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LEAF EXPLORATORY PROJECTS 2021

FUNDED PROPOSALS

BOOK OF ABSTRACTS

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2021

LEAF EXPLORATORY PROJECTS

These are annual projects for the exploration of research ideas and the consolidation of knowledge that will be explored later, in more depth, in applications for National or International Project Calls. In each call, 30 projects are funded with 5k€.
The development of these projects must result in at least one publication, preferably Q1, as a way of evaluating the quality of the work produced.

There is no limit to the number of researchers that make up a project team, but at least two LEAF members must be involved, preferably from different groups, and may be integrated into a PhD plan. The promotion of synergies between researchers with a higher productivity rate and beginners, to create a dynamic of quality production in LEAF, is valued.

For the 2021 call, 32 proposals were funded. The abstracts of proposals that were approved are provided in this document.

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ALGAEGG

The effects of using microalgae as a sustainable feed ingredient in laying hen diets on egg production and quality parameters

PI: Madalena Lordelo

Microalgae are a promising alternative feed ingredient for animal diets because they are capable of capturing carbon dioxide from the atmosphere, which is necessary to mitigate climate change. In addition, microalgae can be easily produced in bioreactors and therefore do not compete with arable land used for human food production. Microalgae have high protein, carbohydrate and fat contents, in most cases comparable or even higher than conventional feedstuffs, such as soybean meal. The objective of the current study is to compare a number of performance and egg quality parameters of layers fed *Spirulina platensis* and/or *Chlorella vulgaris.* Furthermore, it is our goal to gain insight into the sensory traits associated with eggs produced by hens fed microalgae.

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AlgaeUSExtraction

Fractionation of microalgal biomass using ultrasound process

PI: Anabela Raymundo

The use of microalgae as a food ingredient has deserved special attention recently, taking into account its nutritional richness, particularly in terms of proteins, minerals, vitamins, lipids, antioxidants, pigments, and dietary fibres. There are several works highlighting the possibility to use microalgal biomass in different food matrices. Although consumer's appeal for microalgae is increasing, there is still a long way to go before microalgae-based products become mainstream. One of the main drawbacks for the industrial scale-up is related to the new tastes, colors, flavors and aromas that are present in microalgae-based products. Alternative strategies to engage the consumers have been considered by several authors, based on complementary approaches. Removing pigments from microalgae, using ethanol and utilizing the bleached biomass in wheat breads, was recently studied by our research group. This approach showed promising results during sensory evaluations, followed by the improvement in dough rheology (increased elasticity) and in the subsequent baking performance (increased bread volume). However, the extraction process can be considerable improved using ultrasound technics in order to minimise the use of solvent (becoming a more eco-friendly process) and maximise the extracted biocompounds, with potential for further valorisation as added value ingredients. It is intended to optimise de ultrasound extraction process of *Chlorella vulgaris* and *Tetraselmis chuii*, promoting the full recovery of the two fractions, in a circular economy logic - zero waste; the protein-rich fraction will be incorporated into bread and the bioactive-rich fraction will be incorporated into mayonnaise.

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AquaGrape

A study of the role of miRNAs in the regulation of Aquaporins in grapevine berries: potential impacts on berry water relations

PI: Luísa Carvalho

Fruit growth is mainly the result of water accumulation, and thus the maintenance of fruit growth calls for the concerted action of long-distance water and solute transport through the vascular tissue, and short-distance water and solute uptake of individual cells. Aquaporins (AQPs) are water channel proteins that are found in most living organisms. Plant AQPs are present in multiple isoforms reflecting a high diversity of cellular localizations, transport selectivity, and regulation properties. They can be localized in the plasma membrane, endoplasmic reticulum, vacuoles, plastids and, in some species, in membrane compartments interacting with symbiotic organisms. Knowledge of the hydraulic control of berry growth by AQPs is of extreme relevance in viticulture, and little is known on the subject. Recently, miRNAs have been identified as targeting AQPs in animals, but their possible involvement in AQP regulation in plants hasn't been studied yet. Therefore, we propose to study the expression of AQPs and regulatory miRNAs in leaves and berries of grapevine plants well irrigated and subjected to water stress, to find patterns of co-expression. In addition, we will exhaustively monitor water mass fluxes in shoots, leaves, and to and from the berry (through sap flow and hydraulic conductance measurements), in order to understand the model by which water availability impacts on grapevine water relations both at the molecular and the physiological levels.

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BACHALOPH

Cultivation of edible halophytes ex situ and in situ using halosymbiontic plant growth promoting bacteria using saline irrigation water

PI: Ana D. Caperta

Saline soils are unsuitable for conventional crops but represent a unique opportunity to exploit saline agriculture that uses saline water irrigation for plant cultivation. The genus *Limonium* is remarkable for having halophytic species which are rich in antioxidants with commercial interest that grow in conditions of high salinity. This project aims to find solutions to cultivate *Limonium algarvense* Erben using biofertilizers (Plant Growth Promoting Bacteria – PGPB) and saline drip irrigation. Previous studies under the scope of the project MycoHaloph funded by LEAF (2020) allow isolation and characterization of halophilic PGPB from *Limonium* species grown in saline habitats. A multidisciplinary approach will be employed for elucidation of how saline irrigation and PGPB inoculations influence plant growth *ex situ* and *in situ* (i), and physiological and developmental processes (ii); localization of PGPB in the root endosphere (iii); and for development of a protocol for *in situ* propagation in saline conditions. The obtained results will allow scientific support to the use of PGPB inoculum and saline drip irrigation for this species cultivation and to establish a preliminary design for its cultivation.

