–1.50 mg/mL), fructose (0.13–80.55 mg/mL), glucose (0.10–23.64 mg/mL), trehalose (not detected–0.76 mg/mL), turanose (1.91–13.76 mg/mL), maltose (1.76–5.60 mg/mL), and isomaltose (1.83–9.14 mg/mL). The alcohol content ranged from 8.7% to 14%, and the Meads' Brix degrees varied between 6.0 and 17.0. Sweet meads showed higher values of glucose, fructose, maltose, and lactose, as well as higher Brix degrees and alcohol content, and lower glycerol values than dry meads. Inositol was detected in mead for the first time, this compound not having been previously described in this beverage.

Hyperspectral imaging and AI-driven chemometrics: a new frontier for rapid, non-destructive honey authenticity assessment

Fatjon Hoxha[1], Sony George[2], Dolores Pérez-Marín[3]

[1]Agricultural University of Tirana, Faculty of Biotechnology and Food, Department of Agri-Food Technology, Tirana, Albania
[2]Norwegian University of Science and Technology (NTNU), Faculty of Information Technology and Electrical Engineering, Department of Computer Science, Gjøvik, Norway
[3]University of Cordoba (UCO), Faculty of Agriculture and Forestry Engineering (ETSIAM), Department of Animal Production, Cordoba, Spain

CORRESPONDING AUTHOR: fhoxha@ubt.edu.al

Honey authenticity remains one of the most challenging areas in food integrity control, due to the complexity of the matrix, the diversity of botanical origins, and the increasing sophistication of adulteration practices. Current official and instrumental methods-melissopalynology, physicochemical testing, IRMS, NMR, HRMS-provide essential reference data but are often slow, destructive, and require specialised expertise. Against this backdrop, Hyperspectral imaging (HSI) combined with advanced chemometrics, and artificial intelligence (AI) is emerging as a powerful supplementary approach capable of offering rapid, high-resolution insights into honey composition and origin. HSI unites imaging and spectroscopy, capturing both spatial and spectral information across hundreds of wavelengths. When applied to liquid matrices such as honey-a known challenge due to viscosity, crystallisation, and limited optical penetration-HSI offers new opportunities for detecting subtle chemical and physical variations linked to botanical origin, geographical signatures, and dilution with sugar syrups. Recent advances in VNIR and SWIR cameras (400–2500 nm), together with optimised reflectance and transmittance acquisition, provide unprecedented sensitivity to spectral fingerprints that distinguish genuine honey from adulterated samples. To fully exploit this rich spectral

information, AI-enabled chemometric workflows are applied, including PCA for dimensionality reduction, multivariate discriminant analysis, and *learning-based models* capable of classification and prediction. These tools allow the extraction of informative wavelengths, identification of key spectral features, and development of robust models that can screen honey authenticity rapidly and non-destructively. Furthermore, the integration of portable spectral devices extends these possibilities to field inspections, cooperatives, and producer associations. Overall, the convergence of HSI, chemometrics, and AI represents a transformative opportunity to enhance early detection, support official control strategies, and strengthen consumer trust in the honey value chain.

Honey as a source of entomological environmental DNA: metabarcoding insights into plant-sucking insects across agricultural and forest landscapes

Valeria Taurisano, Anisa Ribani, Samuele Bovo, Giuseppina Schiavo, Valerio Joe Utzeri, Francesca Bertolini, Luca Fontanesi

University of Bologna, Department of Agricultural and Food Sciences, Animal and Food Genomics Group, Bologna, Italy

CORRESPONDING AUTHOR: valeria.taurisano2@unibo.it

Aphids play a crucial role in honeydew honey production, as their sugary excretions provide an important carbohydrate-rich source for honey bees. Honeydew honey contains valuable entomological signatures that reflect the plant-sucking insect communities inhabiting agricultural and forest landscapes. Because many of these insects, exhibit host-plant specialisation, their presence can provide indirect information on the botanical and geographical origin of honey. In this study, DNA extracted from honey was used as a source of environmental DNA (eDNA) to characterise the entomological signature of honey, focusing on honeydew-producing Hemiptera. We designed two primer pairs targeting mitochondrial DNA regions of Hemiptera. More than 70 honey samples, including citrus blossom, polyfloral, and honeydew honeys, that were produced in Italy and Türkiye, were analysed with a next-generation sequencing (NGS) approach combined with a dedicated bioinformatic pipeline. This approach allowed the recovery of entomological signatures indirectly derived from honeydew-producing plant-sucking insects and provided valuable information for identifying the botanical and, indirectly, the geographical origin of honey. The obtained results contribute to a better understanding of honey bee foraging preferences and demonstrate the potential of honey-derived eDNA. The combination of honey bees environmental sampling capacity, honey eDNA-based analysis and the high throughput of NGS technologies represents a powerful tool for honey authentication and for characterising entomological communities inhabiting agricultural and forest landscapes.

Royal jelly vesicles as a new drug-delivery system for liquid metal nanoparticles

Mario Kurtjak[1], Ena Kramarić[2], Anna Placci[3,4,5], Valentina Marassi[3,4,5], Vanja Baričević[6], Lana Arsić[6], Dario Stipanić[6], Iva Lovrović[7], Anja Harej Hrkać[2], Mladenka Malenica[8]

[1]Jožef Stefan Institute, [1]Advanced Materials Department, Ljubljana, Slovenia
[2]University of Rijeka, Faculty of Medicine, Department of Basic and Clinical Pharmacology and Toxicology, Rijeka, Croatia
[3]Department of Chemistry 'G. Ciamician', Bologna, Italy
[4]byFlow s.r.l, Bologna, Italy
[5]Biostructures and Biosystems National Institute (INBB), Rome, Italy
[6] University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia
[7]University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia
[8]Clinical Hospital Centre Rijeka, Rijeka, Croatia

CORRESPONDING AUTHOR: *mladenka.malenica@uniri.hr*

Liquid metal nanoparticles exhibit remarkable antimicrobial, anticancer, and anti-inflammatory properties, but their instability and poor solubility under biological conditions hinder such applications. Although protective inorganic coatings or encapsulation into liposomes can mitigate this, the low biocompatibility of many synthetic carriers has increased interest in naturally derived alternatives. Vesicles from royal jelly (RJ) present an innovative and promising carrier system with the aim of increasing bioavailability, selectivity and targeted release. RJ vesicles morphologically resemble extracellular vesicles, membranous structures that transport lipids, proteins, and nucleic acids, cross physiological barriers, and internalise into cells. Despite their known antibacterial and regenerative properties, application as nanocarriers for the encapsulation of bioactive molecules remains unexplored. RJ-derived vesicles with a maximum Martin diameter ranging from 50 to 100 nm were isolated using multidetection asymmetric flow field-flow fractionation, a mild and environmentally

friendly technique that preserved their native characteristics. Gallium nanoparticles (GaNPs) were synthesised by thermal decomposition and coated with oleic acid. GaNPs with around 15 nm size were encapsulated into RJ vesicles *via* sonication. Transmission electron microscopy and microanalysis confirmed the presence of GaNPs within membranous vesicular structures. These preliminary results demonstrate a first step towards the investigation of bioavailability, biocompatibility and stability of GaNPs and other liquid metals-based nanoparticles, functionalised with different ligands, when encapsulated in RJ vesicles. This approach opens new perspectives for the development of biocompatible drug-delivery systems with potential applications in future *in vitro* and *in vivo* studies. Moreover, modification of RJ vesicle membrane proteins may enable the development of personalised therapeutic strategies for a range of diseases.

Acknowledgments: this work has been supported by the University of Rijeka, uniri-mzi-25-34, Nano-delivery particles from bee products for better bioavailability of bioactive compounds with antitumor activity, BEE-nano.

Assessment of extraction strategies for bioactive compounds recovery from propolis of the Montesinho Natural Park (Portugal)

Sandra Barbosa[1,2,3,4], Maria Fernanda Sousa[1,5,6], Nicolas Justus[1,5,6], Ana R. Circuncisão[1,3], Maria Carolina Ribeiro[6], Olívia Pereira[5,7], Cristina Almeida-Aguiar[4], Susana M. Cardoso[3]

[1]Montesino – Organic Beekeeping, Bragança, Portugal
[2]University of Trás-os-Montes and Alto Douro, CITAB, Vila Real, Portugal
[3]University of Aveiro, Department of Chemistry, LAQV-REQUIMTE, Aveiro, Portugal
[4]University of Minho, Department of Biology, CBMA, Braga, Portugal
[5]Instituto Politécnico de Bragança, Bragança, Portugal
[6]Federal University of Technology – Paraná, UTFPR, Ponta Grossa, Brazil
[7]Polytechnic Institute of Bragança, Research Centre for Active Living and Wellbeing (LiveWell), Bragança, Portugal

CORRESPONDING AUTHOR: susanamcardoso@ua.pt

Propolis, a bee product rich in bioactive compounds, exhibits strong antioxidant, antimicrobial, and anti-inflammatory properties, making it increasingly attractive as an ingredient for food and cosmetic products. To ensure phytochemical stability and industrial viability, optimised extraction methods are essential. This work evaluated propolis from the Montesinho Natural Park (Portugal) by comparing the extraction efficiency of maceration (conventional method) and ultrasound-assisted extraction (UAE) (alternative method) in the recovery of bioactive compounds, in particular phenolics. Extractions were conducted at a sample-to-solvent ratio of 3:10 (*w/w*) at room temperature, using ethanol/water mixtures (70% and 80% *v/v*) and monopropylene glycol (MPG). UAE kinetics were monitored every 5 min for up to 30 min with amplitudes between 60–100%, while maceration kinetics were evaluated every 2 days over 30 days to determine the point of maximum total phenolic content (TPC). Distinct kinetic behaviours were observed: maceration reached the highest TPC

after 5 days, with similar values across solvents-MPG [65.9 mg gallic acid equivalents (GAE)/mL], followed by 70% EtOH (64.1 mg GAE/mL) and 80% EtOH (62.2 mg GAE/mL). UAE allowed faster extraction but achieved a TPC approximately 50% lower than maceration, with the highest result for MPG (33.8 mg GAE/mL) after 5 minutes of sonication at 100% amplitude. Based on these results, 70% EtOH and MPG extracts obtained via maceration were selected for further characterisation, showing total flavonoid content of 18.4 and 23.5 mg quercetin equivalents/mL, and antioxidant activity by DPPH [78.1 and 88.4 mg ascorbic acid equivalents (AAE)/mL] and ABTS (75.4 and 86.1 mg AAE/mL), respectively. UHPLC-MS analysis revealed similar phenolic profiles for both solvents, dominated by flavonoids (e.g. chrysin, pinobanksin derivatives, galangin) and flavanones (e.g. pinocembrin, naringin), followed by phenolic acids (e.g. caffeic acid, *p*-coumaric acid, ferulic acid) and their esterified derivatives. Overall, these results highlight maceration as a simple and efficient method to obtain propolis extracts from Montesinho Natural Park, with potential to be used as sustainable ingredients for the food and cosmetic industries.

