# APPLIED AGRO -BIO TECH NOLOGY International Meeting

NEW APPROACHES FOR A QUALITATIVE VALORIZATION OF THE OLIVE OIL SECTOR

30-31 MAY 2023

**BEJA**, PORTUGAL

**BOOK OF ABSTRACTS** 









**KEYNOTE SPEAKER** 



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### **KEYWORDS**

Sustainable Intensification Indicators Complexity Uncertainty Landscape Discourses

### SUSTAINABILITY OF OLIVE GROVES AND OLIVE OIL IN ALENTEJO - WHAT DO INDICATORS TELL US?.

Planting of olive groves and production of olive oil have exponentially increased in Alentejo over the past 20 years, becoming the prime producer in Portugal. This has been underpinned by a rapid process of agro-technological intensification and global market strategies. Contrary to some discourses, more surface planted with olive trees could be found in Portugal over 50 years ago than today. Despite of this, it is self-evident that the current model of financialization and intensification in the sector is unprecedented and impactful, and thus is fairly raising concerns about its current and future sustainability. In response to such a complex and largely uncertain situation, multiple discourses are arising in the public sphere. Each of these discourses seems to over-simplify an intrinsically complex and heterogeneous reality. To respond to the main sustainability challenges and concerns arising, SMART indicators are crucial. It is in this context that over the past 4 years we have been developing, testing and monitoring sustainability indicators for olive grove cultivation and olive oil production in diverse types of olive groves, including super-intensive to extensive, and biodynamic to conventional. Aspects monitored at scales from the plot to the farm include integrated pest management, C sequestration and CO2 storage, nutrient circulation, Life-Cycle and Social-Metabolism, climate change mitigation, water quality and demand, soil functionality and erosion, and olive oil quality, and the effects on ach of these of sustainable technological solutions. Results obtained bring up a series of key conclusions synthesized in the following key-points: a) specific land-management actions, rather than olive grove densities, affect the sustainability of olive groves, b) tradeoffs between diverse sustainability indicators are unavoidable, and diverge across olive grove types c) although a farm-based assessment of sustainability indicators is crucial, this must necessarily be upscaled to the landscape level, so that it can inform more effective strategies and instruments towards achieving regional and national sustainability goals.

### Acknowledgement and Funding

This work was funded by the SUSTAINOLIVE project (Grant nº 1822; PRIMA EU Programme), and by Project UIDB/05183/2020. SP and CB acknowledge the CEEC contracts from FCT (CEECIND/01473/2020 and CEECIND/00093/2018, respectively). AARS, acknowledges a Margarita Salas Postdoctoral Contract for the Training of Young PhDs funded by UCM through the Ministry of Universities, Government of Spain and the European Union – NextGenerationEU.



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### **KEYWORDS**

Sustainability, Circular Economy, Olive Oil Sector, Waste and Surplus Valorization

### SUSTAINABILITY OF OLIVE OIL CHAIN IN THE FRAME OF THE PSAA (ALENTEJO OLIVE OIL SUSTAINABILITY PROGRAM)

The Alentejo Olive Oil Sustainability Program (PSAA) it is an project (ALT20-01-0853-FEDER-000113) which results from a partnership between Olivum - Association of Olive Growers and Mills of Portugal and the University of Évora. It aims to reinforce the image and value of olive oils from Alentejo region. For this, it is extremely important to offer olive oils produced in a sustainable way in their environmental, social and economic dimensions. This involves supporting economic agents in improving the sustainable performance of the olive oil sector, recognizing this improvement. Hence, building a referential with a simple and transparent formulation, which has regional coverage and meets intra-regional variability and is inclusive, universal and equitable, with applicability to all organizations in the sector in Alentejo, regardless of their size and degree of specialization, it is fundamental. The referential has two valences. One strategic, as a planning tool and work guide, and the other, for valuing and differentiating olive oil in the markets, by objectively contributing to sustainability certification in a credible way and with a continuous improvement management model adjusted to standards and typologies existing nationally and internationally. Thus, the PSAA integrates 11 primary intervention chapters and 9 secondary intervention chapters. The PSAA Referential is thus a practical guide to help economic agents in the olive sector to promote and apply the general principles of sustainability, in the environmental, economic and social dimensions, including cultural and nutritional heritage. All these dimensions are contained and respected in the organization and implementation of the PSAA. It is aimed essentially at organizations directly involved in the production of olive products, from olives (table and olive oil) to olive oil.



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#### **KEYWORDS**

olive viruses antimicrobial peptides virus-induced gene silencing plant protection

# OLIVE VIRUSES: PLANT PATHOGENS OR PLANT PROTECT AGENTS?

Harmful symptoms in olive trees such as chlorotic lesions, defoliation, fruit and leaf deformation, stem and tree death, and poor olive oil quality are currently associated with viruses. In Portugal, virus affecting olive trees have been studied since the 90's, and to date eleven virus have been detected in Portuguese olive orchards, out of a total of 17 detected worldwide in this host. Viral diseases are not treatable in an agronomic context and knowledge of these agents, and their epidemiology, is mandatory to establish and improve sanitary regulations and early detection tools. In dozens of olive orchards sampled in the north and south of Portugal, infection rates reach up to 100%. In these surveys, virus belonging to the Alpha- and Beta-necrovirus genera were the most prevalent. Viral genomes were molecular characterized, and their replication strategies have been studied, allowing the design of molecular tools for accurate diagnosis of olive viruses. Despite their harmful effects, viruses have the ability to move inside the plants and can be useful vectors to be developed for general biotechnology. The well-known viral genomes of necroviruses, mainly in the context of their pathogenic interactions with host plants, led us to design vectors for plant protection use i) based on virus-induced gene silencing (VIGS), using the natural plant gene silencing strategy to protect plants against viruses; ii) based on the expression of antimicrobial peptides (AMPs), using the ability of virus genome replication to produce antibiotics and antifungal molecules to prevent diseases caused by bacteria and/ or fungi; and iii) based on CRISPR technology for gene editing. The presence of these viral vectors in plants confers them protection against specific untreatable or harmful diseases, leading us to reduce chemical treatments and to increase production.

### Acknowledgement and Funding

M. Patanita and J.A. Ribeiro are supported by Portuguese National Funds through FCT/ MCTES under the PhD scholarships (SFRH/BD/145321/2019 and 2022.13638.BD, respectively). This work is also funded by National Funds through FCT under the Project UIDB/05183/2020. PANEL 2 - OLIVE AND OLIVE OIL CHEMISTRY

# **KEYNOTE SPEAKER**



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### **KEYWORDS**

adulteration health benefits Mediterranean diet Olea europaea L.

### OLIVES AND OLIVE OIL: INFLUENCE OF THE CHEMICAL COMPOSITION ON QUALITY AND AUTHENTICITY EVALUATION

Olives and olive oil are typical foods of the Mediterranean diet, strongly related to beneficial effects on the health of their consumers. These effects result from the presence of specific chemical compounds in their composition, determined not only by the cultivar/variety (genetically) but also by the edaphoclimatic conditions of the region of origin, type of processing and storage of the final product.

Given the importance of these products in the diet and economy of producing countries (mainly in the Mediterranean basin), there are quality parameters that must be respected in order to market them. The high economic value of olive oil makes it very susceptible to adulteration. Therefore, some parameters used to assess possible fraud will also be discussed.

#### Acknowledgement and Funding

This work has received financial support from Portuguese National Funds (Fundação para a Ciência e Tecnologia e Ministério da Ciência, Tecnologia e Ensino Superior, FCT/MCTES) through project UIDB/50006/2020, AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by European Regional Development Fund (ERDF) through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020) and SYSTEMIC "an integrated approach to the challenge of sustainable food systems: adaptive and mitigatory strategies to address climate change and malnutrition", Knowledge hub on Nutrition and Food Security, funded by national research funding parties in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (no. 696295)

### **PANEL 2 - OLIVE AND OLIVE OIL CHEMISTRY**

# **INVITED SPEAKER**



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### CENTENARIAN OLIVE TREES IN THE CÔA VALLEY REGION: AN OLIVE GROWING HERITAGE TO BE PRESERVED

The Côa Valley region is known for its important archaeological heritage, but there is also a rich plant heritage of which the olive tree is a part. In recent decades, the productive structure of the region was changed with more intensive systems. Nevertheless, a considerable number of centenarian olive groves exist and need to be characterized, to protect the genetic heritage to be valorised. In order to identify and characterize the olive heritage well-adapted to the continental Mediterranean conditions of the Côa Valley, the OliveCoa project aims to survey, identify and characterize the centenarian olive trees in the region to proceed with their valorisation. In this sense, a total of 150 centenarian olive trees were selected, where each plant was characterized in terms of diameter at breast height, height, crown diameter and diversity, with its leaves, fruits and endocarps being morphologically characterized; fruits were collected for the preparation of table olives and oil extraction and their physicochemical and organoleptic characteristics were evaluated, and leaves were collected for the quantification of their bioactive compounds. The information obtained will be used to develop itineraries of centuriesold olive trees and to develop a range of differentiated products that aim to enhance the region's olive trees in an integral way, together with local agents.

