

# LE STUDIUM WORKSHOP

VIRTUAL MEETING | 2020

## 3 December 2020

# Exploring the molecular diversity of grape, a source of natural ingredients

### LOCATION

VIRTUAL MEETING

### CONVENORS

**Dr Magdalena Malinowska**

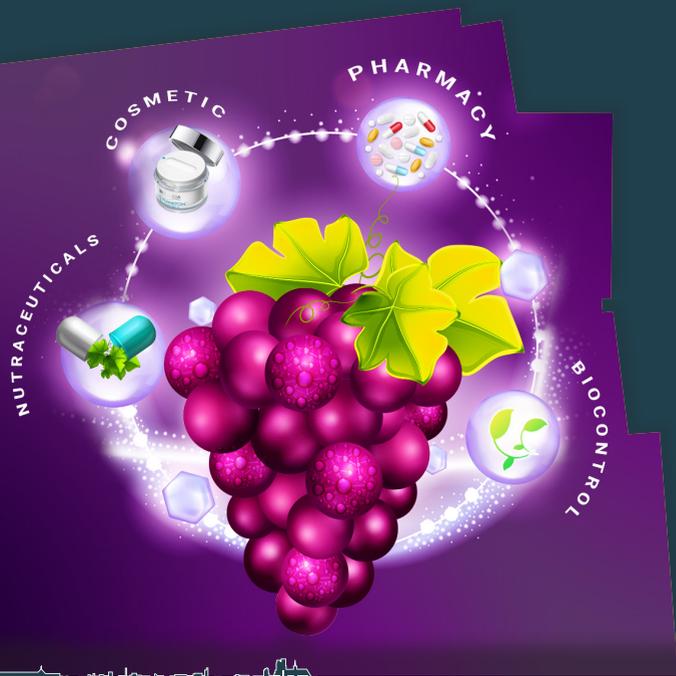
LE STUDIUM Guest Research Fellow / ARD  
2020 Cosmetosciences Programme

FROM Cracow University of Technology - PL

IN RESIDENCE AT Biomolecule and Plant  
Biotechnology (BBV), University of Tours - FR

**Dr Arnaud Lanoue**

Biomolecule and Plant Biotechnology (BBV),  
University of Tours - FR



## CONVENORS

**Dr Magdalena Malinowska**, LE STUDIUM GUEST RESEARCH FELLOW  
FROM Cracow University of Technology - PL  
IN RESIDENCE AT Biomolecule and Plant Biotechnology (BBV), University of Tours - FR

**Dr Arnaud Lanoue**,  
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## ORGANIZING COMMITTEE

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LE STUDIUM Loire Valley Institute for Advanced Studies • Région Centre-Val de Loire • FR

**LE STUDIUM**

# **WORKSHOP**

VIRTUAL MEETING | 2020

**PROGRAMME & ABSTRACTS**

## **Exploring the molecular diversity of grape, a source of natural ingredients**

# EDITO

Created in 1996 on the CNRS campus in Orleans La Source, LE STUDIUM has evolved to become a multidisciplinary Loire Valley Institute for Advanced Studies (IAS), operating in the region Centre-Val de Loire of France. LE STUDIUM has its headquarters in the city centre of Orleans in a newly renovated 17th century building. The amazing facilities are shared with the University of Orleans. In 2014 new developments and programmes linked to the smart specialisation of the Centre-Val de Loire region came to strengthen existing IAS cooperative relationships with the local and the international community of researchers, developers and innovators.

LE STUDIUM IAS offers to internationally competitive senior research scientists the opportunity to discover and work in one of the IAS's affiliate laboratories from the University of Tours, the University of Orleans, National Institute of Applied Sciences (INSA) Centre Val de Loire and ESAD Orléans, as well as of nationally accredited research institutions located in the region Centre-Val de Loire (BRGM, CEA, CNRS, INSERM, INRAE). Our goal is to develop and nurture trans-disciplinary approaches as innovative tools for addressing some of the key scientific, socio-economic and cultural questions of the 21st century. We also encourage researchers' interactions with industry via the IAS's links with Poles of Competitiveness, Clusters, Technopoles, and Chambers of Commerce etc.

LE STUDIUM has attracted over two hundred and twenty LE STUDIUM RESEARCH FELLOWS and LE STUDIUM RESEARCH PROFESSORS for long term residencies. In addition to the contribution in their host laboratories, researchers are required to participate in the scientific life of the IAS through attendance at monthly interdisciplinary meetings called LE STUDIUM THURSDAYS and gathering members of the regional scientific community and industries.