Acknowledgments: this work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 and UIDP/50006/2020. The authors acknowledge the financial support from PRR, Recovery and Resilience Plan - Adult Impulse Program of The Mountain Consortium for Knowledge, under the Innovation Scholarship awarded to Maria Fernanda Sousa and Nicolas Justus in collaboration with the entity Montesino and BioCosMap project (COMPETE2030 – FEDER - 02162400), co-financed by FEDER through COMPETE2030 under the PT2030 program. The work also received funds from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the projects: UID/50006 - LAQV and UID/6157/2023 - LiveWell). S.M. Cardoso thanks FCT/MCTES for the funding provided through the Institutional Call to Scientific Employment Stimulus.

Application of front-face fluorescence spectroscopy combined with chemometrics for the characterisation and adulteration detection of honey

Dany El-Obeid[1], Ghenwa Abou Chrouch[1], Dany Yammouni[2], Jad Rizkallah[1]

[1]Lebanese Dekwaneh Campus, Lebanese University, Faculty of Agriculture and Veterinary Sciences, Mount Lebanon Governorate, Lebanon
[2]Fanar Main Campus, Agriculture Research Institute (LARI), Mount Lebanon Governorate, Lebanon

CORRESPONDING AUTHOR: dany.elobeid@ul.edu.lb

In Lebanon, honey is not only considered a valuable food product but is also widely used for its healing properties and in skin care. However, Lebanese honey consumers face growing distrust due to the misconception that most Lebanese honey brands are adulterated. This study aimed to evaluate the use of front-face fluorescence spectroscopy (FFFS), a non-destructive, fast, and inexpensive technique, combined with chemometric analysis for the authentication of Lebanese honey, with a focus on botanical origin characterisation and adulteration detection. A total of 344 samples were analysed, including 212 honey samples from different botanical origins (oak, thyme, citrus, cedar, black locust, spring flowers, mixed flowers, and others), 32 common adulterants (fruit juices, high-fructose syrups, and glucose/fructose syrups), and 100 intentionally adulterated honey samples prepared using different adulterant types and concentrations. The proposed technique interpreted fluorescence data and successfully differentiated pure honey samples from adulterated honey samples and enabled reliable classification of honeys according to their botanical origin. In addition, intentionally adulterated honeys were clearly distinguished based on both the type and concentration of adulterant. The combination of FFFS and chemometrics also allowed

the prediction of key chemical parameters of honey, including fructose, glucose, electrical conductivity, and moisture content. These results highlight the potential of FFFS coupled with chemometrics as an effective tool for honey authentication and quality control.

PP-01.

Using pesticide residues to determine the geographical origin of honey: requirements, limitations, and integrative approaches

Michela Bertola, Arrigo Moro, Marianna Martinello, Chiara Manzinello, Nicoletta Dainese, Elena Albertin, Carmela Zacometti, Alessandra Tata, Franco Mutinelli

Istituto Zooprofilattico Sperimentale delle Venezie, NRL for honey bee health, Legnaro, Italy)

CORRESPONDING AUTHOR: michela11392@gmail.com

Determining the geographical origin of honey has become increasingly important in food quality control and fraud prevention, as honey prices are strongly influenced by both botanical and geographical provenance. While melissopalynological analysis remains the gold standard for botanical authentication, it is labor-intensive, operator-dependent, and susceptible to manipulation. These limitations have initiated the development of complementary analytical approaches, including physicochemical profiling, mineral analysis, volatile organic compounds (VOCs) characterisation, and contaminant-based methods. Honey is also a valuable bioindicator of environmental exposure, reflecting pesticides applied both within the hive and in surrounding agricultural landscapes. In this study, we investigated whether pesticide residues could support the determination of honey's geographical origin. A validated LC-MS/MS method, based on FMOC-Cl derivatization and HLB-SPE clean-up, was applied to 126 Italian honey samples. Glyphosate was detected in 21.4% of samples (27/126), with concentrations ranging from 10.7 to 518.7 µg/kg. Pesticide-positive samples differed significantly across macro-areas [$\chi^2(2) = 11.06$, $p = 0.004$], with the highest frequencies in

Northern Italy, particularly in regions characterised by intensive agriculture such as Veneto (44%) and Emilia-Romagna (67%). These findings are consistent with land-use patterns. However, the absence of a statistically robust association between macro-area and contamination intensity highlights that pesticide data alone are insufficient to determine provenance. A reliable contaminant-based attribution model requires: (i) a comprehensive multi-residue panel capturing crop-specific active substances; (ii) knowledge of crop-dependent plant protection protocols; and (iii) GIS-based land-use information to contextualize exposure pathways. Integrating these components, supported by multivariate or machine-learning approaches, can enhance the discriminatory power of pesticide profiles. Overall, these results reinforce the value of honey as an environmental biomonitor and indicate that pesticide-based authentication of geographical origin is feasible only when embedded within a spatially explicit, multi-component analytical framework.

PP-02.

Spatial offset Raman spectroscopy for the botanical authentication of heather honey from across the UK

Mable Chungu, Sophie Dodd, Tatiana Stead, Zoltan Kevei, Maria Anastasiadi

Cranfield University, Faculty of Engineering and Applied Sciences, Centre for Soil, Agrifood and Biosciences, Cranfield, UK

CORRESPONDING AUTHOR: tatiana.stead@cranfield.ac.uk

Heather honey, collected from *Calluna vulgaris*, is a highly desirable monofloral honey. It displays distinct physicochemical properties, such as a dark colour and a unique thixotropic consistency. Furthermore, it has a high nutritional value and several potential health benefits, including a strong antimicrobial activity. These characteristics make heather honey a premium product, highlighting the need for a reliable authentication method. In this study, we assessed the suitability of the non-destructive spatial offset Raman spectroscopy (SORS) combined with machine learning for the botanical authentication of UK heather honey. A total of 215 heather and 32 non-heather samples from across the UK and Ireland were collected. The honeys were characterised by measuring physicochemical properties according to the harmonised methods. Heather honeys showed significantly higher moisture, pH, colour and fructose/glucose ratio, and significantly lower °brix, glucose, maltose, sucrose, and glucose/moisture ratio compared to the non-heather honeys. To investigate heather botanical authentication, SORS data was generated on the heather and non-heather samples, and used to train, optimise, and validate three machine learning classification algorithms: PLS-DA, random forest, and XGBoost. Random forest and XGBoost showed the best performance, achieving accuracies of 91.34% and 91.1% and sensitivities of 91.78% and 91.41% respectively. The suitability of SORS for geographical classification was also investigated. However, there was

insufficient variability between honey profiles to discriminate between UK regions. These results demonstrate that SORS data combined with machine learning algorithms can accurately and precisely be used for heather honey botanical authentication, highlighting the potential for real-world application in quality assurance, fraud prevention, and premium product verification across the supply chain.

PP-03.

Insights into pollen representation in honey through the analysis of bees at the hive entrance

Rafaela Costa[1], Irailde Lima[1], Miguel Vilas-Boas[2], Paulo Russo-Almeida[3]

[1]Universidade de Trás-os-Montes e Alto Douro, Departamento de Zootecnia, LabApis, Vila Real, Portugal
[2]Campus de Santa Apolónia, Instituto Politécnico de Bragança, LA SusTEC, CIMO, Bragança, Portugal
[3]Universidade Trás-os-Montes e Alto Douro, UTAD, Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Quinta de Prados, Vila Real, Portugal

CORRESPONDING AUTHOR: prusso@utad.pt

Nectar production for a given plant species depends of environmental conditions and time of the day. The pollen content in the nectar is related to the position of the flowers, the development and structure of their stamens, the position nectaries position on the flower, the nectar/pollen production potential, among others. In addition, the distance travelled by the bees back to the colony, the size of the pollen and the nectar flow, influences the pollen concentration in honey, due to the partial removal of pollen in their proventriculus. Therefore, it is not surprising that the interpretation of the melissopalynological data raises several challenges. Considering the complexity of the aforementioned factors and that bees demonstrate a high degree of fidelity to the plant species they visit in a single flight, we pretended to establish, at least comparatively, the pollen/nectar contribution ratio of the flora in Trás-os-Montes region (Portugal). In this study, 10 µL crop nectar of 1500 honeybees collected at the hive entrance (50 bees x 10 hives x 3 apiaries), were analysed on microscope. Some bees had empty crop. Others did not reveal the presence of pollen, which was linked with the collection of water instead of nectar. Nevertheless, more than 60% allowed us to evaluate the pollen content in the

nectar. One would expect that the nectar collected from each bee would contain only one type of pollen, but this rarely happened, although one species predominated in most bees. The bees carrying *Cistus* pollen on their legs did not contain nectar on crop, consistent with the fact that this species is nectarless. The concentration of pollen in the collected nectar was highly variable among the botanical origin. For example, more than 2,000 pollen grains of *Echium* spp. were counted per 10 µL of nectar, while for *Erica* spp. the count was around 200 grains per 10 µ, which illustrates the usefulness of this kind of information in the interpretation of melissopalynological data.

Acknowledgements: the authors are grateful to the program *Promove* from Fundação "*La Caixa*", in collaboration with BPI and the Foundation for Science and Technology (FCT, Portugal) within the scope of project BeeSustain, PD23-00019. Thanks also for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020, UIDP/00690/2020), SusTEC (LA/P/0007/2021) and UID/04033/2023: Centre for the Research and Technology of Agro-Environmental and Biological Sciences and LA/P/0126/2020 (https://doi.org/10.54499/LA/P/0126/2020).

PP-04.