### Acknowledgement and Funding

The authors would like to thank the Foundation for Science and Technology (FCT, Portugal) financial support from national FCT/MCTES funds to CIMO (UIDB/00690/2020), and SusTEC (LA/P/0007/2020); to the Project "OLIVECOA – Centenary Olive Trees in the Côa Valley Region: rediscovering the past to value the future" (ref. COA/BRB/0035/2019). Nuno Rodrigues thanks the funding FCT- Foundation for Science and Technology, P.I., through the program contract institutional scientific employment.

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### **KEYWORDS**

ancient olive trees heritage genetical diversity olive oil characterization olive tree valorisation **PANEL 2 - OLIVE AND OLIVE OIL CHEMISTRY** 

# **INVITED SPEAKER**



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### UNLOCKING THE POTENTIAL OF PORTUGUESE EVOO REGARDING THE HEALTH CLAIM ON PHENOLIC COMPOUNDS

The research on extra virgin olive oil (EVOO) has brought forth compelling scientific evidence regarding its beneficial properties for human health. Extensive studies have investigated factors influencing its quality, yield, sensory attributes, and potential threats arising from climate change. However, the consumer knowledge and recognition on olive oil health claims remains largely unexplored, with nutritional attributes largely shaped by cultural trends.

One pivotal aspect that sets olive oil apart from other commercial vegetable oils is its phenolic composition and the associated health claim. However, the complete realization of this health claim in Portuguese samples has yet to be achieved.

Our focus over the past decade has been on evaluating the quality of Portuguese EVOO, particularly from the northern regions, with an emphasis on factors contributing to its maximization. We have also conducted an extensive national survey on Portuguese commercial EVOO recently. Additionally, recognizing the complexity surrounding olive oil phenolics and their analysis, prompted us to optimize simplified, consistent, and affordable analytical methods. These methods aim to make the analysis to support the health claim accessible to most analytical labs, thereby supporting a national chain of services to aid producers in realizing the potential of this health claim.

In this communication, we will highlight the potential of Portuguese olive oils in attaining this health claim and outline some of the steps necessary to convert it into a viable source of economic revenue for producers.

### **KEYWORDS**

olive oil phenolic compounds analytical methods survey

### Acknowledgement and Funding

The authors are grateful to FCT for financial support from national funds FCT/ MCTES to REQUIMTE-LAQV (UIDB/50006/2020), CIMO (UIDB/00690/2020), SusTEC (LA/P/0007/2020), to project COA/BRB/0035/2019, and the researchers Nuno Rodrigues and Rebeca Cruz through the institutional scientific employment program contract. Funding from Project AgriFood XXI, (NORTE-01-0145-FEDER-000041), co-financed by FEDER through NORTE 2020) is also acknowledge.

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#### **KEYWORDS**

biorefinery bioenergy biofuels bioproducts extraction

### VALORIZATION OF OLIVE-DERIVED BIOMASSES: A PORTFOLIO OF PROCESSES AND BIOPRODUCTS WITHIN BIOREFINERIES

The sustainable exploitation of biomasses from the olive oil sector is key to fulfilling circular bioeconomy principles. Particularly, in Spain, the olive oil sector and their associated industries generate prunings, olive pomace, olive stone, residual olive pulp, exhausted olive pomace (deoiled or dry olive pomace), and wastewater. Most of the olive-derived biomasses are typically burned as low-value biofuels. However, these biomasses can be reinserted into the economy as new bioresources of multiproduct biorefineries to obtain bioactive compounds, bioenergy, biofuels, and/or other value-added chemicals. This work will review the chemical composition of these biomasses, which is crucial to be considered in biorefinery cascading schemes to take full advantage of their chemical composition and select the best valorization route. It will exemplify biorefinery processes based on olive-derived biomasses and the bioproducts obtained. The implementation of biorefineries could represent a step towards a decarbonized, circular, and more sustainable bioeconomy and support the UN's sustainable development goals (SDGs) such as SDG 7 ("Affordable and clean energy"), SDG 12 ("Responsible Consumption and Production"), and SDG 13 ("Climate action").

### Acknowledgement and Funding

M.d.M.C. appreciate the Ramón y Cajal grant (RYC2020-030546-I/ AEI / 10.13039/501100011033) and the European Social Fund. The grant TED2021-132614A-I00 funded by MCIN/AEI/ 10.13039/501100011033 by the European Union NextGenerationEU/PRTR is also acknowledged.



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### **KEYWORDS**

Biorefinery by-product bioactives phenolics energy

### BIOACTIVE COMPOUNDS AS THE KEY TO A REAL BIOREFINERY OF OLIVE OIL BY-PRODUCTS

The evolution in the olive oil extraction system, from three to two phases, has reduced the volume of wastes, but has not reduced its high phytotoxic load, that has avoided the application of bioprocesses, being the combustion the only alternative after extraction of the pomace oil. These components have also a high biological activity. To recover then thermal treatments are needed. These treatments are accompanied by the application of a three-phase system in the pomace extractors, obtaining pomace oil enriched in minority components, a less humid solid with fewer phenolics, and a liquid fraction rich in these bioactive compounds. After the phenolic extraction the liquid is rich in sugars and free of phytotoxins which, like the solid fraction, that can now be applied to a bioprocess for its full use. That makes possible the application of anaerobic digestion to obtain energy (methane) and a substrate to obtain fertiliser or compost. It can also be saccharified to obtain bioethanol or it can be directly composted or used for animal feed. Once recovered, phenolics are used in different types of industry (food, cosmetics, nutraceuticals, pharmaceuticals or in agriculture). Thus, the use of simple treatments enables the extraction of the most important bioactive components from the olive, detoxifying the rest of the fractions and making possible a real biorefinery of the main byproduct of olive oil extraction.

#### **Acknowledgement and Funding**

Part of this research was supported by the incentives of the Andalusian knowledge system, aid under the Andalusian research, development and innovation plan (PAIDI 2020) (project P18-TP-616).



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#### **KEYWORDS**

biorefinery bioenergy biofuels bioproducts extraction

### LIFE BIOAS PROJECT: A NEW APPROACH TO THE CIRCULARITY IN THE OLIVE OIL SECTOR

The BIOAs is a LIFE project aiming to demonstrate the environmental and economic feasibility of a process for the production of an innovative bio-adsorbent and, simultaneously, its use for the purification of drinking water from arsenic and other pollutants. The focus of the project will be the demonstration of a process to produce an innovative bio-adsorbent by the hydrothermal carbonization of the olive pomace (a byproduct of the olive oil production industry) at a cost at least 50% lower, as compared to the conventional employed adsorbent GFH (Granular ferric hydroxide) and, simultaneously, its use for the water purification in line with the EU Directive of drinking water from arsenic.

The LIFE BIOAs project involves six partners in Italy and Portugal.

The planned actions of the LIFE BIOAs project will contribute to the implementation of the 6, 9 and 12 ONU objectives for a Sustainable Development for the Agenda 2030.

#### Acknowledgement and Funding

- LIFE Programme of the European Union for funding the LIFE BIOAs project (LIFE19ENV/ IT/000512).

- National Funds through FCT/MCTES from MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Research Centres.



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### KEYWORDS

biomass bioenergy by-products gasification wet olive pomace

### VALORIZATION OF BY-PRODUCTS OF THE OLIVE OIL INDUSTRY THROUGH GASIFICATION TECHNOLOGY: AN EXAMPLE OF CIRCULAR BIO-ECONOMY

The olive grove and its industries, mainly olive mills and olive pomace oil extractors, generate a significant volume of by-products which can be valued to generate bioenergy and bio-products, with high added value for the olive sector as a whole and under circular economy models.