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Bamboo4Iberia

Setting the Environment: Mapping the current and potential distribution of bamboo plantations in the Iberian Peninsula

PI: Luís Goulão

Bamboo is recorded to be the fastest-growing land plant on the earth. High potential for cropping and managing this multi-purpose grass is highlighted due to its value for atmospheric carbon sequestration, environmental impact reduction and source of raw material for a number of industrial sustainable and circular economy-based applications. Bamboo utilization is an emerging topic in Europe, asking for scientific evidence and knowledge on plantation and utilization potential to support value chains development and management activities. Bamboos are not native to Europe, but some species can thrive in France, Italy, Portugal and Spain, where small areas of healthy bamboo plantations exist. With the main objective of promoting bamboo as a sustainable material in Europe, this exploratory research project aims at delivering i) maps and dedicated georeferenced databases of the existing different bamboo plantations in Iberian Peninsula with information on genetic material / cultivated species and management practices and ii) undertaking a preliminary agroecological zoning for sustainable bamboo production. We expect that the results obtained will provide scientific evidence in favour of the development of a sustainable value-chain for bamboo in Portugal and Spain.

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BIO-PHYTOFABs

Eco-friendly solutions for BIOcides production against PHYTOpathogens and Food and Fermented Alcoholic Beverages spoilage microorganisms

PI: Patrícia Branco

Nowadays to prevent the microbial spoilage, chemical biocides are commonly applied by Food and Fermented Alcoholic Beverages (FABs) and by crop production industry. However, most of these biocides/preservatives are hazardous to human health and to the environment as they can provoke illness, e.g., allergies, asthma, cancer, and some of them cannot be eliminated from water by wastewater treatments. Even though the use of chemical biocides in crops and food products has become frequent worldwide, consumer awareness of health hazards and environmental pollution resulting from chemical biocides production and application is increasing, favouring the consumption of food products with natural preservatives. Therefore, agriculture and food scientists are confronted with a tough challenge: to produce secure, healthy, and sustainable foods. Thus, with emerging of foodborne diseases and in line with the increased interest for minimally processed and more natural and eco-friendly products, the search of green biocides/biopreservatives, being non-toxic to the environment and human health and at the same time allowing the elimination of microbiological contamination in crops and FABs, is a sustainable and pressing alternative. Several bioactive metabolites, resulting from yeasts and bacteria metabolism with antimicrobial activity (e.g., mycocins, bacteriocins and antimicrobial peptides) have been reported. However, many of these natural antimicrobials when used alone have a limited spectrum of activity; a possible solution for obtaining a natural biocide with a large spectrum of antimicrobial activity may be the combination of various bioactive microbial metabolites.

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BrettVBNC

Brettanomyces bruxellensis energy management strategies during "viable but not culturable" (VBNC) state

PI: Manuel Malfeito-Ferreira

Brettanomyces bruxellensis is the most important spoilage yeast due to the production of "horse sweat" taint in the bulk or the bottled red wines. This yeast species produces off-odor volatile phenols resulting from metabolization of hydroxycinnamic acids (HCAs) found in grapes, musts and wines. Even under the recommended inhibitory concentrations of sulfur dioxide, this yeast species has ability to enter the "viable but not culturable" (VBNC) state where cell remains metabolically active and synthesizes volatile phenols. Whether it is cell's state that influences volatile phenols production capacities, or the volatile phenols synthesis that confers better surviving capabilities to the yeast cells, has been remained unexplained so far. To answer these questions, it is important to show if VBNC cells are able to utilize precursor (HCAs) and intermediates (vinylphenols) of volatile phenol synthesis, to manage its energy requirements. We plan to sequentially quantify the precursor and intermediates, during the VBNC state, by HPLC equipped with photodiode-array (PDA) detector at different time intervals. Simultaneously, we plan to reveal the ATP generation by the cells while metabolizing the HCAs into volatile phenols by assaying the plasma membrane ATPase activity and the intracellular ATP content and its action as a proton-pump. This study intends to highlight the role of precursors and intermediates of volatile phenol synthesis in the energy management of the yeast cell under different inhibitory concentrations of sulfur dioxide and ultimately to design better *B. bruxellensis* spoilage control protocols.

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CAPRI-PluS Meat

Serpentina chevon dry-aged in bag: process yield, microbiological status, nutritional traits and consumer preference

PI: Luísa Louro

The "*Cabrito do Alentejo*" (Alentejo's kid), a PGI (protected geographic indication) meat product obtained from autochthonous *Serpentina* breed goats (30-120 days of age), is tender, juicy, low in fat, with a high proportion of muscle, light colour with little intense red and a pleasant flavour. However, meat from heavier, older animals (or chevon), is not appreciated, being normally commercialized at very low prices when compared with that of younger animals, or used to process. For sustainability and profitability reasons, farmers, industry and government, are interested in increasing chevon value once its breeding is strategically important for the maintenance of biodiversity, mainly in marginal areas. Thus, in order to add economic value and to improve meat characteristics of chevon, different technologies have been studied, namely vacuum-ageing and dry-ageing. Dry-aged meat, besides showed to have more *umami* compared to vacuum-aged, leads to large amounts of waste and lower yields in meat cuts. This project will contribute to the valorization of chevon meat, from older and cull goats from *Serpentina* breed with an exploratory approach on the development of the novel dry-ageing in bag process. We will contribute to an increase on meat quality and economic value, and ultimately to the UN Sustainable Developmental Goals (sustainable consumption and production patterns (1) and life on land preservation (2)).