European Honey Contest 2024—Koper, Slovenia: establishing the Biennial framework for 2026 and beyond

Aljaž Debelak[1], Jasna Bertoncelj[2], Mojca Korošec[2]

[1]Slovenian Beekeeper's Association, Lukovica, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

CORRESPONDING AUTHOR: *aljaz.debelak@czs.si*

Consumers face an increasingly wide choice of honey, accompanied by growing concerns about authenticity. A 2023 European Commission study reported that 46% of imported honey is suspected of adulteration, undermining consumer trust and creating unfair competition for beekeepers producing high-quality honey. To ensure fair market value for premium products, reliable mechanisms for quality assurance and authentication are essential. International honey competitions, such as the European Honey Contest, address this challenge through objective and professional sensory evaluation combined with laboratory analyses to verify quality, safety, and authenticity. This approach provides beekeepers with a credible marketing tool while offering consumers a reliable guarantee of authentic honey. Honey samples were evaluated by a 10-member international panel of honey experts following the Rules of the Board of the Slovenian Beekeepers' Association for the sensory evaluation of honey. Appearance, odour, taste, and aroma in relation to declared botanical origin were scored (maximum 30 points). Honey type categories were defined only when at least seven samples of the same type were submitted; otherwise, honeys were classified as forest (honeydew) or multifloral based on electrical conductivity. All samples that received certificates of participation met strict quality requirements (water content ≤ 18.6% and hydroxymethylfurfural (HMF) ≤ 15 mg/kg),

highlighting the importance of these criteria for all participants and providing a solid foundation for future editions. In total, 114 honey samples from 11 European countries were assessed across five categories: black locust, multifloral, forest, linden, and chestnut honey. To highlight the best honeys, gold, silver, and bronze medals were awarded only to the top three samples per category. The 15 award-winning honeys were additionally tested for authenticity and acaricide residues. Only after the high quality and safety standards were proven, the best honey samples were announced. The established methodology, constructive feedback and good attendance were a solid foundation for the next European Honey Contest in 2026.

PP-05.

Assessment of the original qualities of Lebanese honey according to geographical origin

Amelia V. González-Porto[1], Chadi Hosri[2], Rita Yaacoub[2], Dalida Darazy[2], Eduard Tabet[2], Raquel Martin-Hernández[1]

[1]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRIAF)- CIAPA, Marchamalo, Guadalajara, Spain
[2]Lebanese University, Faculty of Agriculture (LU), George Matta, Lebanon

Corresponding authors: avgonzalezp@jccm.es; chadi.hosri@ul.edu.lb

Honey is a natural product derived from nectar and plant sap, transformed by bee enzymes. The growing demand for natural products necessitates rigorous assurance of their quality, authenticity, and protection against fraud. Characterisation based on geographical origin, botanical composition, and physicochemical properties is essential for authentication, traceability, and the establishment of official controls to ensure purity, quality, and food safety. The physicochemical properties of honey are largely determined by its composition and origin, making the identification of its compositional profile crucial for assessing specific attributes and detecting potential adulteration or improper handling. This study is framed within the definition of honey produced in Mediterranean Basin countries and explores the development of a distinctive quality brand through the MEDIBEES project, funded by PRIMA. The present study aims to analyse the physicochemical characteristics (colour, electrical conductivity), pollen spectrum, and sensory properties of honeys produced along north-south transects of Lebanon, across different altitudes and seasons. The objective is to provide a scientific basis for the development of a differentiated quality label that supports official control measures and safeguards against possible fraud. The samples included a diverse representation of herbaceous and shrubby plants typical of spring and

summer in the Mediterranean Basin (*Cirsium*, *Scabiosa*, *Echinops*, *Lavandula*, *Origanum*, *Salvia officinalis*, *Thymus*), as well as fruit trees such as *Citrus* and *Persea*, and honeydew derived from *Cedrus* and *Quercus* species. This preliminary study provides a foundation for achieving the stated objectives and enhancing the added value of honey production, thereby supporting its competitiveness in the market.

Acknowledgements: this research was supported by the PRIMA Programme (MEDIBEES-2011-PRIMA S1 2020 FARMING SYSTEMS (RIA), sub-topic A) under the auspices of the European Union, and the authors sincerely acknowledge the invaluable contributions of the beekeepers from all partner institutions

PP-06.

Honey quality assessment: 2020 vs 2025

Nedjeljko Karabasil[1], Dejan Bosančić[2], Ivan Vićić[1], Milena Gutić[2], Silvana Stajković[1]

[1]Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Belgrade, Serbia
[2]Centre for Food Testing, Belgrade, Serbia

CORRESPONDING AUTHOR: *nedja@vet.bg.ac.rs*

This study evaluated the quality of honey samples in an accredited laboratory, which were analysed no later than 24 h after sampling, employing standard IHC methods. The analysis focused on selected parameters: sucrose content, reducing sugars (glucose and fructose), hydroxymethylfurfural (HMF), and diastase activity. All samples in 2025 and in 2020 originated from different districts of Serbia, except for two imported samples in 2020 (orange and manuka). The sample set in 2025 included 45 samples: black locust (15), multifloral (18), linden (7), two sunflower honeys, and one each of orange, honeydew, and manuka honeys. Results in 2025 showed sample conformance for diastase value and sucrose, and reducing sugar levels. A diastase value ranging from 8.1 to 36.5 DN, a sucrose level between lower than 0.1 and 4.23 g/100 g and reducing sugars from 64.9 to 79.4 g/100 g. HMF values were recorded between 7.5 and 50.8 mg/kg. Four samples (8.8%), specifically two multifloral and two sunflower honeys, did not comply with regulatory limits for HMF (> 40 mg/kg). The honey quality testing conducted in 2020 included a larger number of samples (157): black locust (42), multifloral (71), linden (24), 15 sunflower honeys, and five honeydew samples. At that time, 8.3% of the analysed honeys had HMF values exceeding the permitted limit. Deviations were also observed in other parameters: sucrose content exceeded the allowed values in 1.9% of samples, reducing sugars were below the required threshold in 5.7% of samples, and diastase activity

was lower than permitted in 6.3% of the analysed honeys. Compared with the results in 2020, five years later, the situation has improved, as reflected in the simple numbers, indicating progress in quality control and production processes. Nevertheless, continuous education for all stakeholders, from producers to packaging facility staff, remains essential to ensure full compliance with regulations and to maintain honey quality.

Acknowledgements: the study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-136/2025-03/200143).

PP-07.

Analysis of volatile compounds in the process of honey adulteration using maple syrup, sugar, and bee pollen

Zvonimir Marijanović, Dolores Klobučar, Mladenka Šarolić

University of Split, Faculty of Chemistry and Technology, Department of Food Technology and Biotechnology, Split, Croatia

CORRESPONDING AUTHOR: *zmarijanovic@ktf-split.hr*

In this study, the chemical composition of volatile compounds in the process of adulteration of honey was analysed. Samples of bee pollen, honey, maple syrup, sucrose solution, a mixture of bee pollen and maple syrup, and a mixture of bee pollen and sucrose solution were taken for analysis. Multi floral honey and bee pollen, which were the basis for the adulteration investigation, were taken from the same beekeeper, for comparison with adulterants. Commercial maple syrup and sucrose solution, which was prepared in the laboratory, were used for adulteration. The isolation of volatiles compounds was carried out using a manual SPME fiber with the layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The obtained samples were analysed by gas chromatography-mass spectrometry (GC-MS). The purpose of this work was to prove whether honey can be adulterated with bee pollen and sucrose solution, as well as with bee pollen and maple syrup. By analysing adulterated honey made from a mixture of bee pollen and maple syrup, as well as a mixture of bee pollen and sucrose solution, a number of volatile compounds that may or may not be present in honey were detected. Volatile compounds, which are characteristic only for maple syrup and sucrose solution, can indicate their presence in honey, as well as the adulteration itself.

PP-08.

Determining the geographical origin of black locust honey using pollen analysis

Soon Ok Woo, Moon Seon Lee, Sam Gyul Lee, Seonmi Kim, Pureum Im, Suwon Park, Yungon Son, Hongmin Choi

Rural Development Administration, National Institute of Agricultural Sciences, Department of Agricultural Biology, Republic of Korea

CORRESPONDING AUTHOR: *wooso1@korea.kr*

This study aimed to determine the geographical origin of black locust (*Robinia pseudoacacia* L.) honey by comparing the morphological characteristics of pollen from black locust honey collected from seven regions in Korea with the pollen characteristics of local honey-producing plants. To this end, black locust honey samples and pollen from surrounding flowering plants were collected from 21 apiaries across seven Korean regions. Honey samples were pretreated with acetic acid to remove impurities and decolorise them. Pollen morphology was then observed using light and scanning electron microscopy. Furthermore, considering that honeybees' typical foraging range is approximately 1,500–1,700 meters, the correlation between pollen collected from nearby nectar plants and pollen found in honey was limited. Results revealed that the predominant pollen in the honey was mostly trispherical. In certain regions, specific pollen from local honey-producing plants was detected in honey, indicating geographical specificity. The results of this study contribute to identifying the geographical origin of black locust honey and provide basic data for future usage of pollen morphology for this purpose.

Acknowledgements: this work was carried out with the support of Research Program for Agricultural Sciences (Project No. PJ017457)" Rural Development Administration, Republic of Korea.

PP-09.

Elemental composition of propolis ethanolic extracts from western Croatia

Irena Brčić Karačonji[1,2], Mateo Jakac[3,4], Paula Žurga[5], Andreja Jurič[1], Marin Glad[4,5], Darija Vukić Lušić[4,5], Nediljko Landeka[3], Arijana Cenov[4,5], Dražen Lušić[2,4,5]

[1]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2]University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
[3]Teaching Institute of Public Health of Istria County, Pula, Croatia
[4]University of Rijeka, Faculty of Medicine, Rijeka, Croatia
[5]Teaching Institute of Public Health of Primorje-Gorski Kotar County, Rijeka, Croatia

CORRESPONDING AUTHOR: *ibrcic@imi.hr*

There is a general lack of scientific data regarding the element composition of propolis ethanolic extracts despite the fact that their presence can significantly affect the pharmacotherapeutic properties of such propolis preparations. In this study, the concentrations of 17 major and trace elements were determined in 104 propolis extract samples. Raw propolis samples were collected from the Istria and Primorje-Gorski Kotar County regions in western Croatia. Propolis extracts were prepared following beekeepers' instructions: ground propolis was extracted with 70% ethanol (3:7 *w/w*) for 40 days. The concentrations of Al, As, B, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr, and Zn were quantified using inductively coupled plasma mass spectrometry (ICP-MS), while Ca, K, Mg, and Na were determined by flame atomic absorption spectrometry (FAAS). Among the analysed elements, K, Ca, Mg, Na, Zn, and Fe were the most abundant in the propolis extracts. Regarding toxic elements (As, Cd, Hg, and Pb), Pb showed the highest concentrations, while Hg levels were below the limit of detection in all samples. The measured concentrations of toxic elements do not pose a health risk to consumers. These findings highlight the importance of propolis extracts as a source of essential

elements, particularly given that ethanolic extracts represent the primary dosage form of propolis and are widely used in the production of various pharmaceutical preparations, including tablets, capsules, and syrups.