The main by-products generated by the olive sector are the following: pruning in the olive grove exploitations (wood chips), the olive pits and the wet olive pomace in the olive mills and the dry olive pomace in the extractors.

The most important of all is the wet olive pomace, which accounts for around 80% by weight of the olive and whose main destination is to obtain crude olive pomace oil in the extracting industries.

However, due to its high humidity (> 70%) and its low olive oil residual and pits content, it is becoming a problem for the mill-extractor pairing. There are currently several alternatives for its recovery and use, such as composting or anaerobic digestion to obtain biogas and biomethane.

However, gasification technology is a very interesting option to generate thermal and electrical energy in "self-consumption" mode for the industries themselves. In addition, a high added value co-product is obtained, biochar, which can be used as a fertilizer or soil improver, due to its high porosity and water retention capacity, as well as being a true CO2 sink.

An example is the company Aceites Guadalentín, S.L. in the municipality of Pozo Alcón, Jaén, in the south of Spain (Andalusia region), which has installed a gasification system for dry olive pomace that allows it to generate electrical and thermal energy that it demands for its production process.

# PANEL 1

SUSTAINABILITY, ECOLOGY AND CROP SANITY



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### **KEYWORDS**

dispersal, Neophilaenus campestres, Philaenus tesselatus, spontaneous vegetation, sweeping technique PANEL 1 - SUSTAINABILITY, ECOLOGY AND CROP SANITY

### MONITORING *XYLELLA FASTIDIOSA* WELLS *ET AL.* POTENTIAL VECTORS, IN SEVERAL OLIVE ORCHARDS IN BEJA

*Xylella fastidiosa* is a phytopathogenic bacteria present in several European Union countries, like Italy, France, Spain and Portugal. The dispersal risk for *Xylella fastidiosa* is considered high in Portugal, because of the inter-regional circulation of infected propagation material, and the presence of potential vectors, that feed on the xylem of infected plants.

*Xylella fastidiosa* infection in olive trees is associated with symptoms as twig and branch desiccation, leaf fall and fast tree decline. In Alentejo, olive orchards have a vital importance for the economic tissue, representing at present up to 199 803 ha. This study aimed the monitoring of *Xyllela fastidiosa* potential vectors in several olive orchards in Baixo Alentejo.

Six full-productive olive orchards cultivar Galega were selected, in the Beja region, and insects were captured between April and May 2022, through sweeping techniques. All the insects collected were identified by order, and the potential *Xylella fastidiosa* vectors were separated, photographed, and taxonomically identified.

Two *Xylella fastidiosa* vector species were identified, namely *Philaenus tesselatus* and *Neophilaenus campestris*. *Euscellis* spp. specimens were also identified as potential vectors. A large number of insects were captured in olive orchards covered with spontaneous vegetation, contrasting with the low number captured in herbicide strip treated orchards.

It would be interesting to expand the *Xylella fastidiosa* potential vectors monitoring period to follow the annual variability, as well as each species' importance and distribution in different types of spontaneous or sowed vegetation.

### Acknowledgement and Funding

This work is a contribution to the project GeoBioTec UIDB/GEO/04035/2020, funded by Fundação para a Ciência e a Tecnologia (FCT).



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### **KEYWORDS**

biodiversity; circular economy; ecosystem management, soil and water conservation PANEL 1 - SUSTAINABILITY, ECOLOGY AND CROP SANITY

### PROMOTION OF ECOSYSTEM SERVICES ASSOCIATED WITH OLIVE ORCHARD IN ALENTEJO

Biodiversity plays a major role in providing critical ecosystem services. In olive orchards, in addition to production services (olive and oil production), we must also consider regulation services (habitat maintenance, pest and disease control), support services (soil formation and nutrient cycle) and recreational services (tourism, recreation and culture).

Identification and promotion of the best methods to promote the ecosystem services in olive farming systems are the key goals of this work.

The challenge that the olive sector is facing now, of ensuring its sustainability, goes a long way toward the conservation and promotion of biodiversity in this ecosystem.

The sustainability of the territory will be significantly improved by the preservation and development of the services this agroecosystem can offer.

### Acknowledgement and Funding

This work is a contribution to the project GeoBioTec UIDB/GEO/04035/2020, funded by Fundação para a Ciência e a Tecnologia (FCT).



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### **KEYWORDS**

metabarcoding, microbiota, ngs, olive tree, rhizosphere

### RHIZOBACTERIAL COMMUNITIES ON PORTUGUESE TRADITIONAL OLEA EUROPAEA CULTIVARS COBRANÇOSA AND NEGRINHA DO FREIXO

The cultivated Olea europaea L., or olive tree, is a widely cultivated crop with significant economic value in Mediterranean countries, particularly Portugal. The rhizosphere microbiome, comprising microorganisms in the soil surrounding the plant roots, is well recognized for its role in plant health by facilitating growth, disease resistance, and nutrient uptake. This study investigated the prokaryotic communities associated with the rhizosphere of two traditional organic orchards located in the Douro Valley, corresponding to two Portuguese cultivars - Cobrançosa and Negrinha do Freixo. Using high-throughput sequencing and physicochemical soil parameters, we characterized the microbial communities associated with the rhizosphere of these cultivars and analyzed relations with abiotic soil factors in two sampling areas. Higher prokaryotic richness and diversity characterized bacterial rhizosphere communities in the Negrinha orchard, where soil presented high organic matter content and water-holding capacity. In contrast, soils of the Cobrançosa orchard presented higher pH and electric conductivity. SIMPER test identified the 28 amplicon sequence variants (ASVs) most important to the total dissimilarity of prokaryotic communities associated with these two cultivars. Canonical correspondence analysis (CCA) correlated ASVs belonging to Bacillus, Gaiella, Acidothermus, Bradyrhizobium, and uncultured ASV from Xanthobacteraceae family to the Negrinha cultivar, and Streptomyces and Sphingomonas to the Cobrançosa cultivar. Previous studies have shown the link between some of these genera and high carbon-to-nitrogen ratios in soils or plant-growth-promoting effects. Our results confirm that the structure of rhizosphere bacterial communities is shaped by plant- and soil-related factors. These results contribute to a better understanding of the interactions between biotic (cultivar) and abiotic factors (soil parameters) acting on the rhizosphere of Olea europaea.

### **Acknowledgement and Funding**

This work was funded by FCT through project Halius (PTDC/BIA-MIC/3157/2020) and CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) and GEOBIOTEC (UID/GEO/04035/2020). The authors are grateful to CARB-Casa Agrícola Rui Batel for the access to the olive orchards.



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### **KEYWORDS**

cover crops, herbicides; biodiversity, soil management, sustainability

### WHAT KEY FACTORS AFFECT SOIL BIODIVERSITY IN THE OLIVE GROVES OF ALENTEJO (PORTUGAL)?

Rural landscapes in Alentejo (S Portugal) have changed greatly along the past 15 years, largely due to the expansion and intensification of olive groves, and lately also of almond groves. This process of intensification (i.e., increase in olive tree density), if managed inadequately, may compromise the quality, biodiversity, habitat and performance of soils. It is, therefore, essential to foster and enact the sustainable intensification of agricultural production to avoid jeopardizing crop production and soil use in the future. Monitoring ecological functions and soil biodiversity will allow to determine how the sustainability of olive groves is impacted by different agricultural management options and decisions.

In this work, we hypothesize that different management practices, such as herbaceous cover crops, tillage or use of herbicides, can significantly affect soil biodiversity in olive groves. We tested this hypothesis in six distinct olive groves and four adjacent "montados" across Alentejo. The soil microbial, mesofauna and insect diversity was assessed for different: i) olive management practices, ii) land use mosaics (montado, intensive and superintensive olive groves) and iii) olive grove microsites (tree row vs interrow). Three composite soil samples were collected from each site and processed for amplicon sequencing targeting the 16S rRNA gene for bacteria and the Internal transcribed spacer (ITS) for fungi. Soil mesofauna diversity was assessed using the Berlese-Tullgren method in three soil samples from each test site, and insects' diversity was assessed within three pitfall traps in each test site. Preliminary results confirm that implementing cover crops instead of tillage or herbicides can significantly increase microbial abundance, soil mesofauna (mites) and insect diversity (ants and coleoptera) in olive groves regardless their land uses. The results of this work will contribute to defining which management practices best preserve biodiversity and soil functionality, thus ensuring the sustainability of olive groves.