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CarryGreen

Improved packaging strategies for the preservation of fresh-cut vegetables and fruits shelf life

PI: Vitor Alves

The minimally processed fruit and vegetable market has grown significantly and is likely to continue to develop in the future. This rapid growth is mainly due to consumer demand for fresher, healthier, and more convenient products. In what concerns packaging, the most common materials are usually films made of polyethylene (PE) and/or Polypropylene (PP) polymers, whose main characteristics are high strength, flexibility, transparency, lightness and selective permeability. As these polymers are not biodegradable, they represent a severe environmental problem. The use of biodegradable materials and, if possible, biodegradable materials with antimicrobial properties to produce packaging for minimally processed products is of significant importance. In this work, new active and biodegradable packaging systems for the preservation of minimally processed leafy vegetables and highly perishable small fruits are studied for the first time.

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Coffee&Sugar

Functional characterization of a sugar transporter from the group early-responsive to dehydration 6-like (ERDL6) from Coffee hybrid Kawisari involved in the resistance to *Hemilea vastatrix*

PI: Maria do Céu Silva

Coffee is the second most transactable product in the world, second to petroleum. Diseases affecting coffee production are a heavy burden on farmers' income with serious social consequences. Since 2013, several devastating outbreaks of Coffee Leaf Rust (CLR) have impacted Central America. CLR is caused by *Hemileia vastatrix* a biotrophic basidiomycete fungus that colonizes coffee leaves in all coffee growing regions of the world. In plantpathogen interactions, sugars are under a tight control, since plants try to decrease sugar content from their apoplastic and cytoplasmatic spaces to "starve" pathogens. RNAseq data from Kawisari, a Coffee hybrid used in Indian breeding programs due to its high resistance to CLR, leaves showed upregulation of a sugar transporter from group ERDL6 connected with the resistance response to *H. vastatrix*. The *ERDL6* gene from Kawisari will be functional characterized; for its capability to transport sugar, which sugars does it transport, if it is an exporter or an importer, and where in coffee leaf cells does it localizes. This data will provide an insight into the role played by *ERDL6* in the resistance against CLR. *ERDL6* gene showed some variability in the mRNA and protein sequences between *Coffea* spp. species that will be correlated with its function. The activity of invertases and SuSy enzymes in infected coffee leaves will be analysed to disclose the dynamics between sucrose and the product of its degradation, glucose and fructose, which will be correlated with the transporter activity. Overall the project completion will contribute to a better understanding of the role of sugars in plant-pathogen interactions, in particular in the interaction between Coffee and *H. vastatrix*, and of special interested in the resistance to *H. vastatrix*, which can be used to select better coffee genotypes that will resist to CLR.

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CORKWATERCHANNEL

Identifying the role of aquaporins in the challenging ecosystem of cork oak woodland: A molecular device to balance the water and nutrient status in plants

PI: Farzana Sabir

The cork oak species is a major component of Portugal's unique savannah-type ecosystem *montado*, providing high ecological, social, and economic values. However, the sustainability of this ecosystem is being compromised by many external factors, principally by drought stress. The water channels, aquaporins (AQPs), are integral in water transport in the soil–plant–atmosphere continuum. They provide rapid and reversible changes to cells hydraulic conductance by modulating membrane water permeability. Hence, the studies related to AQP function and regulation under stress have always deserved interest. Lack of information regarding cork oak AQPs hampers the broad understanding of water transport and associated stress tolerance mechanisms at the molecular level. However, our previous promising results identified and characterized several cork oak AQPs, transporting water and other small molecules when expressed in yeast cells, which further inspired this proposal. The project aims at identifying the regulatory mechanisms of selectivity and gating of previously characterized cork oak AQPs expressed in *aqy-null Saccharomyces cerevisiae*. Point mutation at key amino acid residues of ar/R constriction, or other putative regulatory positions like the N- and Ctermini, loops, and helices, will be performed. The obtained mutants will be further functionally characterized by stopped-flow spectroscopy. The study will establish the structure-function relationship of the selected cork oak AQPs. The effect of other putative regulatory factors like intracellular pH, cations, and inhibitors on the aquaporin's functionality will also be assayed. The perceived functional relevance of cork oak AQPs obtained in yeast will be established at the whole plant level. The real-time PCR technique will reveal the differential expression pattern of cork oak AQPs, especially under drought stress. Moreover, the immunolocalization of AQPs with the group-specific antibodies in different tissues, with or without stress conditions, will provide information on aquaporin distribution in the membrane and their preferential expression site.

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CROPinMINE

Adaptation of crop systems to mine areas recovered with designed Technosols

PI: Erika Santos

The recovery of mine areas with Technosols is a real cost-effective option for environmental recovery and valorisation of non-productive soils. These marginal areas are a promising niche to cultivate non-food crops such as aromatic and medicine plants, contributing to regional sustainability and economy. On the other hand, the amounts of wastes produced at (agro)industrial scale are extreme and generate a high environmental and economic problems because of their management. However, increasing the knowledge about their properties would let to create soils that achieve the local weather, crop demands and mine soil requirements. This is supported by the preliminary results obtained with designed Technosols (previously evaluated) that have improved even tailings (even sulfide-rich), letting the development of a crop system. Nonetheless, some adjustments related to agronomic techniques and plant performance under field conditions are needed to get a significant biomass production for extraction of bioactive plant extracts or other plant-based products. This project intends to adjust the crop system and its management under field conditions promoting a stakeholders network for all crop value-chain and circular economy. The advantages of the development of this project are several due to it will be developed in an active mine located in a strong agro-industrial region, and the project has a multidisciplinary team from LEAF (G1, G2), Universidade de Aveiro and Los Frailes mine (Spain).