Acknowledgements: this research was funded by the European Union—Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. No. 533-03-23-0006), the Croatian Academy of Sciences and Arts, European Union – NextGeneration EU – CCBE-Propolis – uniri-iz-25-164, the University of Rijeka (grants uniri-iskusni-biomed-23-2472 and Prometheus Fund), and supported by the European Regional Development Fund (project RecIMI, KK.01.1.1.02.0007).

PP-10.

Nectarless plants in European honeys: a reference for melissopalynological analysis

Maria Dimou[1] & Panagiota Gotsiou[2]

with the collaboration of: Juliana Atanasova, Waltraud Auer, Patricia Beaune, Gudrun Beckh, Katharina Bieri, Katja Bohm, Marzia Bo, Florence Britis, Dragan Bubalo, Edith Bucher, Maria Carmen Seijo Coello, Francesca Corvucci, Tuğçe Dastan, Einar Etzold, Paola Ferazzi, Francesca Grillenzoni, Claudine Guinet, Susanne Hanewinkel-Meshkini, Helmut Heigl , Sophia Karabournioti, Andreja Kandolf, Birgit Lichtenberg-Kraag, Antonio Manzanares, Carine Massaux, Rudolf Moosbeckhofer, Monique Morlot, Giorgos Moschidis, Katharina von der Ohe, Maria Lucia Piana, Nadine Raum, Asli Elif Sunay, Dariusz Teper

[1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Apiculture & Sericulture, Greece*
[2]*CIHEAM-Mediterranean Agronomic Institute of Chania, Greece*

CORRESPONDING AUTHOR: mdimou@agro.auth.gr

Melissopalynological analysis of honey plays a crucial role in determining its botanical origin. However, this type of analysis is often challenging, as it requires considerable expertise from the specialist performing it. One of the major difficulties arises from the presence of pollen from plants-species that do not produce nectar and therefore should not contribute directly to the composition of honey. Their pollen may appear in honey as a result of environmental contamination, bee foraging behaviour, incidental contact within the hive, or processes occurring during honey harvesting. Without knowledge of which plant species are nectarless, analysts may mistakenly assign incorrect botanical origins or misclassify monofloral honeys. Therefore, understanding the distribution and identity of nectarless plants, which are widespread across Europe, is essential for the accurate interpretation of pollen spectra in honey samples. In this study, we compiled data on the occurrence of pollen from nectar-

less taxa in European honeys based on contributions from experienced melissopalynologists—members of the International Honey Commission (IHC)—and verified these records through a comprehensive literature review. More than 300 taxa were verified, and a list of approximately 150 taxa representing more than 50 families is presented.

PP-11.

Chemical diversity of bee bread across countries: a collaborative study

Soraia I. Falcão[1], Javier Barreiro[2], Mila Arapcheska[3], Nebojsa Nedic[4], Ester Ordonez[5], Katarina Savikin[6], Marian Hýbl[7], Naceur M'Hamdi[8], Adel Hatem[9], Dılhun Keriman Arserim-Uçar[10], Marco Ciulu[11], Ceren Mutlu[1], Carolina Nebot[2]

[1]Campus de Santa Apolónia, Instituto Politécnico de Bragança, CIMO, LA SusTEC, Bragança, Portugal
[2]Campus Terra, Universidade de Santiago de Compostela, Departamento de Química Analítica, Nutricion y Bromatología, Laboratorio de Higiene, Inspeccion y Control de Alimentos (LHICA), Lugo, Spain
[3]St. Kliment Ohridski University, Faculty of Biotechnical Sciences, Bitola, North Macedonia
[4]University of Belgrade, Faculty of Agriculture, Department of Breeding and Reproduction of Domestic and Farmed Animals, Belgrade, Serbia
[5]Agrupación Apícola de Galicia, Ourense, Spain
[6]Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia
[7]Czech Academy of Sciences, Biology Centre, Ceske Budejovice, Czechia
[8]Institut National Agronomique de Tunisie, Tunis, Tunisia
[9]The Agricultural Research Center, Giza, Egypt
[10]Bingöl University, Selahaddin-i Eyyubi Neighborhood, Bingöl, Türkiye
[11]University of Verona, Verona, Italy

CORRESPONDING AUTHOR: *sfalcao@ipb.pt*

Bee bread is a naturally fermented hive product resulting from the enzymatic and microbial transformation of bee pollen by honeybees (*Apis mellifera*). Due to its nutritional and functional properties, bee bread has gained increasing scientific and commercial interest. Its chemical composition is strongly influenced by the botanical origin of the pollen collected by honey bees, which varies significantly according to regional floral diversity and the season of harvest. In addition to high protein levels and essential amino acids, bee bread contains diverse bioactive compounds, including sugars, vitamins, phenolics, pigments, lipids, dietary

fiber, enzymes, and minerals. The aim of this work was to evaluate the chemical composition of bee bread samples obtained from COST members from 10 different countries during 2024, in a total of 36 samples. After the reception, the samples were lyophilised and homogenised. Different parameters were evaluated such as water content, ash, pH, total proteins, total lipids, and sugars. Bee bread samples showed water content between 6.07 and 9.57%, pH between 3.9 and 4.3, and ash content between 1.6 and 2.9%. Monosaccharides predominated, with fructose and glucose ranging from 16.14 to 24.21 g/100 g and 8.73–15.75 g/100 g, respectively, while sucrose ranged from 0 to 4.80 g/100 g. Variations in sugar profiles were associated with botanical diversity, regional factors, and the extent of lactic acid fermentation. The chemical composition of bee bread is crucial for determining its nutritional value, bioactivity, quality, and authenticity, as it reflects both botanical origin and fermentation processes within the hive.

Acknowledgments: this work was supported by national funds through FCT/MCTES (PIDDAC): CIMO UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020. Thanks to the program Promove from Fundação "La Caixa", in collaboration with BPI and FCT, Portugal, within the scope of project BeeSustain, PD23-00019. National funding by FCT- Foundation for Science and Technology, through the institutional scientific employment program-contract with Soraia I. Falcão (2023.11031.TENURE.021). The authors acknowledge and thank the European Cooperation in Science and Technology (COST) for funding COST Action 22105 and the members of this COST Action for their motivation and support during the development of this project.

PP-12.

Comparative phenolic composition and antioxidant capacity of Croatian and Italian strawberry tree (*Arbutus unedo* L.) honey

Andreja Jurič[1], Irena Brčić Karačonji[1,2], Uroš Gašić[3], Dušanka Milojković-Opsenica[4], Carlo I. G. Tuberoso[5]

[1]Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
[2]University of Rijeka, Faculty of Health Studies, Department of Basic Medical Sciences, Rijeka, Croatia
[3]Institute for Biological Research "Siniša Stanković", Belgrade, Serbia
[4]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia
[5]University of Cagliari, Department of Life and Environmental Sciences, Cagliari, Italy

CORRESPONDING AUTHOR: ajuric@imi.hr

Strawberry tree (*Arbutus unedo* L.) honey (STH) is a rare monofloral Mediterranean honey known for its characteristic bitterness and high phenolic content. With the aim of evaluating regional differences in the phytochemical profile of this honey, this study compared the total phenolic content (TPC), antioxidant capacity (DPPH and FRAP), and polyphenolic composition of Croatian (n = 8) and Italian (n = 5) STH. As homogentisic acid (HGA) concentrations below 200 mg/kg do not meet the recommended minimum for confirming the authenticity of STH, three Croatian samples with HGA values < 200 mg/kg were excluded from our analysis, and only samples above this threshold were considered in the final evaluation. The Italian STH exhibited higher TPC levels [average 1618.85 ± 57.36 *vs*. 1340.12 ± 63.84 mg gallic acid equivalents (GAE)/kg] with stronger DPPH scavenging activity [average DPPH values 3.20 ± 0.06 *vs*. 2.21 ± 0.04 µmol Trolox equivalents (TE)/kg] and stronger ferric reducing antioxidant power [average FRAP values (1.19 ± 0.06 *vs*. 0.96 ± 0.09 mM $FeSO_4 \cdot 7H_2O$)], confirming the overall higher antioxidant potential

of Italian STH. HGA, the primary phenolic acid and authenticity marker of STH, was present in all of the samples in a fairly wide range. Italian honey exhibited substantially higher HGA levels (231.69 – 616.09 mg/kg), while Croatian honey showed a narrower range (202.0–306.8 mg/kg) after sample selection. Regarding the phenolic profile, the Italian samples showed higher gallic acid level, whereas Croatian honey was relatively enriched in *p*-hydroxybenzoic, protocatechuic and *p*-hydroxyphenylacetic acid. Acacetin was detected in the STH from both countries, even though the Croatian STH exhibited slightly higher levels. Taken together, these findings underscore the distinct phenolic differences between the Croatian and Italian STHs. The Italian honey exhibited higher TPC, DPPH, FRAP and HGA levels, while the Croatian STH contained greater amounts of phenolic acids and acacetin. This assessment contributes to a more detailed understanding of the regional phytochemical variability of this uncommon monofloral honey.

Acknowledgements: the study was supported by institutional funding provided by the Ministry of Science, Education and the Youth of the Republic of Croatia (no. 106-F25-00045), European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox), and the European Regional Development Fund project KK.01.1.1.02.0007.

PP-13.