### **Acknowledgement and Funding**

This work was funded by the SUSTAINOLIVE project (PRIMA EU Programme) and by Project UIDB/05183/2020. SP and CB acknowledge the CEEC contracts from FCT (CEECIND/01473/2020 and CEECIND/00093/2018, respectively), AARS acknowledges a Margarita Salas Postdoctoral Contract for the Training of Young PhDs funded by UCM through the Ministry of Universities, Government of Spain and the European Union – NextGenerationEU.



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cover crops, herbicides; biodiversity, soil management, sustainability PANEL 1 - SUSTAINABILITY, ECOLOGY AND CROP SANITY

### CARBON BALANCE IN OLIVE GROVES OF ALENTEJO UNDER DIFFERENT LAND MANAGEMENT PRACTICES.

Rural landscapes in Alentejo (Portugal) have changed greatly during the last 15 years, largely due to the expansion and technological intensification of olive groves. The application of agrochemicals (fertilizers and herbicides) along with crop intensification (increasing tree density) likely affect the C balance of these groves.

Hereby we assess the C contents of different farming system components and flows affecting the farm, tree and soil C balances during one hydrologic year. Seven olive groves were selected with different soil management practices and production models, including organic vs integrated, as well as contrasting tree densities, 100, 300 and more than 1000 trees per hectare. The annual C inflows comprise the C which accumulates annually in the trees, olive leaves/flowers, tree pruning, olive fruits and cover crop biomass, and also the inflow due to organic fertilizer applications, whereas annual C outflows comprise harvested olive fruits plus olive leaves, soil erosion and soil CO2 emissions.

Preliminary results show that C balances are mostly positive mainly due to the accumulation of C in the tree permanent structure. Some of the farms showed negative farm C balances, indicating that more C was lost than entered. This was the case mainly in the farms which applied herbicide and, consequently, exhibited both lower annual CO2 entries in the biomass of cover crops, and higher annual C erosion losses. Results indicate that: i) olive cropping contributes to climate change mitigation, and ii) there is a great potential to enlarge this contribution by, for instance, reducing herbicide application to allow positive C balances in the olive grove.

### Acknowledgement and Funding

This work was funded by the SUSTAINOLIVE project (Grant nº 1822; PRIMA EU Programme), and by Project UIDB/05183/2020. SP and CB acknowledge the CEEC contracts from FCT (CEECIND/01473/2020 and CEECIND/00093/2018, respectively). AARS, acknowledges a Margarita Salas Postdoctoral Contract for the Training of Young PhDs funded by UCM through the Ministry of Universities, Government of Spain and the European Union – NextGenerationEU.



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#### **KEYWORDS**

olive production sustainability, anthracnose disease resistance, gene expression analysis

### ALTERNARIA ALTERNATA AN EMERGING PATHOGEN WITH GREAT IMPACT ON OLIVE GROVES

Anthracnose is a disease that affect the olive tree caused by fungi of the genus *Colletotrichum*. These fungi are responsible to premature fruit drop and a consequent decrease in the oil quality, and also cause defoliation of trees compromising the production of the following years. Currently the best control strategy is based on application of synthetic fungicides, with a regulatory pressure in agriculture worldwide to limit its use.

Looking for a sustainable disease management, the present study reports the transcriptional changes of olive genes that encode enzymes directly associated with the maintenance of the balance of oxidative oxygen species (ROS), in response to Colletotrichum sp. infection. The cultivar selected for the studies was 'Galega vulgar', known to be extremely susceptible to this disease. Plants used in the experiments were from in vitro culture (to warranty their healthy status), transplanted to pots and maintained under controlled conditions. Leaves of the olive plants were inoculated with a spore suspension of Colletotrichum sp., and leaf samples were collected before fungi inoculation (T0) and at 10 days (T1) and 35 days (T2) after inoculation. Confirmation of the presence of the fungi in inoculated plants was performed following a real-time qPCR approach. The selected target genes for expression analysis were Superoxide dismutase (SOD), Endochitinase\_EP3-like (CHI2), Glutathione S-transferase L3-like (TransFL3), Glutathione peroxidase 2 (PEROX2), Glutathione S-transferase THETA 1 (THE-TA), Glutathione S-transferase DHAR2-like (TransfDHAR), Glutathione peroxidase 5 (PEROX5) and Glutathione peroxidase 8 (PEROX8). Following a gPCR approach, a general up-regulation of the target genes was detected, but only CHI2 revealed a significantly up-regulation (p < 0.005) between T0 and T2 in response to Colletotrichum sp. infection, being a promising candidate to be later used in functional analysis. We emphasize the importance of this study for the identification of candidate genes to incorporate new sources of resistance of olive trees to anthracnose with the promotion of the development of sustainable management strategies.

### Acknowledgement and Funding

M. Patanita and J.A. Ribeiro are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships (SFRH/BD/145321/2019 and 2022.13638.BD, respectively). This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.



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olive production sustainability, anthracnose disease resistance, gene expression analysis PANEL 1 - SUSTAINABILITY, ECOLOGY AND CROP SANITY

### IDENTIFICATION OF CANDIDATE GENES INVOLVED IN OLIVE RESPONSE TO ANTHRACNOSE FOR A SUSTAINABLE DISEASE MANAGEMENT.

Anthracnose is a disease that affect the olive tree caused by fungi of the genus *Colletotrichum*. These fungi are responsible to premature fruit drop and a consequent decrease in the oil quality, and also cause defoliation of trees compromising the production of the following years. Currently the best control strategy is based on application of synthetic fungicides, with a regulatory pressure in agriculture worldwide to limit its use.

Looking for a sustainable disease management, the present study reports the transcriptional changes of olive genes that encode enzymes directly associated with the maintenance of the balance of oxidative oxygen species (ROS), in response to Colletotrichum sp. infection. The cultivar selected for the studies was 'Galega vulgar', known to be extremely susceptible to this disease. Plants used in the experiments were from in vitro culture (to warranty their healthy status), transplanted to pots and maintained under controlled conditions. Leaves of the olive plants were inoculated with a spore suspension of Colletotrichum sp., and leaf samples were collected before fungi inoculation (T0) and at 10 days (T1) and 35 days (T2) after inoculation. Confirmation of the presence of the fungi in inoculated plants was performed following a real-time qPCR approach. The selected target genes for expression analysis were Superoxide dismutase (SOD), Endochitinase\_EP3-like (CHI2), Glutathione S-transferase L3-like (TransFL3), Glutathione peroxidase 2 (PEROX2), Glutathione S-transferase THETA 1 (THETA), Glutathione S-transferase DHAR2-like (TransfDHAR), Glutathione peroxidase 5 (PEROX5) and Glutathione peroxidase 8 (PEROX8). Following a gPCR approach, a general up-regulation of the target genes was detected, but only CHI2 revealed a significantly up-regulation (p < 0.005) between T0 and T2 in response to Colletotrichum sp. infection, being a promising candidate to be later used in functional analysis. We emphasize the importance of this study for the identification of candidate genes to incorporate new sources of resistance of olive trees to anthracnose with the promotion of the development of sustainable management strategies.

### Acknowledgement and Funding

M. Patanita and J.A. Ribeiro are supported by Portuguese National Funds through FCT/MCTES under the PhD scholarships (2022.13638.BD and SFRH/ BD/145321/2019, respectively). This work is also funded by National Funds through FCT under the Project UIDB/05183/2020.



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### **KEYWORDS**

olive production sustainability, anthracnose disease resistance, gene expression analysis

### EVALUATION OF THE EFFICIENCY OF THE CHEMICAL PRECIPITATION PROCESS IN OLIVE OIL MILL WASTEWATER

The wastewater from olive oil mills, namely olive mill wastewater (OMW) represents a serious environmental problem, especially in the Mediterranean regions, constituting nowadays an important sector of the economy with a great exponential growth. The exhaustive exploitation of water resources makes it essential to search for new technologies and treatment approaches to reduce the impact of effluents on aquatic ecosystems and improve the possibility of their reuse. The most used processes to treat OMW are biological processes but, due to the low biodegradability, their organic removal efficiency is very low. So, the aim of this work was to evaluate a new chemical treatment process based on the use of Ca(OH)2 followed by natural carbonatation to drop the treated wastewater's pH to 8. The results showed a biodegradability increase due to the higher reduction in organic matter (COD, as well as BOD5). Related with nutrients, it was also observed phosphorus and Kjhedal Nitrogen removal of 99%±0.2 and 87%±0.8, respectively. This treatment process also allowed the obtention of a low turbidity effluent, with a removal efficiency of 99±0.2%. Regarding the inhibition of algae growth, the results showed a decrease in toxicity after the treatment.