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DigitalFood

Exploring the digital food environment in Lisbon: focus on meal-delivery apps (MDAs)

PI: Gabriela Albuquerque

The “Food Environment” is the dimension of the Food Systems framework in which food policies are more targeted for reshaping. The “digital food environment” is an innovative concept, arising from the rapid transformation occurring in food systems, due to the increased use of technologies to improve food production and distribution. However, digital food environments are seldom characterised and their regulation is currently absent. This project aims at conducting an exploratory assessment of the digital food environment in Lisbon, Portugal, with a particular focus on meal-delivery apps (MDAs). We will map the food availability and its nutritional value, and marketing attributes of online food outlets available through MDA among Lisbon dwellers and compare these outcomes across three parishes of different socioeconomic characteristics. We expect to identify opportunities for improvement and gaps in food policy in support of sustainability, health and nutrition, including regulation, also delivering insights capable to be scaled to “glocal” contexts.

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EpiRus

O ciclo de vida e a virulência de ferrugens são controlados por mecanismos epigenéticos?

PI: Pedro Talhinhas

As doenças das plantas são responsáveis por avultadas perdas na agricultura e os agentes patogénicos colocam desafios inesperados aos fitopatologistas. As ferrugens causam doenças relevantes desde a antiguidade, com disseminação intercontinental e podendo quebrar os esforços de melhoramento para resistência. São agentes patogénicos biotróficos obrigatórios e especializados, que estabelecem diálogos histológicos e moleculares intrincados com os seus hospedeiros. Os seus grandes genomas oferecem oportunidades únicas para estudos citogenéticos. A rápida evolução e elevada adaptabilidade dos fungos causadores de ferrugens são insuficientemente explicadas pelas vias clássicas de reprodução e este projeto visa elucidar o papel de mecanismos epigenéticos no controlo do ciclo de vida/ciclo nuclear, bem como na relação entre os membros do heterocário verificado ao longo do ciclo dicariótico dos fungos causadores de ferrugens. Anteriormente esta equipa identificou a presença de núcleos diploides em fases do ciclo de Pucciniales que deveriam ser estritamente haploides e caracterizou este fenómeno a nível citogenómico e citogenético. As células dos fungos Pucciniales são dicarióticas nas fases ecídicas, uredospóricas e teleutospóricas e os distintos alelos presentes nos dois núcleos (heterocário) mostram-se relevantes na genética destes organismos. A análise bioinformática destes dois núcleos separadamente é uma tarefa hercúlea, mas a descoberta recente feita pela nossa equipa usando Citometria de Fluxo (FCM) mostrou que os núcleos do heterocário do fungo *Coleosporium tussilaginis* f.sp. *senecionis-silvatici* têm tamanhos de genoma diferentes (*Twin Peaks*), permitindo a separação física de cada população nuclear por seriação celular. Esta característica única será explorada para estudar as relações entre núcleos no heterocário e as expectáveis diferenças nos padrões epigenéticos. Uma vez combinadas, as abordagens previstas nesta proposta que se enquadra no programa de doutoramento da Rita Carvalho (LEAF/ISA) aprovado pela FCT, fornecerão uma perspetiva global da regulação epigenética de características-chave na patologia de Pucciniales, nomeadamente o ciclo de vida/ciclo nuclear e as relações entre os membros do heterocário. Para tal, juntamos uma equipa LEAF abrangendo as valências de Citogenética, Fitopatologia e Gestão de Recursos Vegetais.

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FILMAWAS Filters made by wastes

Capture of nitrogen gases from slurry through filters made by inorganic wastes for crop fertilization

PI: David Fangueiro

Slurries are an important source of nitrogen GHGs, and there are limited available strategies to reduce or capture them to diminish the ecological fingerprint, partially because the complexity of the N cycle. Thus, the European Union (EU) is boosting different strategies to either reduce or capture GHGs emissions, because on human health and environment. The EU is also promoting projects about recycling and reutilization of wastes due to the lack of natural resources, as well as the unsustainable amount of residues produced. Thus, this project combines two priority lines of the EU Green Deal and the 2020-2030 Agenda, using wastes to reduce the GHGs emission and synthetizing fertilizers, recovering waste material currently sent to landfills. The project will evaluate different inorganic wastes use as filters (ashes and iron oxides, mainly) that can react with NOx and NH3 gases, transforming them (by chemical or photocatalytic reactions) into NH4 and/or NO3, and so, enrich them in N. In addition, different types of slurry and other variables such as filtering time will be evaluated. On the other hand, filter materials enriched in N will be tested, in mesocosm assays and under controlled conditions, in crop/s (e.g. Rye grass) as fertilizer. Firstly, plant nutritional characteristics such as fiber, energy, ashes and protein —chosen accordingly to feed livestock and thus close the circle— will be analyzed. Secondly, it will be evaluated the change on soils chemical and physical properties. Finally, this project will also measure N gas forms produced through the filter and so, quantifies the effectiveness of the filters and the N forms that are evading it.