For the period 2015-2021, LE STUDIUM operates with an additional award from the European Commission in the framework of the Marie Skłodowska-Curie Actions (MSCA) with the programme MSCA-COFUND for the mobility of experienced researchers. LE STUDIUM is also the official partner of the

Ambition Research and Development 2020 (ARD 2020) initiated by the Region Centre-Val de Loire, that supports the specialisation strategy around 5 main axes: biopharmaceuticals, renewable energies, cosmetics, environmental metrology and natural and cultural heritage.

Researchers are also invited and supported by the IAS to organise, during their residency and in collaboration with their host laboratory, a two-day LE STUDIUM CONFERENCE. It provides them with the opportunity to invite internationally renowned researchers to a cross-disciplinary conference, on a topical issue, to examine progress, discuss future studies and strategies to stimulate advances and practical applications in the chosen field. The invited participants are expected to attend for the duration of the conference and contribute to the intellectual exchange. Past experience has shown that these conditions facilitate the development or extension of existing collaborations and enable the creation of productive new research networks.

The present LE STUDIUM WORKSHOP named «Exploring the molecular diversity of grape, a source of natural ingredients» is the 107th in a series started at the end of 2010 listed at the end of this booklet.

We thank you for your participation and wish you an interesting and intellectually stimulating conference. Also, we hope that scientific exchanges and interactions taking place during this conference will bring opportunities to start a productive professional relationship with presenting research laboratories and LE STUDIUM Loire Valley Institute for Advanced Studies.

**Yves-Michel GINOT**

Chairman  
LE STUDIUM



## INTRODUCTION

Grapevines and their by products produce a broad range of specialized metabolites, including flavonoid- and stilbenoid-type compounds, that exhibit diverse biological activities highly sought by biomass-based industries. Among the large molecular diversity present in grape, only few lead molecules are exploited. This is the case for resveratrol; currently exploited as food supplements and cosmetics ingredient for its anti-aging properties and for oligomeric pro-anthocyanidins (OPCs) that are used for several health purposes including the prevention of cardiac and Alzheimer diseases. Beyond these two well-known lead molecules, grapevine is a natural source for a myriad of other biomass-derived molecules that remains underexplored. The objective of the present workshop is to build-up a transversal platform for the development of novel grape biomass-derived chemicals with outputs in pharmacy, cosmetics and sustainable agriculture. Several aspects will be covered in a top-down approach such as impact of climate change on phytochemicals, green extraction technologies, comprehensive analytical tools and biological activities.

This LE STUDIUM Workshop is organised in the framework of the regional research COSMETOSCIENCES Programme

# Programme

**THURSDAY 3<sup>RD</sup> DECEMBER 2020**

08h30 Official opening - **Prof. Nathalie Giglioli-Guivarc'h** (Head of Biomolecule and Plant Biotechnology (BBV), University of Tours) & **Sophie Gabillet** (General Secretary of LE STUDIUM Loire Valley Institute for Advanced Studies)

09h00 **Prof Régis Gougeon**

Messages in a bottle: from oenolomics to the structural resolution of the transient chemistry of wine aging

09h40 **Dr Arnaud Lanoue**

Molecular diversity of grape explored with semi-targeted metabolomics

10h20 Coffee break

10h35 **Dr Viviana Martins**

Potential of vineyard calcium sprays to improve grape berry and wine quality

11h15 **Dr Paweł Siudem**

Aronia melanocarpa Fruits as a Dietary Source of bioactive compounds: 1H-NMR, HPLC-DAD, and Chemometric Studies

11h55 **Dr Magdalena Malinowska**

Grape cane extracts a natural ingredients with cosmetic activities

12h35 Lunch

13h30 **Dr Elżbieta Sikora**

Supercritical CO<sub>2</sub> plant extracts as raw materials in modern cosmetic formulations

14h10 **Prof Hernâni Gerós**

From the French Paradox to nanoencapsulation of wine bioactive compounds

14h50 Coffee break

15h05 **Dr Christophe Hano**

Screening for youth : from *in vitro* to *in vivo* assays with a particular emphasis on the contribution of baker yeast for anti-aging assays

15h45 **Dr Helena Kandarova**

In Vitro Phototoxicity Testing of Food Supplements and Cosmetics

16h25 General discussion

16h35 Meeting between speakers

# ARD 2020

## COSMETOSCIENCES PROGRAMME



In an international environment characterised by changing regulatory regimes and increasing harsh competition, research and innovation are key factors to ensure smart specialisation and sustainable economic development of territories and stakeholders. In the very well established perfume and cosmetic industry of region Centre-Val de Loire, the COSMETOSCIENCES programme aims at giving a significant impetus to research projects with a strong character of innovation to unlock industrial development blockages by opening the door to new concepts and enable new startups. It fosters French leadership in the sector and the leadership of the region Centre-Val de Loire, particularly with regard to sustainable cosmetics.