#### Acknowledgement and Funding

This work was developed under the NETA project - New Strategies in Effluent Treatment (POCI-01-0247-FEDER-046959) funded by PORTUGAL2020.

PANEL 2 OLIVE AND OLIVE OIL CHEMISTRY



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### **KEYWORDS**

fatty acids varietal olive oils

### FATTY ACID PROFILE OF VARIETAL OLIVE OILS FROM ALENTEJO, PORTUGAL

The aim of this work is to obtain a full characterization of the fatty acid composition of varietal EVOOs, seeking for a better discrimination of varietal OO. Two sets of olive oils were used: 82 varietal olive oils produced with olives piked up in Alentejo region, from one crop year, and 72 olive oils over 3 consecutive crop years produced from olives picked up in the same orchard.

Fatty acid profile of olive oils was performed through transesterification with cold methanol solution of KOH according to the Official method. FAME composition and quantification was performed by gas chromatography using a Hewlett Packard (6890 series) system equipped with a flame ionization detector (GC-FID). In total 12 fatty acids were identified.

A linear discriminant analysis was performed, and results obtained with this analysis, shows that fatty acids allows a good discrimination of "Galega Vulgar", "Arbequina", "Blanqueta" and "Madural" olive oils. "Picual" and "Verdeal Alentejana" cultivars are also fairly discriminated. This result was evident either considering OO from one crop year or from 3 consecutive crop years, regardless the geographic origin. Crop year also influenced the discrimination of olive oils.

### Acknowledgement and Funding

Por3O - Portuguese Olive Oil Omics for traceability and authenticity - PTDC/ AGRPRO/2003/2014, by National Funds through FCT - Foundation for Science and Technology under the Projects This work is funded by National Funds through FCT - Foundation for Science and Technology under the Project UIDB/05183/2020



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### **KEYWORDS**

volatile compounds varietal olive oils

### VOLATILE PROFILE OF VARIETAL OLIVE OILS FROM ALENTEJO, PORTUGAL

Chromatographic techniques, particularly those coupled to mass spectrometry are widely used to fully characterized EVOOs samples and to establish varietal characterization profiles.

The aim of this work is to obtain a full characterization of the volatile composition of varietal EVOOs, using this information to build a database of the compounds characteristics of each cultivar and establishing a fingerprint pattern that can be used to assess EVOO authenticity.

Volatile profile of olive oils was performed by head-space solid phase micro-extraction hyphenated with gas chromatography/ mass spectrometry (HS-SPME-GC/MS). The HS-SPME extractions was done using a fiber CAR/DVB/ PDMS. The analyses were performed on a gas-chromatography/mass spectrometer (GC/MS) system consisting of a Bruker GC 456 with a Bruker mass selective detector Scion TQ and a CTC Analysis autosampler CombiPAL. Data were acquired with MSWS 8.2 Bruker and analyzed with Bruker MS Data Review 8.0.

A principal component analysis allowed us to verify that volatile data can be useful to discriminate olive oils according to variety. Graphical representation of chromatographic compounds were also performed by the construction of surface density van Krevelen plots

### **Acknowledgement and Funding**

Por3O - Portuguese Olive Oil Omics for traceability and authenticity - PTDC/ AGRPRO/2003/2014, by National Funds through FCT - Foundation for Science and Technology under the Projects This work is funded by National Funds through FCT - Foundation for Science and Technology under the Project UIDB/05183/2020



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### **KEYWORDS**

extra-virgin olive oil Galega cultivar olive oil quality pulsed electric fields semi-industrial scale

### GALEGA CULTIVAR OLIVE OIL SEMI-INDUSTRIAL PRODUCTION ASSISTED BY PULSED ELECTRIC FIELDS IMPACT ON EXTRACTION YIELD, PHYSICOCHEMICAL AND SENSORIAL QUALITY

The olive oil extraction process employed significantly impacts the overall quality of the final product. Technological innovations aim to balance process efficiency and product quality. Existent industrial processes successfully demonstrated that pulsed electric fields (PEF) are a potential technology for increasing extraction yield without compromising olive oil quality. By inducing electroporation of plant cell membranes, PEF enhances the effective disruption of olive fruit tissue which can facilitate the extraction of olive oil and its minor constituents.

This work investigated the impact of PEF technology on the extraction of Portuguese Galega cultivar olive oil by applying a shortened malaxation time, with focus on the extraction yields, physicochemical characteristics and sensory properties, including the main legal quality parameters of extra virgin olive oil.

The olive oil production was carried out at semi-industrial scale (350 kg/h). PEF treatment (2.0 kV/cm; 8.5 kJ/kg; monopolar pulses of 40  $\mu$ s; 100 Hz) was conducted in continuous mode (flow rate of 2242 kg/h), before malaxation during 30 min at 32.0  $\pm$  0.7 °C. In parallel, olive oil was produced without PEF application, as a control, with 45 minutes of malaxation at 32.0  $\pm$  0.7 °C. Olive oil extraction yields, acidity, peroxide value, K232, K268, K270,  $\Delta$ K, oxidative stability, total polyphenols, tocopherols and tocotrienols content and sensorial analyses were performed.

PEF treatment maintained the same extraction yield while reducing the malaxation time by 33% compared to control sample, resulting in an increased production capacity. In addition, both olive oils produced were classified as extra-virgin by considering the physicochemical and sensory analyses. Acidity and K232 values were lower in PEF sample compared to control sample. However, PEF samples showed lower oxidative stability. In conclusion, PEF can be applied in the production of high-quality olive oil Galega cultivar, with a positive impact on production efficiency, maintaining the extra-virgin classification.

### **Acknowledgement and Funding**

This work was financially supported by the project INOVC+ (CENTRO-01-0246-FEDER-000044), WINBIO (POCI-01-0246-FEDER-181335), TAGUSVALLEY2030 IT (CENTRO-01-0246-FEDER-000032) and TAGUSVALLEY2030 RHaq (CENTRO-04-3559-FSE-000143), under European Social Fund from the European Union managed by COMPETE 2020, CENTRO 2020 and PORTUGAL 2020.



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### **KEYWORDS**

co-extraction phenolic compounds oxidation

### NEW OLIVE OIL FLAVORED PREPARATIONS FOR THE VALORIZATION OF OLIVES WITH LOW FRUITINESS INTENSITY

Virgin olive oil (VOO) is considered as a "healthy fat" due to its considerable amounts of natural antioxidants and has unique sensory characteristics. However, when the olive oil is extracted from olives in late harvest time or that were subjected to frost, a total absence of fruity aroma can occur, and this product can no longer be classified as "extra virgin olive oil". Enrichment of VOO by co-extraction is a method based on the addition of herbs during olive oil extraction. It can have several benefits, not only from the sensory point of view, but also, to improve bioactive compounds and oxidative stability. Moreover, ultrasound assisted extraction (USAE) can be beneficial to promote the aromatization process, due to the mechanical effect generated by the cavitation phenomena, and thus enabling the extraction and dissolution of bioactive compounds into the oil.

The present work presents several studies that have been performed by our team for the development of new products by co-extraction of ripen olives of cv. 'Cobrançosa', with low fruitiness flavor intensity, with aromatic plants, namely thyme, oregano, and rosemary. Aromatic plants addition trials were carried out during the extraction process in the malaxation step with and without previous ultrasound application. Olive oil quality criteria, as well as total phenols and phenolic profile, were evaluated. US application did not significantly improve the phenolic content in oregano and thyme preparations. Oregano preparations showed the highest increase in phenolic compounds when compared to the other counterparts.

### **Acknowledgement and Funding**

This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.



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### **KEYWORDS**

Fatty acids Leucocarpa oleaster Phenols

### CHEMICAL COMPOSITION OF OLIVE OILS FROM WILD CULTIVARS

The valorization of wild cultivars can be a contribution to maintain olive biodiversity in the Mediterranean Basin. The natural distribution of the Mediterranean olive tree includes the var. sylvestris (also named oleaster) and the cultivated tree var. europaea. In turn, Olea europaea L. Leucocarpa cv. is characterized by the predominance of the white-ivory color of the fruits, known in Europe as "Maltese pearls". 'Galega Vulgar', the main traditional cultivar in the center of Portugal is very well adapted to adverse cultivation conditions and is the reference of Portuguese olive oils.