Establishing the phenolic signature of Beira Baixa honey

Beatriz Oliveira Marques[1], Odete Gonçalves[2], Paulo Russo[3], Ofélia Anjos[2,4,5], Eugénia Gallardo[1], Soraia Inês Pedro[4,6]

[1]University of Beira Interior, Faculty of Health Sciences, RISE-Health - Department of Medical Sciences, Covilhã, Portugal
[2]IPCB – Polytechnic University of Castelo Branco, Castelo Branco, Portugal
[3]Universidade Trás-os-Montes e Alto Douro, UTAD, Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Quinta de Prados, Vila Real, Portugal
[4]Polytechnic University of Castelo Branco, Research Centre for Natural Resources, Environment and Society, CERNAS-IPCB, Castelo Branco, Portugal
[5]CBP-BI – Plant Biotechnology Centre of Beira Interior, Castelo Branco, Portugal
[6]University of Beira Interior, Faculty of Health Sciences, RISE-Health - Department of Medical Sciences, Covilhã, Portugal

CORRESPONDING AUTHOR: ofelia@ipcb.pt

Phenolic compounds are key bioactive constituents of honey, known for their antioxidant, antimicrobial, and anti-inflammatory properties. This group mainly comprises phenolic acids and flavonoids, whose presence and concentration depend strongly on botanical origin and geographical conditions, making them valuable markers of honey quality and authenticity. In this study, the phenolic composition and palynological profile of honeys from the Beira Baixa region were investigated to support their differentiation and potential recognition as a product with Protected Designation of Origin (PDO). A total of 24 honey samples were analysed. Phenolic compounds were extracted and determined using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD), allowing separation, identification and quantification based on retention times and UV-Vis spectral characteristics. Three g of honey were extracted using 3 mL of water, with the samples shaken for 30 minutes, after which the organic phase was collected for analysis. Several phenolic compounds were detected, with the most frequently

identified compounds including gallic acid, catechin, chlorogenic acid, *p*-coumaric acid, epicatechin, rutin, protocatechuic acid, phloridzin, and ellagic acid belonging mainly to the groups of phenolic acids, and concentrations ranging [2.63–15.45 mg/g], [0.18–2.99 mg/g], [0.35–0.93 mg/g], [0.28–1.26 mg/g], [1.37–3.39 mg/g], [0.51–3.33 mg/g], [0.26–4.30 mg/g], [0.60–0.96 mg/g], and [0.42–2.12 mg/g], respectively. Palynological analysis, performed using the acetolysis method, confirmed the botanical origin of the honey samples and revealed a predominantly multifloral profile. The dominant pollen types identified were *Lavandula* sp., *Echium* sp., *Rubus* sp., *Cistus ladanifer* and *Cytisus* sp. The relative abundance of these pollen types showed good agreement with the phenolic profiles obtained, reinforcing the link between floral sources and bioactive composition. Overall, the establishment of a robust phenolic fingerprint provides scientific evidence supporting the high quality and authenticity of Beira Baixa honey.

Acknowledgements: thanks for financial support by national funds FCT/MCTES through the projects UID/00681/2025 (DOI: 10.54499/UID/00681/2025) of Research Centre for Natural Resources, Environment and Society (CERNAS) and the project BioLivingLABS, Collective Actions – Transfer of scientific and technological knowledge, COMPETE2030-2024-6, Project Number 17807, co-financed by the European Regional Development Fund (ERDF) throughCOMPETE2030 (Thematic program for Innovation and Digital Transition).

PP-14.

Sugar profile of Istrian *Hedera helix* honey

Dušanka Milojković-Opsenica[1], Mihajlo Jakanovski[2], Itana Bokan Vucelić[3], Dijana Mišetić Ostojić[4], Dražen Lušić[5]

[1]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia
[2]University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia
[3]Teaching Institute of Public Health of Primorje-Gorski kotar County, Rijeka, Croatia
[4]Croatian Veterinary Institute, Veterinary Center Rijeka, Croatia
[5]University of Rijeka, Faculty of Medicine, Rijeka, Croatia

CORRESPONDING AUTHOR: *dusankam@chem.bg.ac.rs*

Honey is the natural sweet substance produced by honeybees from the nectar of plants (floral honey) or from secretions of living plants or excretions of plant sucking insect on the living parts of plant (honeydew honey). From a chemical standpoint, honey is defined as a supersaturated aqueous solution of sugars, with fructose and glucose as the dominant monosaccharides, accompanied by smaller amounts of disaccharides, trisaccharides, and higher oligosaccharides. The sugar profile, including total sugar content, the abundance of individual carbohydrates, and their characteristic ratios, is widely recognised as a robust indicator of honey authenticity, production practices, and botanical and geographical provenance. In this study, the sugars profile of *Hedera helix* honey was investigated using forty-one samples collected from the Istrian region (Croatia). Analysis of sugars was performed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). In addition to glucose, fructose, and sucrose, five further carbohydrates: trehalose, melibiose, isomaltose, maltose, and raffinose were quantified. Total sugar content, the sum of fructose and glucose, and their characteristic ratio were also evaluated. The average contents of the main sugars were 31.9 g/100 g of honey for glucose and 33.2 g/100 g for fructose, resulting in a mean sum content of 65.1 g/100 g and F/G ratio

of 1.04. Sucrose was present at low levels (0.6 g/100 g), while trehalose, melibiose, isomaltose, maltose, and raffinose were detected at average concentrations ranging from 0.2 to 0.4 g/100 g. Principal component analysis (PCA) revealed a moderate variability among samples, primarily driven by differences in mono- and disaccharide composition, confirming the relevance of detailed carbohydrate profiling for the characterisation of *Hedera helix* honey from the Istrian region.

Acknowledgements: this research was funded by the project European Union – NextGeneration EU – CCBE-Propolis – uniri-iz-25-164.

PP-15.

Comparative study of vibrational spectroscopy techniques applied to honey characterisation

Soraia Inês Pedro[1,2], Beatriz Oliveira Marques[3], Ana Daniela Dias[4], Fátima Peres[4,5], Ofélia Anjos[1,2,4]

[1]Polytechnic University of Castelo Branco, Research Centre for Natural Resources, Environment and Society, CERNAS-IPCB, Castelo Branco, Portugal
[2]CBP-BI – Plant Biotechnology Centre of Beira Interior, Castelo Branco, Portugal
[3]UBI – University of Beira Interior, Covilhã, Portugal
[4]IPCB – Polytechnic University of Castelo Branco, astelo Branco, Portugal

CORRESPONDING AUTHOR: ofelia@ipcb.pt

This study evaluated the potential of three vibrational spectroscopy techniques, namely FT-Raman, FTIR-ATR and FT-NIR, as rapid and accurate tools for assessing honey quality in accordance with legal standards, as well as for developing methodologies to distinguish between honeys from different regions with Protected Designations of Origin (PDOs). Principal component analysis (PCA), discriminant analysis (LDA) and partial least squares regression (PLS-R) models were constructed for both qualitative discrimination and the quantitative prediction of electrical conductivity, free acidity, pH, moisture content, colour, and viscosity. Robust calibration models were obtained for all quantified parameters. In general, the models showed high coefficients of determination (R^2 values exceeding 0.92 for most attributes), high residual predictive deviation values (higher than 4) and low mean square errors. FT-Raman and FTIR-ATR provided the strongest predictive performance. Qualitative analyses revealed partial discrimination among PDO regions, although some regions exhibited greater similarity, likely due to shared floral sources in the surrounding landscapes. In conclusion, the findings confirm that vibrational spectroscopy is an effective approach for controlling the quality of honey, as well as for its chemical characterisation and authentication. Additionally, the

ability to differentiate PDO regions reinforces the cultural and geographical identity of these honeys. This supports their commercial positioning and contributes to the protection, enhancement and sustainable marketing of distinctive regional apicultural products.

Acknowledgements: thanks for financial support by national funds FCT/MCTES through the projects UID/00681/2025 (DOI: 10.54499/UID/00681/2025) of Research Centre for Natural Resources, Environment and Society (CERNAS), by the European Union's NextGenerationEU recovery funds under Project BeeLand (10/C05-i03/2021.PPRR-C05-i03-I-00008) and the project BioLivingLABS, Collective Actions – Transfer of scientific and technological knowledge, COMPETE2030-2024-6, Project Number 17807, co-financed by the European Regional Development Fund (ERDF) throughCOMPETE2030 (Thematic program for Innovation and Digital Transition).

PP-16.

Physicochemical profile and pollen spectrum of common ivy (*Hedera helix* L.) honey

Saša Prđun[1], Lidija Svečnjak[1], Barbara Stjepanović[2], Ana Večenaj[2], Dragan Bubalo[1]

[1]University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia
[2]Andrija Štampar Teaching Institute of Public Health, Department of Food Safety and Quality, Zagreb, Croatia

CORRESPONDING AUTHOR: sprdjun@agr.hr

Common ivy (*Hedera helix* L.) honey is a late-season unifloral honey characterised by specific physicochemical properties, which reflect its composition and quality. Its botanical origin is commonly assessed by melissopalynological analysis, based on the identification and quantification of pollen grains and/or accompanying physicochemical analyses but the records on the properties of this rare honey type are generally scarce. This study aimed to investigate selected physicochemical and melissopalynological properties of ivy honey. A total of 32 honey samples were collected from the beekeepers in Croatia during 2023–2025. Selected physicochemical analyses, including water content and electrical conductivity, were performed according to standard analytical methods. The botanical origin was determined by means of qualitative pollen analysis. The results have revealed that the average water content of the investigated honey samples was 18.0% (14.0–19.9%), while the average electrical conductivity was 0.52 mS/cm (0.37–0.78 mS/cm). Pollen analysis confirmed the dominance of *H. helix* pollen grains (> 45%) in all samples, with percentages ranging from 54% to 98%, depending on geographical location, climatic conditions, and foraging activity of honey bees. In addition to *H. helix* pollen, common ivy honey contained pollen from other flowering species, categorised as accompanying (16–45%),

secondary (3–15%), and rare (< 3%) according to relative abundance. The accompanying pollen group (16–45%) was dominated by *Asparagus* spp. (26%) and *Frangula alnus* (18%). Secondary pollen types (3–15%), present in approximately half of the samples, were mainly from Asteraceae, Apiaceae, Fabaceae, Rhamnaceae, Lamiaceae, Poaceae, Brassicaceae, and Boraginaceae families. Rare pollen (< 3%) types included *Ericaceae* (2%), *Amorpha fruticosa*, Liliaceae, *Trifolium* spp. and *Centaurea jacea* (1% each). The observed differences in pollen profiles suggest that common ivy honey may contain nectar contributions from various late-blooming plant species which largely influenced by the local flora. A pollen spectrum plays a key role in verifying origin of unifloral honeys, and it is also very important for maintaining the product quality, and assessing the presence of bioactive compounds associated with specific botanical sources.