Anchored in the region Centre-Val de Loire, this project articulates around the structuring of research at the national level on this cosmetic theme, including through the research group (GDR) Cosmactifs, created by CNRS in January 2015. It brings 48 laboratories together and is driven by the University of Orléans. Focused on economic development, this project shares in the international influence of the French cosmetics industry across the region Centre-Val de Loire.

Together with the Cosmetic Valley competitiveness cluster and in conjunction with the cosmetic industry the programme creates the Centre of Expertise for the Cosmetics Industry. Located at the very heart of the territory covered by Cosmetic Valley, the centre's mission is to support business growth in the perfume and cosmetics sector with research, training and development activities and services specifically targeting very small and medium sized enterprises (VSEs and SMEs). The centre focuses on three complementary developmental axes:

1. Cosmétopée and Sustainable Cosmetics,
2. Glycochemistry and Glycobiology
3. Innovation in Formulation, Cellular Tools and Technologies.

**Cosmeto**  
ciences

*Recherche & Industrie, innovons ensemble*

# Abstracts

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## Dr Arnaud Lanoue

CONVENOR

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Dr. Arnaud Lanoue is a phytochemical analyst at University of Tours (France). He obtained a joint Ph.D. at the universities of Amiens (France) and Geneva (Switzerland) in 2002, where he studied the bioproduction of alkaloids in transgenic roots grown in bioreactors. He conducted postdoctoral research at the Juelich Research Center (Germany) on plant natural products as signaling molecules within the Biorhiz project (Marie Curie actions-Research Training Networks). He is currently Associate Professor in the Department of Pharmaceutical Sciences at the University of Tours. Since 2007, he participates and/or coordinates several research projects on plant bioactive molecules including polyphenols, alkaloids and triterpenoids. He has co-authored 60 peer-reviewed papers (h-index 20) with more than 1400 citations. He is supervising the analytical platform at Laboratory "Biomolecules and Plant Biotechnologies" where he developed metabolomics tools coupled to multivariate statistics to screen metabolotypes of plant biomass issued from natural resources and biotechnological processes for applications in agroecology, human health and cosmetic purposes.

### Molecular diversity of grape explored with semi-targeted metabolomics

Grapevine produces a myriad of bioactive phenolic compounds, with abundant health benefit and organoleptic properties that could be retrieved from grape fruits but also from different byproducts of viticulture including leaves and wood biomass. It is assumed that the polyphenol content in grape might change according to the cultivar but also according to environmental parameters including biotic stress and soil composition which is usually regarded as terroir effect. Considerable advances have been made in the chemical characterization of wines, particularly volatiles and polyphenolic compounds using advanced metabolomics tools. However the metabolotyping of grapevine organs, i.e. the characterization of metabolic phenotypes, is very limited. Analytical methods using Ultra High Performance Liquid Chromatography in tandem with Diode Array Detection and Mass Spectrometry in combination with chemometric analyses have been developed offering rapid and comprehensive metabolomic analyses of grape chemical composition. In this way, field-based metabolomics studies have been designed to finely grasp the question of metabolic variations depending on varietal diversity or difference in soil composition as well as following pathogen infection.

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Magdalena Malinowska works as an Assistant Professor at Cracow University of Technology (CUT), the Faculty of Chemical Engineering and Technology. She has the experience in organic synthesis, compounds purification and analysis. Her research is focused on the isolation, identification of a new natural active ingredients as well as their chemical modification for the development of novel active agents for the topical application in cosmetic and pharmaceutical industry. The new structures, screened for their bioavailability, skin permeability and toxicity are dedicated for skin care and regeneration. Dr Malinowska has also experience in the formulation and quality control of modern forms of skin care formulations.