The present work evaluates fatty acid composition, total phenols and chlorophyll pigments, of oleaster and Leucocarpa olive oils from the central region of Portugal and stablish a comparison with Galega oils extracted from olives produced in adverse conditions.

Preliminary results reveal that oleaster and Leucocarpa oils have high contents of saturated fatty acids (> 24%) and total phenols higher than 700 mg GAE/kg. Galega oils showed the lower content of linoleic acid (~ 4%). Although one of the samples of Galega oils had a phenolic content higher than 1000 mg GAE/kg, the characteristics of the oils are very dependent on the degradation by pests and diseases of the olives.

### **Acknowledgement and Funding**

This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.



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### **KEYWORDS**

ioactive compounds Extra virgin olive oil Health Nutraceutical

### EXTRA VIRGIN OLIVE OIL BIOACTIVE PROPERTIES FOR HEALTH PROMOTION

Extra virgin olive oil (EVOO) has been widely known as a major component in Mediterranean cuisine with important properties associated with its intricate profile. This work aims to study the bioactive compounds, specifically the fatty acids composition and phenolic profile, as well as the antioxidant activity of a Northern Portuguese organic EVOO and to evaluate the health-nutritional lipid indices (atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic:hypercholesterolemic FA-ratio (HH)) linked with health promotion and disease prevention. Identification and quantification of bioactive compounds were performed on 15mg of organic EVOO, which was collected and examined for its lipid profile by GC-FID, in triplicate. Phenolic compounds were extracted in methanol:water (80:20) and analyzed using the Folin-Ciocalteu assay and HLPC-DAD. Antioxidant capacity was analyzed using the ABTS scavenging assay. Results showed that the fatty acid profile of EVOO was composed mainly by monounsaturated (Oleic acid, 74.48%), followed by saturated (Palmitic acid, 16.97%), and polyunsaturated fatty acids (Octadecadienoic acid, 8.41%). Using the three nutritional quality indices, the nutritional value of EVOO was analyzed showing that the AI (0.14) and TI (0.35) values were relatively lower compared to literature while maintaining a higher HH ratio (6.95) suggesting that this EVOO may decrease cardiovascular risk. The total phenolic compounds concentration was 0.94±0.29 mg GAE/ mL; hydroxytyrosol (>30%), tyrosol (>30%), and oleuropein were identified. The antioxidant capacity was shown to be 235.49±4.42 µmol of Trolox equivalent/mL. Not only is EVOO valued for its unique flavor and versatility in the Mediterranean, but also for its complex composition and functionality providing health benefits. It is a valuable food to be included in a healthy diet, with minor components holding a significant biological activity, paving the way to a fascinating further exploration in the food chemistry and nutrition fields.

### **Acknowledgement and Funding**

This work was supported by project HSoil4Food – Healthy soils for healthy foods (NORTE-01-0145-FEDER-000066), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.



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### **KEYWORDS**

Extractability extraction yield fatty acids olive oil phenolic compounds **PANEL 2 - OLIVE AND OLIVE OIL CHEMISTRY** 

### A NEW LABORATORY SCALE OLIVE OIL EXTRACTION METHOD WITH COMPARATIVE CHARACTERIZATION OF PHENOLIC AND FATTY ACID COMPOSITION

The establishment of operation protocols for olive oil (OO) extraction at non-industrial scale is crucial for research purposes. Thus, the present study proposes a simple and cost-effective method for OO extraction at the laboratory scale (LS) level. To validate the proposed methodology, industrial OO extraction (IS) was performed in parallel, using the same cultivars, 'Galega vulgar' (GV), 'Cobrançosa' (COB) and 'Arbequina' (ARB), collected from the same orchards and within the same time period. Obtained results showed highest extractability for COB and ARB, of about 53%, while GAL showed 50%. All produced OO showed values lower than the regulated limits for the physicochemical parameters (acidity, K232, K268 and  $\Delta K$ ), classifying them as extra virgin OO (EVOO). Highest total phenolic content was observed for COB, with no significant differences (p-value > 0.05) between extraction methods. Regarding fatty acid composition, oleic acid (C18:1) showed the lowest percentage for ARB, with about 66% and 68%, for LS and IS, respectively, and the highest for GV with about 72% for both LS and IS. Furthermore, all samples from both extraction methods were compared to the European Community Regulation, with fatty acid composition within the regulated levels for EVOO. This work showed promising results regarding extraction yields and OO extractability, as well as its quality parameters.

### **Acknowledgement and Funding**

The authors acknowledge Fundação para a Ciência e a Tecnologia for the PhD grant to M.D.F. (SFRH/BD/140083/2018). Authors also acknowledge FCT for Projects PTDC/BIA-CBI/1365/2020, UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and LA/P/0121/2020 to CHANGE—Global Change and Sustainability Institute. J.M.H. is granted under the María Zambrano international talent-attraction program under the Next Generation EU funds. Authors also acknowledge Herdade do Esporão, for making the sampling possible, and ACOS—Associação de Agricultores do Sul, for all the help with LS olive paste production and NIR analysis.

PANEL 3 BY-PRODUCTS AND ECONOMIC VALORIZATION



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### KEYWORDS

animal feed antioxidant activity by-products valorization nutritional value olive pomace

### OLIVE POMACE IN ANIMAL FEED – CHEMICAL COMPOSITION AND NUTRITIONAL VALUE

Olive pomace (OP), the main olive oil by-product, is composed by pulp, stones, skin, and residual oil, representing 35-40% of total olive processed. In Portugal, during 2021, were used 1.35 million ton of olive for olive oil production (INE, 2022), which represented an OP production comprised by 473000 and 540000 ton. Olive pomace is normally subject to a second extraction, where olive pomace oil and extracted olive pomace (EOP) are obtained. The OP can also be destoned (destoned olive pomace (DOP)). The chemical composition of olive pomaces is greatly variable, depending on olive oil extraction system, culture conditions, maturation stage of olive and the proportion of solid components. The OP and DOP present higher moisture content (60-70%) than EOP (7-14%). The OP and DOP presented higher ether extract (9-14.9% dry matter (DM)) than EOP (1.7% DM), being the oleic acid the major fatty acid present in olive pomaces (26-57 g/kg DM). Olive pomaces had lower levels of crude protein (5.7-13.6% DM) and the cell wall is highly lignified (acid detergent lignin (ADL): 18.9-36.5% DM), which strongly limits the digestibility. Olive pomaces also had high total phenolic content (3.2-20.6 mg gallic acid equivalents/g DM) and high antioxidant activity (Ferric reducing antioxidant power (FRAP): 7.0-115 mg FeSO4/g DM; Trolox equivalent antioxidant capacity (TEAC): 6.6-71.7 mg Trolox/g DM). Olive pomace inclusion in animal feed can be advantageous, mainly because of the bioactive compounds it provides in the diet. However, olive pomace may be used in animal feed but only associated with other feeding resources to complement its nutritional imbalances.

#### Acknowledgement and Funding

This work is funded by Alentejo2020 through the FSE under the "CEBAL Technology Transfer Potentiation Program – Interface Highly Qualified Human Resources" (ALT20-05-3559-FSE-000076); PDR2020 through the FEADER under the project "SubProMais – Use of agro-industrial by-products in animal feed" (PDR2020-101-030993) and by National Funds through FCT under the projects UIDB/05183/2020 (MED), LA/P/0121/2020 (CHANGE), and UIDB/00276/2020 (CIISA), and the PhD studentship awarded to Liliana Cachucho (2020.05712.BD).



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### **KEYWORDS**

hydroponics immediate one-step lime precipitation lettuce crop olive mil wastewater olive stones **PANEL 3 - BY-PRODUCTS AND ECONOMIC VALORIZATION** 

### RECYCLED OLIVE OIL BY-PRODUCTS IN A HYDROPONIC SYSTEM FOR LETTUCE CROP

The Mediterranean countries, being leaders in the olive oil production, produce around 30 million m3 of olive mill wastewater (OMW) annually. OMW is very pollutant, especially due to its high organic load and since a big quantity of these wastewaters is simply left in evaporation ponds or is thrown in the fields, measures must be taken rather quickly. Moreover, as water and food scarcity continues to be a growing problem worldwide, affecting more than 40% of the world's population, another worry arises.