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HybridCoffee

The parental genomes of *Coffea* spp. and their role in the resistance to *Hemileia vastatrix*

PI: Leonor Morais-Cecílio

Coffee is a valuable commodity that provides a way of living for many millions in the world south. *Coffea arabica* although responsible for high quality coffee is very susceptible to coffee leaf rust disease (CLR) caused by *Hemileia vastatrix*. Coffee varieties resistant to CLR resulting from crosses between *C. arabica* and Timor hybrid (HDT) genotypes were produced successfully since 1970. HDT is a natural hybrid between *C. arabica* and *C. canephora*. Kawisari another natural hybrid is also used in breeding programs in India and resulted from crossing *C. arabica* and *C. liberica*. Both hybrids are thought to be tetraploid plants, like *C. arabica* (a hybrid between *C. canephora* and *C. eugenioides*), but unlike *C. arabica* quite resistant to CLR. The contribution of each parental genome has been studied for some *C. arabica* genotypes but was never attempted for HDTs and Kawisari. Genome *In Situ* Hybridization (GISH) will be applied to HDTs and Kawisari genomes to disclose their genome constitution. RNAseq from HDT CIFC832/2 shows an unexpectedly high contribution of genes with higher homology to *C. eugenioides*, a minor parent contributor to HDT genome. *PAD4* is an extreme case of such situation and will be used as a marker for assess chromosome restructuring and parental genome contribution. Furthermore, GISH will be used in association with DNA methylation makers to distinguish methylation patterns related to individual parental genomes. The experimental data produced will be correlated with the resistance/susceptibility phenotype, which can be further used in a more informed decision in breeding programs using HDTs and Kawisari genotypes.

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Metabo-Piglet

Intestinal Metabolome and Mineralome of piglets under dietary inclusion of the microalgae *Chlorella vulgaris*

PI: João Freire

In Europe, meat production is heavily dependent on overseas importation of soybean meal, as it is the primary protein source in feed. Microalgae, such as *Chlorella vulgaris*, can be produced locally, fixate Carbon and shorten the cycle and decrease the Carbon footprint of meat production. Moreover, Microalgae are rich in high value nutrients and bioactive phytochemicals with potential physiological benefits. However, little detail is available on the impact of such diets in relation to piglet physiology, particularly as it concerns the result of microalgae intake on the metabolomic environment of the lower intestine from piglet digestion of microalgae-derived nutrients, microbiota transformation of such compounds and novel host-microbe exchanges in this complex intestinal environment. The recent advent of the Omics techniques allows filling such knowledge gaps as they generate very complete, broad and high-throughput datasets. In this project, we will study the effect of dietary inclusion of microalgae on the metabolome and mineral profiling in the intestinal contents of piglets post-weaning, in a very novel approach. Samples were obtained from a trial previously conducted and we will use an approach based on NMR-based metabolomics and Inductively Coupled Plasma – Optical Emission Spectrometry. Results will be integrated with productive performance and proteomics analysis currently being conducted in the framework of the project of this team's PhD student. With this project, we will determine important metabolic effects of microalgae inclusion in piglet nutrition and establish their significance to swine production.

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MozDiVigna

Vigna crop wild relatives in Mozambique: Unravelling diversity for agriculture and food security

PI: Maria Romeiras

Crop wild relatives (CWR) have important traits that can be used for crop improvement and their conservation in their natural habitats is crucial, as is the study of their potential agricultural use. In Mozambique, there is a substantial number of native legume species, including many CWRs, which can become key elements for the sustainability of local agricultural systems. Among this group, the *Vigna* genus (ca. 20 taxa) stands out as a promising donor of favorable traits but remains poorly studied. This exploratory project aims to assess the diversity of *Vigna* CWR in Mozambique, characterize their chemical and nutritional profile, identify priority areas and CWR taxa for local conservation, and delineate conservation and sustainable use guidelines at regional and local scales.

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Nanofert

Bases para o desenvolvimento de pellets nanofertilizantes

PI: Gonçalo Rodrigues

A utilização de fertilizantes químicos e fitofármacos é uma premissa atual da produção de grandes culturas, promovendo aumentos de produção por hectare, mas representando simultaneamente uma preocupação ambiental crescente. Para dar resposta a esta problemática é proposto neste projeto exploratório, estudar a ecotoxicidade dos componentes envolvidos no desenvolvimento de novos pellets nanofertilizantes e bioestimulantes para a cultura do arroz, servindo de base para a escolha de componentes que farão parte do design final dos pellets. A utilização de imagem térmica para determinação do stress das plantas expostas aos compostos em estudo pretende ser um fator diferenciador e um método expedito de avaliação.

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NutrAlPasta

Nutritive Pasta products enriched with Algae dewatered and dried by different methods

PI: Cristiana Nunes

Microalgae are recognized as a source of numerous phytochemicals with remarkable functional activity and health impact and represent a sustainable food ingredient with great importance in the context of climate changes and food shortage. Worldwide, microalgae have been sustaining a viable multi-billion-dollar industry and continue to grow while providing valuable products for multiple applications in food, nutraceuticals, pharmaceuticals, cosmetics, feed, fertilizer and biostimulants, and other chemicals. Among consumers, there is a growing trend towards natural, healthy food products. Not surprisingly, microalgae have an increasing role in direct use in food products, be it for their health benefits, natural pigments, or as vegan protein source. A strong argument to support the biofortification with microalgae is that bioactive compounds are naturally encapsulated and protected within the microalgae cell. However, it is important to consider the possibility to perform a biomass pre-treatment in order to promote a controlled release of the active compounds trough a partial cell wall disruption. The controlled disruption of cell wall (= cell disruption), resulting from the downstream of biomass production, including drying processes, has an important impact on the bioavailability of microalgae contents. It will influence the nutritional composition, bioactivity and sensory profile. Furthermore, efficient and cost-effective dewatering and drying methods for microalgae strongly affect the overall energy consumption, and thus the sustainability of these ingredients. In this context, microalgae processed by different dewatering and drying methods (solar and spray-drying) will be incorporated in fresh pasta products to study its impact on nutritional composition, bioactivity and technological quality properties. *Chlorella vulgaris* and *Arthrospira platensis* (commonly known as *Spirulina*) produced by Allmicroalgae company (Pataias, Portugal) using different technologies will be used.