### Grape cane extracts a natural ingredients with cosmetic activities

Modern and effective skin care formulations based on a natural ingredients has become the key issue in cosmetic industry. It is well known, that grapes are a rich source of many valuable compounds like E-resveratrol, which exhibits several biological activities for health and beauty. Wine production process generates various biomolecule-rich by-products including pomace, leaves or canes. It creates an opportunity to the exploitation of these natural resources of metabolites in cosmetic formulations. In our studies, we focused on the polyphenolic profiles of unique grape varieties, cultivated in the Loire Valley in France, which over the past decades became extremely rare. The metabolomic screening of the tested grape extracts involved the analysis and data integration of all the metabolites, based on high-throughput, multivariate data. Basing on the results obtained, we evaluated the cosmetic potential of the selected grape varieties as the multifunctional rejuvenating agents. These sources of grape metabolites exhibit various biological activities including skin whitening potential, strong antioxidant activity and the delaying of cellular senescence. The confirmed biological effect was additionally supplemented with the evaluation of the bioavailability and skin permeability of the extracts key metabolites. Importantly, the results allowed for the initiation of in vitro cell cultures of the selected rare grape varieties for their future utilisation in innovative skin care formulations.



## Prof Hernâni Gerós

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## From the French paradox to nanoencapsulation of wine bioactive compounds

Authors:

(Hernâni Gerós) Richard Breia, António Teixeira, Henrique Noronha, Viviana Martins, Artur Conde, Hernâni Gerós

The French paradox. In the 1990s, people's attention was increasingly drawn to the positive effects of moderate wine consumption. Although the French diet is relatively rich in saturated lipids compared to that of other countries, the level of mortality due to coronary heart disease is reduced as a result of daily wine consumption (Renaud and de Lorgeril 1992). Different epidemiological studies suggest that a moderate alcohol and/or wine consumption may protect against the incidence of many diseases of modern society, including cardiovascular disease, dietary cancers, diabetes, hypertension, ischemic stroke, among others. Key role of wine compounds as antioxidants. The particular case of resveratrol (RVS). Mechanism of RVS synthesis in plants in response to stress conditions. Metabolism of administrated RVS. Anticarcinogenic activity of RVS. Lipid based noncarriers for the delivery of RVS. Experimental strategies for the preparation of RVS-loaded nanocarriers (liposomes) and biophysical characterization. RVS-loaded nanocarriers are uptaken by endocytosis in yeast (Barbosa et al. 2019). Conclusions and perspectives.



## Prof. Régis Gougeon

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Régis Gougeon is Professor of Chemistry and Oenology at the University of Burgundy. He is deputy director of the research team on physical-chemistry of food and wine at the University of Burgundy / Agrosup Dijon. He is also co-Chairman of the Scientific board of the Bourgogne Vigne et Vin cluster, a public interest group whose objective is to promote coordination of research, teaching and transfer in the field of vine and wine at the scale of the Bourgogne-Franche-Comté territory. He is particularly interested in the chemistry of wine aging and of oenological interfaces (wine / cork, wine / wood, etc.), through the development of analytical tools for the exploration of the chemodiversity of wine, and its significance regarding environmental factors at work at the time of its elaboration.

### Messages in a bottle: from oenomics to the structural resolution of the transient chemistry of wine aging

Metabolomics applied to vine and wine sciences, has significantly developed over the last ten years. To that respect, high-resolution mass spectrometry has clearly shown unprecedented capability based on ultra-high resolving power and mass measurement accuracy. The introduction of the concept of oenomics, exemplified by the metabo-geography expression of cooperage oak wood in bottle-aged wines, further paved the way for breakthrough investigations of specific chemical fingerprints of wines, considered as transient molecular memories of vineyard-related environmental parameters and/or enological practices, which may have driven their original composition. We thus reported a remarkable and straightforward discrimination of wines according to either the level of SO<sub>2</sub> added to the must at pressing, or the type of stopper used at bottling, the latter being directly correlated to distinct oxygen ingresses during ageing. Through some examples, we will show how high-resolution MS can decipher the extent of the yet-unknown chemistry of wines or spirits. We will show in particular how sets of combined analytical strategies involving molecular and physico-chemical approaches can bring unprecedented molecular signatures of dry white wines antioxidant metabolome.



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Christophe Hano, completed his Ph.D. in 2005 in Plant Physiology, Biochemistry, and Molecular Biology, is currently Assistant Professor at the University of Orleans at Research INRAE Lab LBLGC USC1328 and a member of the Cosm'ACTIFS Research Group (CNRS GDR3711). His research career has focused on applied plant metabolism and plant biotechnology. He has written more than 100 scientific peer-review papers, reviews, and book chapters in internationally renowned journals, and he edited a variety of journal topical issues on plant secondary metabolism, including polyphenols as well as one book on the anti-aging action of plant polyphenols. He is the Assistant Editor and an Editorial Board Member of several renowned Q1 Journals in Plant Biochemistry and Biotechnology. Currently, he is developing research projects aimed at studying plant secondary metabolism to lead to the development of natural products with interests in pharmacology or cosmetics. His research focuses on the elucidation of biosynthetic mechanisms of plant natural products and their exploitation by metabolic engineering approaches.