However, It is known that OMW, although very pollutant, may be a great source of nutrients if efficiently treated. So, to try to solve these problems, this study aims to treat OMW using a fast, eco-innovative and environmentally friendly technology called Immediate One-Step Lime precipitation process (IOSL). This technology is based on the production of precipitates that allow the removal of organic matter and other contaminants. Posteriorly, the treated OMW has its pH level adjusted and is supplemented with nutrients to be used as nutritive solution in a hydroponic system for lettuce crop.

Besides wastewaters, olive stones are another by-product that may end up not being used by this industry and are also rich in sugars, fibers and phenolic compounds. Searching for an appropriate use for the olive stones, they were utilized in the hydroponic system, working as supporting medium. The lettuces were harvested after 7 weeks and were morphologically, chemically and sensorially analyzed.

With IOSL technique it was possible to achieve removal rates of 86% for TSS, 88% for COD and 92% for BOD5 in the OMW. Although some parameters, such as chlorophyl and carotenoids were higher for the control lettuce experiment, the lettuce's survival rate of 100% was attained. It was concluded that the IOSL precipitation process for OMW is an efficient way to reuse wastewater, applying it on hydroponically grown lettuce crop along with olive stones, recycling the olive oil industry's by-products and growing food in a sustainable way, through a circular economy.

### **Acknowledgement and Funding**

This work was financially supported by the NETA project: New Strategies in Wastewater Treatment (POCI-01-0247-FEDER-046959), funded by PORTUGAL2020. We would like to thank Fundação para a Ciência e a Tecnologia, FCT, for the PhD grant awarded to Alexandra Afonso (2020.04822. BD)



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### **KEYWORDS**

olive oil by-products circular economy by-product valorisation industry monitoring indicators

### VALORISATION OF OLIVE OIL BY-PRODUCTS DEVELOPMENT OF MONITORING INDICATORS

The cultivation of olive trees has been a characteristic practice in the Mediterranean basin since ancient times, being one of the most important woody crops for the culture and economy of this region. At present, 69% of worldwide olive oil production occurs in the EU, with Spain, Italy, Greece, and Portugal, accounting for about 99% of the production in the EU. Recently, the traditional olive groves have given way to high-density monoculture olive groves, resulting in significant changes of the agricultural system with important economic, sociocultural, and environmental consequences. The olive oil production process generates a considerable number of by-products, which raises the problem of waste accumulation. Aiming to monitor the evolution of the olive industries, regarding the adoption of sustainable practices compatible with the objectives of the circular economy, monitoring indicators were proposed based on the PC&I method (Sustainability Standards with Principles, Criteria and Indicators). Twenty monitoring indicators were elaborated for the economic, social, and environmental dimensions, directly related to the olive tree cultivation and olive oil production. To gain an understanding of the most widely adopted valorization strategies, interviews with olive oil producers were carried out. It was possible to conclude that most of the interviewees compost their by-products, as it is a cost-effective and technologically feasible method that provides advantages in agricultural productions, such as being used for soil fertilization. In the future, these indicators can be used as a tool to monitor and identify challenges and opportunities in olive production.



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### **KEYWORDS**

olive mil wastewater vertical flow constructed wetland vetiveria zizanioides

### STUDY THE POTENTIAL OF CONSTRUCTED WETLAND FOR TUNING PRE-TREATED OLIVE MILL WASTEWATER AFTER ONE STEP LIME PRECIPITATION)

The olive oil production industry is one of the most important agroindustries in the Mediterranean region, representing 95% of the world production. In the Alentejo region, there is a great focus on the production of olive oil, which represents 70% of national production. The growth of the olive grove area and the construction of new olive oil mills in the region have caused major environmental impacts, namely regarding the liquid effluents produced. These effluents contain high concentrations of contaminants, which have a negative impact on the environment.

In the present work, a sustainable and low-cost technology was developed for the treatment of effluents from the production of olive oil, by applying a one-step lime precipitation process, followed by fitorremediation in an artificial wetland in vertical flow, planted with *Vetiveria zizaniodes* in expanded clay (Leca®NR10/20) to remove organic matter, Kjeldahl nitrogen and phenols. Hydraulic loads between 35 and 237 L m-2 d-1 were applied, which allowed the removal of  $80\pm7$  %,  $44\pm3$  % and  $52\pm7$  %, organic matter (COD) Kjeldahl nitrogen (TNK) and phenols, respectively.

### **Acknowledgement and Funding**

This work was supported by the NETA project: New Strategies in Wastewater Treatment (POCI-01-0247-FEDER-046959), funded by PORTUGAL2020. The authors want to thank Fundação para a Ciência e a Tecnologia, FCT, for the funding of the UID Fiber Materials and Environmental Technologies (FibEnTech-UBI), project UIDB/00195/2020, the funding of the grant 2020.04822.BD, awarded to Alexandra Afonso, and the funding of the contract awarded to Annabel Fernandes.



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### **KEYWORDS**

Bioconversion Entomofertilizer Hermetia illucens Olive pomace Protein production

### OLIVE POMACE VALORIZATION: FROM AN ENVIRONMENTAL ISSUE TO AN ENVIRONMENTALLY FRIENDLY SOLUTION

Hermetia illucens is an insect capable of being reared in various organic substrates. The short life cycle, high protein and fat contents and high feeding conversion rate make Hermetia illucens larvae a suitable bio-converter of organic matter. Therefore, valuable products originated from this bioconversion as protein, chitin, oils and frass (insects' faeces, exuviae and undigested food), which can be potentially used as organic entomofertilizer. Olive pomace (OP) is an organic by-product of the olive oil industry with a strong environmental impact due to the large quantities generated annually and characterized by the high concentration of polyphenolic compounds, low pH and phytotoxic properties. Insects are presented as a possible costeffective and eco-friendly solution to mitigate this problem, acting as a circularity tool in the bioconversion of OP. Therefore, it has been proposed here as an alternative method for OP treatment. This study aims to evaluate the environmental safety of the frass originating from the bioconversion of OP by Hermetia illucens. Consequently, frass was applied to the soil at several application rates and ecotoxicological effects on soil invertebrates were assessed, looking at the reproduction and adult survival of the organisms. Potential phytotoxicity was also evaluated by looking at the germination rate and early stages of plant growth. At the same time, the effects on soil functions were also assessed, looking at possible changes in the soil enzymatic activity related to nutrient cycles. No adverse effects were seen on the evaluated endpoints of the invertebrate species, and no phytotoxic effects were found. The entomofertilizer generally stimulated enzymatic activities in soils. More complex agroecological scenarios have now to be tested further to evaluate the safety and efficacy aspects of this entomofertilizer, matching their fertilization potential against the current mineral fertilizer options.

#### Acknowledgement and Funding

This study was supported by the project ENTOSAFE (PTDC/CTA-AMB/0730/2021), financially supported by national funds (OE), through FCT/MCTES, by CESAMthrough FCT/MCTES (UIDP/50017/2020 + UA/P/0094/2020) national funds. D. Cardoso is hired under the Scientific Employment Stimulus - Individual Call (CEECIND/01190/2018). M. Prodana is a contracted researcher within ENTOSAFE. A. Mostafaie is a PhD grant holder (2021.08001.BD:).