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OneHealth

Impact on health of convenient plant-based foods, with fruit pomaces

PI: Joana Ferreira

Food waste is one of the major pollutants of the environment, and the third biggest generator of CO2 in the world. The valorization of food industry byproducts (e.g., pomaces from the juice industry) is a sustainable approach to reduce food waste significantly. Additionally, consumers are increasingly aware of the impact of food on health; "you are what you eat" is ancient wisdom gaining protagonist as a choice determinant. Health/nutritional claims are effectively used as differentiators to consumers‘ preferences. Therefore, this project aims to upcycle a by-product from the juice industry, mainly apple and carrot pomace, to develop innovative foods with excellent nutritional profile and rich in bioactive compounds. Bioactive potential of the fruit pomaces will be characterized and a food formulation, selected from the best fitted for the purpose, will be designed targeting a specific diet.

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PrEcoLite

The influence of pruning wound protection on grapevine wood microbial ecology and secondary metabolites

PI: Giovanni Del Frari

Esca is a grapevine trunk disease that poses major concerns to worldwide viticulture. Affected plants suffer from reduced longevity, productivity and quality of the yield, altogether causing enormous economic losses. The causal agents are a diverse array of fungi that infect and colonize grapevine woody tissue, inducing the appearance of both internal and external symptoms. Infections may occur both in the nursery and in the vineyard, and they are mainly due to fungal propagules reaching open wounds (e.g. during grafting, yearly pruning). To prevent infections from taking place, at vineyard level, the most common strategy is to apply pruning wound protection (PWP) products, such as fungicides or biological control agents. These control strategies only confer partial protection, and the side effects of applying such treatments on the wood microbial ecology and secondary metabolites have not yet been explored. In this study, we will use next-generation sequencing (NGS; DNA metabarcoding) and HPLC to investigate the endophytic microbiome and the wood phenolic compounds profile of grapevine canes -cultivars Cabernet Sauvignon and Syrah- treated with four PWP products. We will examine the ITS1 region for the fungal community profiling, three, six and nine weeks after canes were differently treated. Through this study, we will be the first group to (***a***) use DNA metabarcoding to examine the grapevine canes endophytic microbiome in response to the application of PWP products, (***b***) assess the effects of treatments on wood phenolic compounds, (***c***) explore the correlation among wood microbiome, phenolic compounds and plant health. The positive outcomes of this study go beyond the pure academic understanding, indeed they will offer valuable insights for viticulturists over the consequences of their choice when adopting control measures against wood pathogens.

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Res4FoodPharma

Production of Functional Compounds from Agro-Industrial Residues and Bioactivity Assessment for Food and Pharmaceutical Applications

PI: Suzana Ferreira-Dias

Functional foods, with recognized improved benefits for human health, have drawn increasing attention to consumers, worldwide. These concerns, together with the need for sustainable valorization of local agro-resources, agro-residues and by-products, using environmentally friendly processes to produce high added-value products, will contribute for the implementation of new strategies, in the frame of the biorefinery context, to decrease the global process costs of functional foods. This project is a follow-up of the exploratory project Bio4FoodRes where olive pomaces (Cobrançosa and Galega cultivars) and grape stems and pomaces (Touriga Nacional and Marselan varieties) were used as sources of biomass and/or oil to obtain the following added-value products, using biocatalysis or environmentally friendly processes: (i) oligosaccharides (OS) with prebiotic activity; (ii) low-calorie structured lipids (SL) and (iii) of phenolic extracts (PHE) with antioxidant activity. In this proposal, the studies will be extended to (i) the valorization of pomaces and stems of other autochthones olive cultivars and grape varieties to produce those added-value compounds, under previously optimized conditions, and (ii) the assay of their bioactivity by *in vitro* techniques (OS: prebiotic and antimicrobial activities; PHE: antimicrobial, anti-inflammatory and anticancer activities; SL: caloric value and bioaccessibility). Knowing their bioactivities and eventual relationships with their chemical composition/production process, it will be possible to have “taylor-made” compounds for different food/pharmaceutical applications.

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SAPPLANT

How water superabsorbents influences plant physiology and genetics

PI: João Lucas Coito

Climate change poses a serious threat to plant yield and crop production. In Europe, this threat is expected to have a greater impact in central Europe European and Mediterranean countries. One way to mitigate the lack of water or improve water use is to enrich the soil with superabsorbent polymers (SAPs). These polymers have a high-water retention capacity, around 100 to 1000 grams of water per gram of SAP, which allows it to retain high amounts of water in the soil after periods of rain or irrigation, leading to less water need for irrigation or overall, less irrigation. We expect, with this project to test the effect in plant physiology, production capacity and effects in the progeny in plants cultivated in an SAP enriched soil. In this work we plan to use the model plant *Arabidopsis thaliana*, already established in our lab. Two population will be established. One population without SAP (SAP-) and one population cultivated in SAP enriched soil (SAP+). Each population will be submitted to drought stress to access the capacity of the SAPs to mitigate this abiotic stress. We will measure several physiological parameters to access plant physiological adaptation and other set of measurements to access plant yield. In addition, we will collect leaf samples to extract RNA and access the gene expression of several genes of interest. These preliminary results will serve as a catapult for a larger project to be submitted to the FCT which will use, besides *Arabidopsis*, *Vitis vinifera* as a crop model plant.