PP-17.

Novel insights into the relationship between ascitic fluid cell dynamics and primary DNA damage levels in blood cells of Ehrlich ascites tumor-bearing mice treated with propolis and gamma irradiation

Vesna Benković[1], Anica Knežević[1], Fabijan Knežević[2], Nevenka Kopjar[3]

[1]Faculty of Science, University of Zagreb, Zagreb, Croatia
[2]Catholic University of Croatia, School of Medicine, Zagreb, Croatia
[3]Institute for Medical Research and Occupational Health, Zagreb, Croatia

CORRESPONDING AUTHOR: *vesna@biol.pmf.hr*

Studies investigating natural radioprotectors in tumor-bearing animal models are limited, yet the data derived from these models are vital for predicting effects in humans. Using model of Swiss albino mice bearing the Ehrlich ascites tumour (EAT) we investigated the effects of ionising irradiation combined with ethanolic extract of propolis (EEP), and a water-soluble derivative of propolis (WSDP) on the ascitic fluid cell dynamics and primary DNA damage levels in the white blood cells. Irradiation was performed using a γ-ray source (^{60}Co), and the absorbed dose was 4 Gy. Each experimental group consisted of five male mice per tested component. Three days after EAT intraperitoneal (*i.p.*) inoculation (1 × 10^6 cells), the effectiveness of the test components was evaluated when administered *i.p.* at a dose of 100 mg/kg for three consecutive days, either before or after irradiation. White blood cells were used to perform the alkaline comet assay 1 and 72 hours after the last treatment. The comet descriptors for evaluation of primary DNA damage were tail intensity and tail length. Tumor cell viability and ascitic fluid cell dynamics were assessed using microscopic slides stained with Trypan Blue. The results demonstrated a higher DNA-protective efficacy of the tested components

when administered preventively. Pre-treatment and therapeutic application of EEP and WSDP differentially affected ascitic fluid cell dynamics. Distinct interaction patterns were identified and correlated with observed DNA damage levels. Overall, the findings provide new insights into the protective potential of EEP and WSDP and suggest their possible synergistic effect with gamma irradiation, warranting further investigation.

Acknowledgements: the study was funded by institutional funding provided by the Ministry of Science, Education and the Youth of the Republic of Croatia (no. 106-F25-00045) and European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox).

PP-18.

Impact of solvent selection on the antibacterial activity of propolis extracts

Ivana Flanjak[1], Katarina Živković[1], Martina Jakovljević Kovač[1], Katarina Gal[1], Ariana Penava[2], Sabina Cviljević[3], Ivan Vukoja[3,4], Blanka Bilić Rajs[1]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia
[2]Instutite of Public Health Požega-Slavonia County, Požega, Croatia
[3]General Hospital Požega, Požega, Croatia
[4]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia

CORRESPONDING AUTHOR: *ivana.flanjak@ptfos.hr*

Antibacterial properties of propolis are well known, but the effectiveness of bioactive components significantly depends on the solvent type and extraction method. Propolis bioactive components are most often extracted using different ethanol: water mixtures, but application of those extracts has some limitations. In order to increase the applications of propolis extracts and expand the range of sustainable propolis-based products, different solvents (e.g., edible oils, water, natural deep eutectic solvents—NADES) assisted with innovative extraction techniques (microwaves, ultrasound, high pressure, supercritical CO_2) are evaluated. In this study, the inhibition potential of NADES extracts of two propolis samples from different regions of Croatia against two bacterial species (*Streptococcus pneumoniae* and *Staphylococcus aureus*) was evaluated. NADES extracts were prepared using choline chloride:lactic acid:water (1:2:2) as solvent assisted with ultrasound while alcoholic extracts used for comparison were prepared with 80% ethanol and ultrasound. Antibacterial activity was determined using the agar diffusion method. The results showed that NADES extracts of both propolis samples had significantly higher antibacterial activity against both bacterial species (the inhibition

zones 35 and 38 mm for *S. pneumoniae*; 33 and 34 mm for *S. aureus*, respectively) compared to the inhibition zone of ethanolic extracts (13 and 14 mm for *S. aureus* while ethanolic extracts had no inhibition potential against *S. pneumoniae*). The obtained results give us a strong incentive to further research for alternative extraction methods that will increase the possibilities of using propolis extracts in cases where the use of an alcoholic solution of propolis is not applicable.

Acknowledgements: this work was supported by Osijek-Baranja County under the project "Biological activity of propolis extracts prepared using green techniques".

PP-19.

Functional and antioxidant characterisation of bee pollen according to botanical origin using composite indices and multivariate analysis

María Shantal Rodríguez Flores[1], María Carmen Seijo Coello[1], Yasmine Saker[1], Sonia Harbane[1], Monserrat Míguez Bernárdez[1], Eva María Lorenzo Iglesias[2], María Lourdes Borrajo Diz[2], Adrián Seara Vieira[2], Olga Escuredo Pérez[1]

[1]Universidade de Vigo, Facultade de Ciencias de Ourense, Grupo de Investigación en Sistemas Agroambientais (GISA), Ourense, Spain
[2]Universidade de Vigo, Escola Superior de Enxeñaría Informática, Grupo de Investigación en Sistemas Informáticos de Nueva Generación (SING), Ourense, Spain

CORRESPONDING AUTHOR: *mariasharodriguez@uvigo.gal*

Bee pollen is increasingly recognised as a functional food due to its rich phenolic composition and antioxidant activity, which are strongly influenced by botanical origin. However, the integration of multiple chemical and biological parameters into a coherent functional evaluation remains a challenge. In this study, bee pollen samples of different botanical origins were functionally characterised using composite antioxidant and global functional indices combined with multivariate analysis. Antioxidant activity was evaluated using complementary assays, and composite Antioxidant Index (AI) and Global Functional Index (GFI) were calculated after data normalisation. Botanical composition was determined by palynological analysis. Principal component analysis (PCA) was applied to explore relationships between functional indices and botanical origin and to assess the discriminant capacity of the variables. The results showed that AI and GFI differed significantly among pollen samples according to their dominant botanical taxa. Pollen rich in *Castanea* and *Rubus* exhibited higher functional and antioxidant scores, whereas samples dominated

by *Cistus* and *Helianthemum* presented lower values. PCA clearly separated pollen samples according to botanical composition, with AI and GFI contributing strongly to sample discrimination, confirming their suitability as integrative descriptors of functional quality. These findings demonstrate that botanical origin is a key determinant of bee pollen functional properties and that composite indices combined with multivariate analysis constitute an effective approach for functional classification. This strategy provides a practical tool for quality assessment, authentication and valorisation of bee pollen as a functional food.

Acknowledgements: M. S. Rodríguez Flores acknowledges support from the INOU 2025 research funding programme. O. Escuredo Pérez acknowledges funding from the VASBEEP project (PID2022-141679OR), supported by the Spanish State Research Agency.

PP-20.

Preparative isolation and characterisation of nanoscale components from royal jelly by asymmetric flow field-flow fractionation

Anna Placci[1,2,3], Stefano Giordani[1,2,3], Mladenka Malenica[4], Virginia Rondinini[1], Pietro Parisse[5], Loredana Cassalis[5,6], Marin Kovačić[7], Dražen Lušić[8,9,10], Barbara Roda[1,2,3], Andrea Zattoni[1,2,3], Pierluigi Reschiglian[1,2,3], Valentina Marassi[1,2,3]

[1]University of Bologna, Department of Chemistry 'G. Ciamician', Bologna, Italy
[2]byFlow s.r.l, Bologna, Italy
[3]Biostructures and Biosystems National Institute (INBB), Rome, Italy
[4]University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia
[5]CNR-IOM – Istituto Officina dei Materiali, Area Science Park, Basovizza, Trieste, Italy
[6]Elettra Sincrotrone Trieste, Basovizza, Trieste, Italy
[7]University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia
[8]University of Rijeka, Faculty of Health Studies, Department of Basic Medical Sciences, Rijeka, Croatia
[9]University of Rijeka, Faculty of Medicine, Department of Health Ecology, Rijeka, Croatia
[10]Teaching Institute of Public Health of Primorje-Gorski Kotar County, Department of Environmental Health, Rijeka, Croatia

CORRESPONDING AUTHOR: anna.placci3@unibo.it

Royal Jelly (RJ) is well known for its biological activities, such as antimicrobial, antioxidant, and antitumoral, yet it represents a highly complex natural matrix composed of molecular and nanoscale components. While molecular and macromolecular constituents, including proteins, lipids, and sugars, have been extensively studied, the particulate fraction of RJ remains poorly characterised. In particular, analytical approaches enabling native isolation and size-resolved characterisation of RJ-derived

nanoparticles are still limited. In this study, we present a multidetection asymmetric flow field-flow fractionation (AF4-MD) approach for the isolation and characterisation of nano-sized components from RJ. The method provides gentle, size-based separation, combined with multiparametric detection, including spectroscopic characterisation, and allows the direct analysis of complex matrices without sample pretreatment. AF4-MD was applied to two RJ samples, resolving four nanoparticle populations with distinct size ranges and sample-dependent distributions. Six fractions were collected, displaying different spectroscopic profiles: early- and late-eluting fractions exhibited absorption typical of nucleic acids, whereas central fractions showed protein spectra. The collected fractions were further analysed by atomic force microscopy (AFM) to characterise size, morphology, and relative particle abundance. AFM revealed a heterogeneous distribution of nanoscale material across the fractions, with clear differences in particle size, morphology, and abundance. The first fraction contained the highest number of nanoparticles, with a median size of approximately 100 nm, together with fibrillar species, consistent with glycolipoproteic aggregates. Nanoparticle populations were also detected in the central and late fractions, with median sizes ranging from approximately 50 to 80 nm and exhibiting round, lobed, and multilobed morphologies. The integration of AF4-MD and AFM enabled the characterisation of the nanosized components of RJ, highlighting its high heterogeneity. These results underscore the value of soft, native separation techniques, which help avoid artifacts from aggregation or particle disruption, and pave the way for functional studies on biological activity and future potential applications.