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### **KEYWORDS**

Circularity Ecotoxicity Safety Wastewater Treatment Waste Repurposing

### AN ECOTOXICOLOGICAL ASSESSMENT OF OLIVE MILL WASTEWATER AFTER TREATMENT WITH CHEMICAL PRECIPITATION TECHNIQUE

Olive mill wastewaters (OMWW) are one of the most common waste products from olive oil production. OMWWs are highly toxic to the environment and must be safely discharged, treated, or reused. The chemical precipitation technique (CPT), through the addition of a single reagent (Lime - calcium hydroxide), is proposed as a single-step process for OMWW treatment that results in treated water while also originating a solid precipitate (sludge) with prospective utility. Depending on its quality and composition, the treated OMWW might be reused for irrigation, and hydroponics, among other usages. At the same time, the obtained sludge has the potential as fertilizer or soil amendment, promoting circularity and valorizing wastes produced in the olive industry sector. However, there is a lack of knowledge about the ecotoxicological effects of CPT-treated OMWW (CPT-OMWW) and respective sludges. This study aimed to assess: 1) the efficacy of CPT for treating olive mill wastewater and 2) the potential of this sludge to be considered as a soil amendment. A battery of freshwater and soil ecotoxicological assays was performed for both matrices. The ecotoxicological evaluation of OMWW and CPT-OMWW was conducted using three aquatic species exposed to a dilution range of each wastewater - the algae Raphidocelis subcapitata, the crustacean Daphnia magna, and the fish Danio rerio). Acute endpoints were derived for each species: wastewater concentrations inducing a 50 % lethality (LC50) and/or wastewater concentration causing a 10 % effect response (EC10). Four species were used for the ecotoxicological evaluation of the obtained sludge: two soil invertebrates, the springtail Folsomia candida and the oligochaete Enchytraeus crypticus, and two plants, the ray grass Lolium perenne and the cabbage Brassica oleracea. Sludge was incorporated at 0, 0.5, 1, 2, 4, and 8% in natural LUFA 2.2 soil. Survival and reproduction endpoints were evaluated for the invertebrate species and germination index and biomass for the plant species. We found that CPT reduced the toxicity of OMWW to D. magna and D. rerio (12.7% and 13.89%) LC50 in OMWW, respectively, to almost 100% survival at all tested concentrations after CPT). Likewise, R. subcapitata's EC10 for average daily growth rate ((Day-1) after 72 hours) increased after CPT treatment (2.95% to 26%). Sludge did not affect either F. candida or E. crypticus adult survival at any tested concentration, yet there was a decrease in reproduction for both 1% and 2% application rates compared with the control soil. Sludge did not affect seedlings (Gemination Index) in either of the tested plant species but decreased biomass in shoot and root in both species was observed when compared to the control soil.

### Acknowledgement and Funding

Thanks are due for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), to FCT/MEC through national funds. This study was supported by the NETA project (POCI-01-0247-FEDER-046959). ARRS received a research contract from the NETA project (POCI-01-0247-FEDER-046959). JP received a research fellowship from the NETA project (BI/UI88/10136/2022). DC was hired under the Scientific Employment Stimulus – Individual Call (CEECIND/01190/2018).



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### **KEYWORDS**

biogas olive pomace sustainability valorisation

### VALORISATION OF FRESH OLIVE OIL POMACE FOR BIOGAS PRODUCTION

Olive oil is a liquid fat obtained by pressing whole olives and directly extracting the virgin oil through mechanical processes. The remaining residue is a liquid/solid mixture called olive oil pomace, containing about 60 to 80% water. The current methods to treat and valorise this residue require an intensive use of energy for removing the excess water to facilitate the chemical extraction of remaining oil still present in the pomace (so-called olive pomace oil). The dry remainder after this secondary oil extraction is then burnt for production of electricity (e.g., through co-generation), for generating thermal energy (e.g., for heating of the olive mills), and for animal feeding. Due to the large increase in olive oil production, there are now several constraints to this process in terms of installed capacity. Alternative and more sustainable methods to valorise this residue were therefore investigated, namely evaluation of the potential for biogas production from fresh olive oil pomace. For this purpose, four samples of olive pomace were collected right after the exit of the decanter/centrifuge from two different mills in Portugal, located in the Alentejo and Douro regions. The preliminary results have shown promising gas yields, also when compared to other types of substrates. Further investigations are necessary to better understand the biogas potential of fresh olive oil pomace, and these experiments have thus provided an initial snapshot of the potential of such olive pomaces to produce biogas.

### Acknowledgement and Funding

Fundação Eugénio de Almeida and Cooperativa de Olivicultores de Valpaços are kindly acknowledged for providing the samples. Fraunhofer Society is kindly acknowledged for the support.



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### **KEYWORDS**

Extracted Olive Pomace (EOP) inhibitor detoxification Lignocellulosic Biomass (LCB) Microbial adaptation

### SYSTEMATIC ANALYSIS OF MICROBIAL ALTERNATIVES FOR LIGNOCELLULOSIC BIOMASS HYDROLYSATES DETOXIFICATION: A REVIEW

The Mediterranean area produces up to 98% of the olive byproducts, where Extracted Olive Pomace (EOP) constitutes 35% of its full production. To access these non-seized sugars contained in the lignocellulosic biomass (LCB), a pre-treatment is always in need. Its production, unfortunately, carries important fermentation inhibitors (furaldehydes, phenolic compounds, organic acids) that depletes the fermentation rate due to its toxic effect on fermentative metabolism. Due to complications to eliminate them by physicochemical means, other strategies are put in practice. Using microorganisms as a way; not only to harvest the available sugars and other compounds contained in LCB, but also as a manner to eliminate the inhibitors from biomass hydrolysates has been showed as one of the most prolific paths to overcome the problem. The different strategies based on microbial adaptation to surpass toxicity of the main inhibitors found in EOP hydrolysates reported within the last 15 years are analysed in the present work.

### Acknowledgement and Funding

A. Arroyo-Escoto aknowledges Fundação para a Ciência e a Tecnologia (FCT) for the Doctoral Grant (UI/BD/153579/2022). M. C. Fernandes aknowledges Reforço de Capital Humano Altamente Qualificado de Interface Project (ALT20-05-3559-FSE-000076), financed by Social European Fund, under Alentejo2020 Programm. MED is funded by FCT under UIDB/05183/2020 project.



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### **KEYWORDS**

Auto-hydrolysis Biorefinery Dilute acid hydrolysis Extracted Olive Pomace Gasification

### THE IMPACT OF PRETREATMENT METHOD ON THE GASIFICATION OF EXTRACTED OLIVE POMACE

The extracted olive pomace (EOP) is the most relevant solid by-product of the olive pomace oil extraction industry. Its current upgrade is mainly focused on the bioenergy field, where it is used as a solid biofuel through its direct combustion. A potential, more efficient, alternative, is its use as a raw material for gasification in the biorefinery framework.

With the aim of increasing the efficiency of the gasification process and to study the integration of the biorefinery's thermochemical and biochemical routes, in this work we studied the effect of diverse pre-treatment methods (autohydrolysis and dilute acid hydrolysis) in the fractionation of biomass and on the gasification potential of the remaining solid fraction. All pre-treatment processes allowed the production of sugars suitable for biotechnological applications and increased the calorific value of the produced gases in comparison with the untreated EOP. The use of sulfuric acid allowed the highest gas yield based on treated biomass (2,9 NI/g), but, acetic acid treatment presented the highest gas production yield considering the overall process, corresponding to a 23% increase in relation to the untreated EOP.

### **Acknowledgement and Funding**

This work was partially carried out under the Refinolea Project (FCOMP-01-0202-FEDER-005450) in the Biomass and Bioenergy Research Infrastructure (BBRI-LISBOA-01-0145-FEDER-022059) that is supported by the Operational Programme for Competitiveness and Internationalization (PORTUGAL 2020), by Lisbon Portugal Regional Operational Programme (Lisboa 2020) and by North Portugal Regional Operational Programme (Norte 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).



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### **KEYWORDS**

adequacy compost distribution manure spreader olive by-products PANEL 3 - BY-PRODUCTS AND ECONOMIC VALORIZATION

### USE OF A MANURE SPREADER FOR LOCALIZED APPLICATION OF COMPOST IN OLIVE GROVES

The economic and environmental sustainability of the olive growing sector requires the use of circular economy principles. One of the most important is the agronomic valuation of olive by-products.

In modern olive orchards, pruning by-products are crushed and left on the soil surface, but the leaves removed from the trees at harvesting haven't any valuation, being burned in the most of the cases. Olive pomace are used for oil extraction, and may have subsequent valuations, namely in the production of heat.

Although composting is a viable technique with low costs, which allows to transform organic by-products into organic correctives, it has a limited use.

In this context, within the framework of the project financed by the PDR2020 programme of the Ministry of Agriculture, entitled GO-TECOLIVE – "Techniques and technology for the valuation of by-products in olive growing", the aim was to evaluate: the technical feasibility of producing compost with olive leaves, dried olive pomace after oil extraction and sheep manure, and evaluate the ability to mechanically distribute a uniform layer of the compost produced, in the olive grove.

This work shows the results obtained with a manure spreader from a Portuguese manufacturer, in the application of compost in two olive groves.

### **Acknowledgement and Funding**

This work was only possible thanks to funding from the Ministry of Agriculture through the PDR2020 program, with the project GO-TECOLIVE, intitled "Técnica e tecnologias para valorização de subprodutos em olivicultura".

Thanks are also due to the former students of Agronomy from University of Évora, Jorge Saragoça, João Rento, Ricardo Pedro e Patrick Donno for their collaboration in the field work.

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