### Screening for youth: from *in vitro* to *in vivo* assays with a particular emphasis on the contribution of baker yeast for anti-aging assays

Aging is a dynamic and complex biological process involving multiple actors and subject to a number of genetic and/or environmental influences. A variety of theories were suggested to explain the aging process, including the free radical theory of aging proposed by Prof. Harman in 1956. Undoubtedly, this theory was the most widely studied and continues to be revised, and so far, it remains a sound theory. The theory explains that aging can be caused by excessive oxidative stress.

Evidence that polyphenols such as resveratrol (RES) have prolonged the lifespan of different species, operating through a well-conserved mechanism, was first described in yeast and then confirmed in many other model species such as *Caenorhabditis elegans*, *Drosophila melanogaster* and mice. Yeast cells prove to be an excellent model for evaluating the *in vivo* antioxidant capacity of polyphenols in the context of cellular oxidative stress. It is also an attractive and stable eukaryotic model, whose mechanisms of defense and adaptation to oxidative stress are well established and can be extrapolated to human cells.

Sirtuins are a conserved family of nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent protein deacetylases, and interestingly some compelling evidence has linked their action to ROS and aging, in particular to the ROS-driven mitochondria-mediated hormetic response. In yeast, SIR2 (sirtuin-2) activation by RES has been proposed both gene expression level and enzyme activation. However, the exact mechanism accounting for the putative longevity effects of RES is still debated. Other potential anti-aging plant polyphenols and their mechanisms of action at the molecular level should be investigated. Such studies may provide important information for the use and development of anti-aging plant sources and derived compounds, and may reveal mechanisms to pave the way for anti-aging drug development.



### **Dr Helena Kandarova**

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Dr. Helena Kandarova, ERT is a Senior scientist at CEM, SAS and Assistant professor at the IBM, FCHPT STU in Bratislava. Before joining academia, Dr. Kandarova held a position of Senior Scientist and General Acting Manager for EU at MatTek Corporation (USA) for 13 years. She established MatTek In Vitro Life Science Laboratories and led the company in the position of Executive Director between 2009 - 2018. Dr. Kandarova is president of the ESTIV, Vice-president of SETOX and Chair of the Slovak National Platform for 3Rs (SNP3Rs). She has been involved in many international projects aiming at the validation of 3D reconstructed human tissue models for topical toxicity and phototoxicity testing of chemicals, cosmetics, pesticides and medical devices. She co-authored of over 50 papers, 7 book chapters (H-index 19, over 1000 citations) and several protocols adopted into the OECD and ISO guidelines.

### **In Vitro Phototoxicity Testing of Food Supplements and Cosmetics**

A crucial step in the safety assessment of cosmetics, pesticides, food and pharmaceuticals that are absorbing UV and visible light is the evaluation of their photonic potential and potency. The validated and regulatory accepted in vitro phototoxicity assay, the 3T3 NRU PT (OECD TG 432), provides a high level of sensitivity and thus also protection for the end-users. However, it has been reported that the assay also generates a high rate of false-positive results due to the lack of barrier properties naturally appearing in the human skin or other targeted tissues. In vitro reconstituted human skin models (RHSM) - pre-validated for phototoxicity testing almost 20 years ago - are increasingly being used in the hazard identification and safety testing of cosmetics. This is due to their organotypic structure with a functional stratum corneum that allows for assessment of the bioavailability of topically applied compounds and mixtures. They are realistically modelling the exposure conditions. RHSM can be used either as a stand-alone methods or in combination with the 3T3 NRU PT (OECD TG 432) and ROS Assay (OECD TG 495), to minimize and/or correct the false positives obtained from the latter mentioned tests. In this way, they help to maintain high sensitivity but also ensure relevance to human response for topically tested compounds. The presentation will describe the in vitro approaches towards in vitro phototoxicity testing of food supplements and cosmetics and will show on several practical examples the successfully applied testing strategies.