Acknowledgments: this work has been supported by the University of Rijeka, uniri-mzi-25-34, Nano-delivery particles from bee products for better bioavailability of bioactive compounds with antitumor activity, BEE-nano.

PP-21.

Predicting bee pollen nutritional quality from botanical composition using a continuous nutritional index

María Shantal Rodríguez Flores[1], Olga Escuredo Pérez[1], Yasmine Saker[1], Sonia Harbane[1], Monserrat Míguez Bernárdez[1], Eva María Lorenzo Iglesias[2], María Lourdes Borrajo Diz[2], Adrián Seara Vieira[2], José Bernal del Nozal[3], María Carmen Seijo Coello[1]

[1]Universidade de Vigo, Facultade de Ciencias de Ourense, Grupo de Investigación en Sistemas Agroambientais (GISA), Ourense, Spain
[2]Universidade de Vigo, Escola Superior de Enxeñaría Informática, Grupo de Investigación en Sistemas Informáticos de Nueva Generación (SING), Ourense, Spain
[3]University of Valladolid Faculty of Sciences, Department of Analytical Chemistry, Valladolid, Spain

CORRESPONDING AUTHOR: *mariasharodriguez@uvigo.gal*

Bee pollen is the main source of proteins, lipids, and micronutrients for honey bee colony development, but its nutritional quality varies widely depending on botanical origin and floral landscape structure. As the nutritional functionality of pollen is largely determined by amino acids and fatty acids, this study proposes a continuous nutritional index that integrates amino acid composition and lipid balance. A total of 36 honey bee pollen samples were collected in Galicia (north-west Spain). Palynological analysis was used to identify the botanical origin. The nutritional characterisation included standard protein content and amino acid profiles, as well as fatty acid composition, with a particular focus on ω-3 and ω-6. All nutritional variables were normalised and integrated into a continuous nutritional index. Protein content showed very high variability (1.7–41.7 g protein eq/100 g dry pollen), confirming the strong dependence of pollen nutritional quality on botanical origin. Nutritional index values ranged from 0.1 to 0.7. The highest values were predominantly associated with samples dominated by *Genista* type pollen, indicating that this pollen type

provides a nutritionally balanced pollen profile. *Eucalyptus* pollen type also showed high nutritional values. In contrast, samples dominated by *Castanea* and *Rubus* generally presented lower or highly variable nutritional index values. Random Forest regression models demonstrated that pollen nutritional quality can be partially predicted from pollen data. Taxonomic dominance and floral diversity emerged as the main explanatory drivers, supporting the idea that nutritional quality is linked to botanical origin. The proposed nutritional index offers a meaningful tool for assessing pollen quality, providing a valuable framework for monitoring nutritional stress and floral resource suitability in changing landscapes.

Acknowledgements: MS. Rodríguez Flores acknowledges the support of the INOU 2025 research funding programme. M. C. Seijo Coello acknowledges funding from the ENCE Social Plan project and the University of Vigo.

PP-22.

Monitoring pesticide residues in beekeeping products to safeguard bee health and food safety in Albania

Kastriot Korro[1], Fatjon Hoxha[2], Richard Bagshaw[3], Mandeep Singh[3], Albert Licollari[3]

[1]Agricultural University of Tirana, Faculty of Veterinary Medicine, Albania
[2]Agricultural University of Tirana, Albania
[3] Nucro – Technics Laboratories, Toronto, Canada

CORRESPONDING AUTHOR: kkorro@ubt.edu.al

Honey and other beekeeping products are highly valued natural foods and effective bioindicators of environmental contamination. Despite the importance of apiculture in Albania, systematic and harmonised data on pesticide residues in bee products remain limited. This study aimed to characterise pesticide residues in honey and propolis from different Albanian regions in order to assess consumer food safety and potential risks to honeybee health. Honey and propolis samples were collected from beekeeping producers across six regions of Albania, including one certified organic protected area, representing both intensively managed agricultural landscapes and semi-natural environments. A validated multi-residue analytical approach based on the QuEChERS method, compliant with USP <561> and European Union regulatory guidance, was applied. Analyses were performed using GC-MS/MS and LC-MS/MS to screen for 95 pesticide active substances belonging to organochlorine, organophosphate, pyrethroid, and other pesticide classes. Compound-specific limits of detection and regulatory decision thresholds were used to ensure analytical reliability. Due to its simplicity, scalability, and broad analyte coverage, the applied methodology is suitable for high-throughput pesticide surveillance in honey, propolis, and other agricultural products. All analysed honey samples complied with USP <561> limits and relevant EU maximum residue levels, indicating a low dietary risk for consumers and

consistency with recent international assessments of honey safety. In contrast, two propolis samples contained aldrin/dieldrin, cyfluthrin, and/or fenpropathrin at concentrations exceeding acceptable limits. Furthermore, multiple legacy and currently used pesticides were detected at low concentrations in hive-related matrices from organically managed colonies and protected park environments, indicating diffuse landscape-level contamination. These findings suggest that Albanian honey is safe for human consumption under current conditions, while highlighting potential chronic and sublethal risks to honeybee colonies associated with highly bee-toxic pyrethroids and persistent organochlorines accumulating in hive materials. The analytical framework presented here supports the establishment of a national pesticide residue monitoring system and reinforces the role of beekeeping products as practical bioindicators for pollinator protection strategies and pesticide risk management in Albania.

PP-23.

Effects of chlorpyrifos incorporated into beeswax on colony population development of Africanized *Apis mellifera*

Ricardo de Oliveira Orsi[1], Guilherme Duarte Figueiredo de Souza[1], Iloran Rosário Corrêa Moreira[1], Victor Pereira Fest[1], Jaine Xavier[1], Laís Tainá Feliciano[1], Flávio Augusto Lourencetti[1], Isabella Cristina de Castro Lippi[2]

[1]UNESP – Univ. Estadual Paulista, Department of Animal Production and Medicine Veterinary Preventive, Center of Education, Science and Technology in Rational Beekeeping (NECTAR), Botucatu, São Paulo, Brazil
[2]Southern Cross University, Faculty of Science and Engineering, Lismore Campus, Australia

CORRESPONDING AUTHOR: ricardo.orsi@unesp.br

Currently, herbicides, insecticides, fungicides, and acaricides are the most widely used classes of pesticides worldwide, with insecticides often being associated with the greatest harm to pollinators. Among these compounds, chlorpyrifos has been reported to cause adverse effects on metabolism, development, and pathogen defence in honeybees. Such detrimental effects on honeybee colonies may negatively impact the production and quality of hive products, including honey. Therefore, this study evaluated the effects of chlorpyrifos, at an environmentally relevant concentration, incorporated into beeswax, on colony population development. For this purpose, beeswax was melted and chlorpyrifos was added at a concentration of 24.95 µg/kg. Combs foundation were produced and placed in experimental colonies (five colonies exposed to chlorpyrifos and five control colonies). After comb construction and the onset of egg-laying by the queen, colony population development was monitored weekly over a 60-day period. Brood frames were photographed, and the areas of open and sealed brood were quantified (cm^2).

Data were analysed by Student's *t*-test ($p < 0.05$). No significant differences were observed between control and treated colonies for either open brood area (250.0 ± 272.8 vs. 177.2 ± 170.0 cm^2) or sealed brood area (346.4 ± 172.8 vs. 306.0 ± 212.8 cm^2). These results indicate that, at the tested concentration, chlorpyrifos did not affect colony population development. Although the findings suggest that honey quality was also not adversely affected, chromatographic analyses were not performed to verify the presence of pesticide residues in honey.

Acknowledgments: FAPESP - São Paulo Research Foundation. Process 2023/14022-8.

PP-24.

Glyphosate negatively affects queen replacement dynamics in Africanized *Apis mellifera* colonies

Ricardo de Oliveira Orsi[1], Larissa Beatriz Costa Oliveira, Flávio Augusto Lourencetti[1], Guilherme Duarte Figueiredo de Souza[1], Iloran Rosário Corrêa Moreira[1], Jaine Xavier[1], Victor Pereira Fest[1], Isabella Cristina de Castro Lippi[2]

[1]UNESP – Univ. Estadual Paulista, Department of Animal Production and Medicine Veterinary Preventive, Center of Education, Science and Technology in Rational Beekeeping (NECTAR), Botucatu, São Paulo, Brazil
[2]Southern Cross University, Faculty of Science and Engineering, Lismore Campus, Australia

CORRESPONDING AUTHOR: ricardo.orsi@unesp.br

Herbicides constitute a group of compounds commonly used for the control of invasive plant species. Among them, glyphosate (N-phosphonomethylglycine) has been one of the most widely used herbicides worldwide over the past century. Furthermore, indiscriminate use of these compounds may result in residues in bee products, such as honey, which can compromise their commercialisation when residue levels exceed regulatory limits. Among the effects associated with herbicide exposure, particular attention has been given to those that are detrimental to bee development during the larval stage, which may directly affect the emergence of new queens. Thus, the objective of this study was to evaluate the effects of the herbicide glyphosate on the production of new queens in colonies of Africanized *Apis mellifera*. Ten honeybee colonies housed in Langstroth nucleus hives were used and fed sugar syrup (1:1, water:sugar) according to the following treatments: T1-control (uncontaminated syrup); and T2-syrup contaminated with glyphosate. The syrup was supplied directly to the colonies in a volume of 500 mL (Boardman feeders a seven-day period), containing 13.69 mg of glyphosate. After seven days, the queens were removed to induce natural queen

cell construction. The number of queen cells produced in each colony was recorded, and a subset of these cells was removed, placed in cages, and maintained in an incubator (32.0 ± 2.0 °C and 70% relative humidity) until queen emergence. Data were analysed using Student's *t*-test for mean comparisons, considering $p \leq 0.05$. The results showed that feeding colonies with glyphosate-contaminated sugar syrup significantly ($p \leq 0.0001$) reduced food consumption (317.9 ± 5.8 mL) compared to the control treatment (463.4 ± 5.8 mL). Following colony orphaning, natural queen cell production resulted in 18 queen cells in the control treatment and 15 in the glyphosate treatment. Of the queen cells maintained in the incubator, six queens emerged in the control group (representing 75% of the incubated queen cells), whereas only two queens emerged in the glyphosate treatment (33.3%) ($p \leq 0.05$). These findings indicate that the evaluated herbicide negatively affects queen replacement in Africanized honeybee colonies.