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SOILVITIMICROBES

Multifunctional microbial inoculum development for vineyards with elevated concentration of copper in the soil

PI: Amaia Nogales

There is an increasing demand for new nature-based solutions to prevent soil deterioration and to sustain crop production in moderately metal-contaminated soils. Since the vineyards with a tradition of Cu-based fungicide applications can present impaired soil functions and grapevines may show a decreased performance, the development of a microbial inocula based on Cu-tolerant multifunctional beneficial microorganisms that act at both, plant and soil levels, promoting a progressive recovery of soil ecosystem and plant health, is an area of special interest in modern sustainable agriculture. At LEAF-ISA, a multidisciplinary team working on agricultural soil microbiology (from G1 and G2) together with Dr. Navarro de la Torre (Universidad de Sevilla) identified two Cu-tolerant microbial consortia based on native arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB) with highly mutualistic traits for plants. However, the mechanisms explaining the microbial-induced metal toxicity in their hosts were not fully understood. An innovative hypothesis is that microbial-inducible aquaporins (AQP), which are membrane transporter proteins of water, osmolytes, metals and H2O2, have a crucial role helping symbiotic plants cope with Cu toxicity. Therefore, the objective of this project is to explore the potential synergistic effect of both functional groups of microorganisms on grapevine growth and development in a Cu-contaminated soil, and to study the mechanisms of microbial-induced metal stress tolerance. For this, the coordinated work of a multidisciplinary team composed by members of the three LEAF groups will be essential. Well-established methodologies of G1, G2 and G3 on microbial culture, propagation and inoculation, plant-soil interactions, plant physiology monitoring, histology, immunolabeling and AQP gene expression analyses will be implemented. This cooperation will strongly promote innovation by providing a tailored consortium-based microbial inoculum to sustain grapevine productivity in degraded soils while minimizing fertilizer and pesticide requirements. It will lead as well to important advances in the scientific knowledge, since the role AQPs on plant-microbial interactions in contaminated soils could be demonstrated for the first time, leading to a high impact publication.

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TARIoT

A aplicação de sistemas IoT no tratamento de águas residuais vinícolas

PI: Margarida Oliveira

O setor do vinho apresenta uma elevada importância no contexto socioeconómico nacional, com Portugal a apresentar-se consistentemente como um dos cinco maiores produtores da União Europeia. A produção de vinho é responsável por um elevado consumo de água e também, nas adegas, pela elevada produção de águas residuais. Um adequado tratamento das águas residuais vinícolas é determinante para o desempenho ambiental do setor e possibilita a sua reutilização, reduzindo-se a dependência deste recurso. Apesar do conhecimento técnico e científico existente verifica-se, hoje em dia, uma existência alargada de problemas associados ao tratamento de águas residuais vinícolas. Este trabalho pretende contribuir para a resolução deste problema, através duma abordagem essencialmente tecnológica, focada no desenvolvimento de soluções IoT (I*nternet of Things*) para o tratamento de águas residuais vinícolas contribuindo para a digitalização em Agroindústrias.

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TomatOmics

Metabolomic analysis of tomatoes produced under metal and salinity stress

PI: Miguel Pedro Mourato

Tomato is one of the most important agricultural crops, both in Portugal and worldwide. The necessity to increase plant production can lead to the use of marginal or contaminated soils. In these conditions plants can be subjected to different abiotic stresses due to environmental factors like the presence of heavy metals or soil salinity. When plants grow in a contaminated medium, they activate different defense mechanisms, and its metabolism can be affected leading to morphological, physiological and biochemical changes. This can lead to changes in the composition of tomatoes eventually changing its flavor. The modern "omics" approaches are able to characterize in detail the molecular mechanisms. In this work we will use a metabolomic approach, using a GCMS technique, to unravel the differences in the composition of different metabolites in tomatoes from two different species (one with high lycopene content, "Nemabrix", and one with normal lycopene content, "Lusitano"), grown in soils contaminated with Cu, Cd and NaCl. The tomatoes were produced from plants grown in soils containing different concentrations of Cu (0, 50, 200 and 600 mg/kg), Cd (0, 1, 4 and 12 mg/kg), Cu+Cd (0, 50+1, 200+4 and 600+12 mg/kg) and NaCl (0, 2 and 5 g/kg). We intend to study how the heavy metals (one essential, Cu, and one non-essential, Cd) and soil salinity (as NaCl) can affect the composition of the tomatoes in sugars, amino acids, organic acids and other compounds. This will allow the identification of biochemical changes induced by the toxic elements that can affect fruit quality and flavor.