## Dr Viviana Martins

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## Potential of vineyard calcium sprays to improve grape berry and wine quality

Calcium (Ca) supplements have increasingly been used for improving fruit resistance to abiotic stress and shelf life, but little was known about the effects of Ca on grape berry quality. Following the demonstration that Ca sprays increased grape berry firmness and cell wall pectin content [1], we hypothesized that exogenous Ca also influenced grape berry polyphenolic composition, given the central role of Ca as secondary messenger. Targeted UPLC-MS analysis was performed in fruits collected from vines cv. "Vinhão" sprayed with 2% (w/v) CaCl<sub>2</sub> throughout the fruiting season, in two consecutive vintages. Results showed that Ca deflected grape berry metabolism towards the production of more stilbenoids and less anthocyanins, in line with the expression patterns of UFGT- and STS-encoding genes [2]. The volatile profile of wines produced from these berries was analysed by GC-FID and SPE/GC-Ion Trap-MS, following vinifications at laboratory scale. Results showed that Ca shifts wine volatile profile, affecting compounds associated to both varietal and fermentative aromas [3].

[1] DOI: [10.1016/j.plaphy.2020.02.033](https://doi.org/10.1016/j.plaphy.2020.02.033)

[2] DOI: [10.1016/j.foodchem.2019.126123](https://doi.org/10.1016/j.foodchem.2019.126123)

[3] DOI: [10.1016/j.foodres.2020.108983](https://doi.org/10.1016/j.foodres.2020.108983)

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## Supercritical CO<sub>2</sub> plant extracts as raw materials in modern cosmetic formulations

New products containing active substances from the plants are systematically appearing on the cosmetics market. Among others natural products, based on raw materials that meet the standards set for natural and organic cosmetics (COSMOS), are becoming more and more popular.

The extracts obtained under supercritical CO<sub>2</sub> conditions (SC-CO<sub>2</sub>) are an interesting alternative to plant extracts obtained by traditional methods. The parameters of the SC-CO<sub>2</sub> process allow to obtain extracts of very high purity, free from contamination with conventional solvents. Additionally, relatively low temperatures and anaerobic atmosphere during the process, avoid oxidation and thermal decomposition of the bioactive components contained in the plant extracts, such as: triglycerides of essential fatty acids (EFAs), phospholipids, tocopherols, carotenoids, phytosterols or phytohormones.

The influence of selected extracts, obtained under supercritical CO<sub>2</sub> conditions, on the physicochemical and user properties of cleansing products and mature skin care emulsions will be discussed. The moisturizing effect of berry fruit seed extracts (blackcurrant, strawberry, blackberry and chokeberry), as well as antibacterial and astringent properties of the hops extract will be described. Moreover, the results of studies on obtaining effective cosmetic formulations, such as microemulsions, nanoemulsions or lipid nanoparticles will be presented.



### Dr Pawel Siudem

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Pawel Siudem, is a pharmacist and scientist working at the Department of Physical Chemistry of the Medical University of Warsaw. He is a part of the team, which is focused on the structural and physicochemical analysis of natural products. In 2020 has defended his PhD thesis entitled Structural and physicochemical analysis of the selected TRPV1 receptor. His scientific interest include NMR, theoretical calculations and chemometric methods in the analysis of the bioactive compounds.

### Aronia melanocarpa Fruits as a Dietary Source of bioactive compounds: 1H NMR, HPLC-DAD, and Chemometric Studies

*Aronia melanocarpa* (Michx.) Elliott's (chokeberry) it is well known for the presence of anthocyanins. The fruits also contain significant amounts of other bioactive compounds, such as chlorogenic acid and its isomer neochlorogenic acid. They exhibit antioxidant, anti-inflammatory, antidiabetic, and antibacterial activities; thus, they can have a significant impact on the health-promoting properties of chokeberry. In our research, we have determined the changes in the content of chlorogenic acids (CGA) and anthocyanins during the development and ripening of fruits. Aronia fruit samples from two organic farms in Poland, were collected every two weeks from July to October. The methods of NMR spectroscopy and HPLC-DAD were used to determine the chemical composition of the prepared methanolic extracts. The obtained results were analyzed using chemometric analysis and multivariate statistics (PCA). The results showed that the content of chlorogenic acids and anthocyanins changes during ripening and depends on the time of harvest and the region of cultivation. A correlation between the time of CGAs reduction and the appearance of anthocyanins was also noticed. Additionally, the antioxidant properties of extracts were determined using three methods: DPPH, FRAP and ORAC.

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**New avenues for the behavioral manipulation of disease vectors**

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10-12 June 2010

## CONTACT

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## LE STUDIUM

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