Acknowledgments: FAPESP - São Paulo Research Foundation. Process 2023/14022-8 and 2024/06729-7.

PP-25.

Does chlorpyrifos added to beeswax persist in Africanized *Apis mellifera* colonies?

Ricardo de Oliveira Orsi[1], Renato Arruda Agostinho[1], Guilherme Duarte Figueiredo de Souza[1], Iloran Rosário Corrêa Moreira[1], Victor Pereira Fest[1], Jaine Xavier[1], Natália Fernanda Carriel Corrêa[1], Isabella Cristina de Castro Lippi[2]

[1]UNESP – Univ. Estadual Paulista, Department of Animal Production and Medicine Veterinary Preventive, Center of Education, Science and Technology in Rational Beekeeping (NECTAR), Botucatu, São Paulo, Brazil
[2]Southern Cross University, Faculty of Science and Engineering, Lismore Campus, Australia

CORRESPONDING AUTHOR: ricardo.orsi@unesp.br

Among beekeeping products, beeswax has the lowest replacement rate and, under natural conditions, combs may remain in the hive for many years. However, under commercial beekeeping management, combs are frequently processed by beekeepers to extract beeswax for sale, and this continuous recycling can lead to the accumulation of pesticide residues. Among these compounds, chlorpyrifos, a widely used organophosphate insecticide and acaricide, has been shown to exert harmful effects on bees and may consequently impair colony productivity. Therefore, the objective of this study was to contaminate beeswax with chlorpyrifos and to assess its recovery using high-performance liquid chromatography (HPLC; Shimadzu). Raw beeswax was obtained from a commercial producer from a single batch, melted and chlorpyrifos was added at a concentration of 24.95 µg/kg. Chromatographic analysis showed that the added pesticide was recovered at the expected concentration. These results support the hypothesis that beeswax can act as a reservoir for pesticides within the hive, potentially leading to chronic exposure of the

colony and negatively affecting bee health and productivity, with possible implications for the beekeeping industry.

Acknowledgments: FAPESP - São Paulo Research Foundation. Process 2023/14022-8.

PP-26.

Environment-driven distribution of heavy metals in honey bees (*Apis mellifera*) developmental stages and their brood combs

Ivana Tlak Gajger[1], Ana-Marija Kovač[1], Snježana Kos[2], Višnja Oreščanin[3], Nikola Čudina[1], Andreja Prevendar-Crnić[1], Alen Slavica[1]

[1]University of Zagreb, Faculty of Veterinary Medicine Croatia
[2]Spadent d.o.o., Zagreb, Croatia
[3]Oreščanin Ltd., Zagreb, Croatia

CORRESPONDING AUTHOR: itlak@vef.unizg.hr

Honey bees and hive matrices are increasingly recognised as sensitive bioindicators of environmental metal pollution. In this study, we quantified chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), and lead (Pb) in adult bees, pupae and brood combs collected from three apiaries in continental Croatia, which differ in levels of anthropogenic activities. The objective was to link environmental pollution gradients with honey bee life-stage specific exposure and the role of brood combs. Heavy metal concentrations were analysed using source-excited energy dispersive X-ray fluorescence (EDXRF), and the data were processed with descriptive statistics and regression models to clarify contributions from location and hive matrices. Across all three apiary locations, dark brood combs consistently exhibited the highest burdens of all investigated metals. Mean Cr concentrations in brood combs reached about 41.00–56.28 mg/kg, compared with roughly 1.23–4.43 mg/kg in adult bees and pupae, while Ni ranged around 12.17–17.83 mg/kg in combs versus about 1.17–1.61 mg/kg in bees ($p < 0.001$). Copper in combs was approximately 21.97–40.93 mg/kg, clearly exceeding values in adult bees and pupae (about 7.46–16.39 mg/kg), and zinc in combs peaked near 93.80–190.80 mg/kg, compared with roughly 66.95–87.52 mg/kg in adult bees ($p < 0.01$). Lead showed

a similarly pronounced pattern, with combs containing about 2.77–5.43 mg/kg, several-fold higher than the sub-mg/kg levels observed in adult bees and pupae (0.45–0.88 mg/kg; $p < 0.01$). Adult bees and pupae displayed lower, but clearly location-dependent concentrations, indicating that foraging activity and developmental physiology modulate metal uptake and retention, with significant differences between more and less polluted sites ($p < 0.05$). Multiple regression analysis identified matrix type as the dominant predictor for Cr, Ni, Cu, Zn and Pb (R up to 0.85; $p < 0.001$), while location remained an important covariate ($p < 0.01$). Targeted comb renewal and careful apiary location emerge as key management tools for mitigating metal exposure in honeybee colonies.

PP-27.

Development of mucoadhesive buccal film with propolis extract

Jelena Mudrić[1], Ioannis Nikolakakis[2], Ivana Kurćubić[3], Zorana Mutavski[1], Jelena Živković[1], Katarina Šavikin[1], Jelena Đuriš[3]

[1]Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade, Serbia
[2]Aristotle University of Thessaloniki, Faculty of Health Sciences, School of Pharmacy, Department of Pharmaceutical Technology, Thessaloniki, Greece
[3]University of Belgrade, Faculty of Pharmacy, Department of Pharmaceutical Technology and Cosmetology, Belgrade, Serbia

CORRESPONDING AUTHOR: *jmudric@mocbilja.rs*

Propolis has been widely used in traditional medicine for its ability to enhance the body's resistance to infections. In recent years, clinical studies have shown promising results for the use of propolis in dental and periodontal therapy. Consequently, this study aimed to develop mucoadhesive buccal films containing propolis extract with prolonged release of bioactive compounds. Buccal films were prepared using the solvent casting method with hyaluronic acid as the film-forming polymer. Hyaluronic acid (2.5%) was dissolved in water (95%) and left to stand for 48 h. Afterward, highly purified propylene glycol propolis extract (2.5%) was added, and the mixture was stirred for 30 min before casting and drying. The obtained films were evaluated for mass, thickness, mucoadhesive force, and mechanical properties. *In vitro* release studies were performed in 10 mL of preheated simulated salivary fluid. The content of chrysin (marker flavonoid) and ferulic acid (marker phenolic acid) was determined using a high-pressure liquid chromatography (HPLC) method. The prepared buccal films had a mass of 94.01 ± 7.62 mg and a thickness of 340 ± 40 μm, indicating that the thickness of the investigated

film was adequate. The mucoadhesive force was 8.86 ± 0.77 N, ensuring prolonged contact with the buccal mucosa. Mechanical properties, including tensile strength (3.15 ± 1.23 MPa), elongation at break (84.89 ± 28.96%), and Young's modulus (6.22 ± 0.49 MPa), were suitable for buccal administration. The release of chrysin (50% for 15 min; 77.05% after 300 min) and ferulic acid (32.16 % for 15 min; 93.50% after 300 min) from buccal films was prolonged compared to the rapid release observed from propolis extract. In conclusion, hyaluronic acid-based buccal films are a promising delivery system for propolis bioactive compounds, intended for use in dental and periodontal therapy. The films exhibited suitable thickness, strong mucoadhesive properties, adequate mechanical strength, and sustained release of chrysin and ferulic acid.

PP-28.

Assessment of bee bread processing waste as a novel protein source

Ceren Mutlu[1], Volkan Aylanc[1,2], Miguel Vilas-Boas[1]

[1]Instituto Politécnico de Bragança, CIMO, LA SusTEC, Bragança, Portugal
[2]Universidade do Porto, Faculdade de Ciências, Departamento de Química e Bioquímica, LAQV-REQUIMTE, Porto, Portugal

CORRESPONDING AUTHOR: *mvboas@ipb.pt*

Protein-based products are vital to a sustainable food system due to their fundamental role in human nutrition. However, current food production systems rely heavily on animal-derived proteins, and this dependence is becoming increasingly unsustainable in the context of population growth, environmental and ethical concerns, and growing awareness of healthy and sustainable nutrition. As a result, research into alternative and sustainable protein sources has accelerated. The beekeeping sector offers considerable potential as a source of alternative proteins, particularly through protein-rich products such as pollen and bee bread (20–25% protein). Furthermore, utilizing processing waste from protein-rich bee products offers a sustainable approach to developing circular economy practices and reducing environmental impacts. This study aimed to evaluate bee bread processing waste as a novel protein source. Proteins were extracted from defatted and ultrasonicated bee bread processing waste via alkaline extraction (pH 10) and isoelectric precipitation (pH 4.5), followed by freeze-drying. Protein isolate powder was characterized in terms of FTIR spectra, bulk density, water and oil binding capacities, foaming capacity and stability, and emulsion activity and stability properties. Overall, this study provides a foundation for transforming underutilized beekeeping by-products into safe, functional, and high-value protein components for next-generation food applications.

Acknowledgments: the authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). Thanks to the program *Promove* from Fundação "*La Caixa*", in collaboration with BPI and FCT, Portugal, within the scope of project BeeSustain, PD23-00019.


IHC
INTERNATIONAL HONEY COMMISSION


Sveučilište u Rijeci
University of Rijeka
MEDICINSKI FAKULTET - FACULTY OF MEDICINE
MEDRI


NASTAVNI ZAVOD ZA
JAVNO ZDRAVSTVO
DR. ANDRIJA ŠTAMPAR
Stvaramo zdraviju budućnost


NASTAVNI ZAVOD ZA
JAVNO ZDRAVSTVO
PRIMORSKO-GORANSKE ŽUPANIJE


upu
Associations of Primorje-Gorski Kotar County
Beekeepers Federation of Primorje-Gorski Kotar


nektar
udruga pčelara
POREČ - PARENZO


MINISTARSTVO POLJOPRIVREDE,
ŠUMARSTVA I RIBARSTVA


ISTARSKA
ŽUPANIJA
REGIONE
ISTRIANA


PLAVA LAGUNA


GRAD POREČ - PARENZO
CITTÀ DI POREČ - PARENZO


Croatian Beekeeping Federation


eba
European Beekeeping Association


HUSAM


imi


National
BEE
THE
NET
Centre
Croatia


CROATIA
Full of life

IHC

INTERNATIONAL
HONEY COMMISSION



ISBN 978-953-8341-80-9