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Trans-LuPig

Supplementing weaned piglet feed with deflamin-containing lupin extract as a model for health and nutrition: a transcriptomics approach

PI: Paula Batista Santos

Matrix metalloproteinases (MMPs), particularly MMP-9, are deeply involved in inflammation and cancer processes. Although studies relating MMP-9 inhibition to clinical reduction of Inflammatory Bowel Diseases (IBDs) are few, they suggest that ingestion of MMP-9 inhibitors (MMPIs) can decrease their incidence. Since IBDs are related to pre-cancer stages, a food-based strategy may be one of the most valid, easy and cost-effective alternative to prevent cancer incidence. Targeting MMP-9 inhibition through food has been difficult, mostly because of the emergence of severe side-effects, lack of specificity, potential toxicity, susceptibility to the digestion process and cost. We have previously identified deflamin, a promising small protein isolated from lupin seeds, with an outstandingly potent and very specific MMP-9 inhibitory activity. Deflamin is a novel type of MMPI that is edible, survives the digestion process in mice and may be used as a nutraceutical or as a functional food ingredient. To determine whether deflamin anti-inflammatory and anti-cancer activities survive the digestive process, and if it is (or not) absorbed into the bloodstream, nutrition studies were recently performed, using weaned piglets as an animal model of digestion. This proposal will provide novel and valuable information on the implications of deflamin incorporation in piglet feeds using a high throughput transcriptomics approach at the level of intestinal mucosa and liver tissues and on the feasibility of using deflamin as a functional food in preventive and curative diets and/or as a supplement to be used in cancer and inflammable bowel diseases patients. Additionally, the multidisciplinary nature of the project is ensured by the participation of both senior and young researchers among LEAF groups 1 and 2 from different areas of the biological sciences, including general animal husbandry, animal nutrition/feeding, animal physiology, agronomy, biochemistry and molecular biology.

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Urine2Fertilizer

Biobased fertilizer from urine collected at source

PI: Rita Fragoso

Phosphorous (P) is a non-renewable resource and the interest in recovering it from alternative sources, such as wastewater or urine, has increased recently. The valorisation of urine collected from hospital patients through urinary catheters could be a starting point to assess the impact and technical feasibility of urine segregation and treatment at source, reducing the environmental impact and costs associated with their treatment. The valorisation of this urine-stream needs to consider the elimination of the risk associated with pathogens and pharmaceuticals, which may be achieved using ozone-based advanced oxidation processes (AOPs), as well as the recovery of nutrients by crystallization of struvite (MgNH4PO4.6H2O), applied with success to sources rich in magnesium, ammonium and phosphate, as urine. The optimization of both processes may evidence the possibility of recovering P as a slow-releasing fertilizer from urine collected at source.

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USCOLIVE

Ultrasound assisted coextraction technologies to obtain new olive oil preparations

PI: Fátima Peres

In recent years, innovation in olive oils has gone through its flavoring with different ingredients, with the aim of improving its sensory and nutritional properties as well as shelf life. Thus, this type of product is highly appreciated especially among consumers outside the Mediterranean countries, reaching prices higher than the ones for extra virgin oils. Ultrasound-assisted co extraction can be used to produce flavored oils from autochthonous olive cultivars using different aromatization agents. This project proposes studying the effect of olive ultrasound-assisted co-extraction with selected aromatization agents, specific of the Mediterranean region, on the quality of the final product. The trials will be performed using overripe fruits with low aromatic potential (Beira Interior traditional cultivars) and different aromatization agents. A complete chemical characterization of all the raw materials will be done as well as the complete analysis of the flavored oils produced (quality, sensory, bioactive and volatile compounds).

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VinumVitaVitis

Grapevine ε-viniferin+resveratrol as a nutraceutical for gut inflammatory diseases: the power of synergy

PI: Ricardo Boavida Ferreira

Grapevine has been used in medicine due to biological activities of several of its secondary metabolites in which stilbenes are included. Viniferins and resveratrol are grapevine bioactive compounds and wine sub-products such skins, stems and seeds, that have a potential as antioxidants and more recently for inflammatory bowel diseases (IBDs). The objective of the proposed work is to assess the synergic effect of trans-resveratrol and ε-viniferin as anti-inflammatory nutraceuticals for IBDs and validate their *in vivo* efficacy in colitis. Several experimental systems will be used to assess its anti-inflammatory activities, using methods well developed in our group and a network of collaborations. Cutting-edge approaches like RNASeq, coupled with biochemical technics and animal models are expected to support significant correlations between stereoisomers of trans-resveratrol oligomers and the prevention of colon diseases. Our work will aim at better understanding the potential of these compounds in IBDs whilst developing curative and preventive approaches that can help mitigate the current burden of IBDs and simultaneously help and solve the disposal problems arising from the large amounts of residues generated by the wine industries.

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WHEY2HEALTH

Bioactivities of fermented whey enriched with antibacterial and anti-inflammatory peptides as a way towards a healthier gut microbiome and a healthier life

PI: Isabel Santos

With increasing health concerns about multi-drug resistant bacterial diseases, the gut microbiome and foodborne pathogens, food-derived antimicrobial peptides are very promising in the field of both health and nutrition. Previous works in our lab using lactic acid bacteria (LAB) and a specific cheese whey fermentation technology yielded the discovery of novel whey bioactive peptides with strong antibacterial and anti-inflammatory activities. Being a byproduct of the food industry and completely GRAS (generally accepted as safe), these whey peptides hold great potential use for human and animal health, either as nutraceutical alternatives to antibiotics or in functional diets for gut inflammatory diseases. Here we propose to test the potential of these whey peptides as a) alternatives to antibiotics including multi-drug resistant bacteria related to gut inflammation and microbiome imbalance such as *Clostridium difficile* and *C. perfrigens*, b) promoters of a healthier microbiome and c) as functional food components against gut inflammatory diseases. A practical approach will be used, using a multidisciplinary team and several collaborations, which are well aligned with both the scope of LEAF as well as the UN goals and the One Health approach. If success is achieved, a commercial exploitation shall be pursued and in the future clinical models will be tested as well.

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