Proceedings of the Final Conference of the FEDKITO project

Towards circular economy in the agri-food sector: strategies and challenges

September 12th, 2023

Aula Magna of the Department of Agriculture, Food and Environment (DAFE)

Via del Borghetto, 80 - 56124 - Pisa

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Session 1.

Bio-based solutions for the agri-food sector

FEDKITO: Fresh food sustainable packaging in the circular economy

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Fresh foods (FF) such as fruit, vegetables, meat and dairy products are highly susceptible to spoilage during harvest, post-harvest and storage. Every year, 15 to 50% of the world's produce is lost due to mechanical damage, fungal contamination, insect infestation and oxidation of unsaturated fatty acids. The lack of adequate hygienic conditions during transport, and storage affects the quality and shelf life of FF. The biological threats not only reduce the market value of the food, but also expose consumers to the risk of ingesting toxic metabolites, including mycotoxins.

The solution proposed by FEDKITO is to create innovative packaging materials using chitosan (CHT), an edible and biodegradable polymer derived from the deacetylation of chitin, alone or flavoured with essential oils (EOs), which are able to protect FF from insect and fungal attack in the post-harvest/farming phase, significantly reducing the use of chemicals. The smart active packages will be enhanced by low-cost paper-based electrochemical biosensors and multiplexed user-friendly smartphone-based biosensors. These are able to monitor the presence of mycotoxins and chemical residues and continuously check food quality characteristics during storage and distribution. This innovative technology establishes new protocols for FF processing, storage and trade to promote food security and waste reduction. Also in line with circular economy criteria, CHT was obtained from the chitin-rich pupae of the black soldier fly, *Hermetia illucens* (Diptera Stratiomyidae), reared in large numbers on FF by-products and waste resulting from the selection of tradable products.

Keywords: post-harvest, food loss, shelf life, chitosan, essential oils

Cascading processes to extract and valorise high-value molecules from agro-industrial residues: the PROLIFIC and AGRILOOP projects

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Globally, a third of all food produced every year (~2.5 billion tons) is lost or wasted and ends up as co-products, residues and waste along the food chain (from field to fork). On the other end the projected global demand for proteins and bioactive compounds will be in 2030 well exceeding current production capacities, given the rising world population. Agro-industrial residual biomass, side streams and food production by-products may represent rich sources of valuable ingredients both for humans and animals. Yet their potential the routes for their full exploitation are still at an early stage.

Two European projects (PROLIFIC and AGRILOOP) are contributing to improve the knowledge on the recovery of significant amounts of proteins, polyphenols, polyesters and other valuable compounds from agricultural and food-processing residues and to develop integrated value-chains aiming at high-value industrial applications. These projects optimize, validate and scale up an integrated array of cascading green extraction processes starting, among others, from legumes, fungi, coffee, tomato, grape and potato feedstocks. The extracted molecules, after being validated for their safety and composition, are tested for techno-functional activities to find several high-value applications in the food, packaging, cosmetic and feed sectors. The projects also assess the economical, environmental, societal, ethical, safety and regulatory implications of the newly created value chains.

PROLIFIC (2018-2022) was funded by BBI-JU under the European Union's H2020 research and innovation programme (GA n. 790157). https://www.prolific-project.eu.
AGRILOOP (2022-2026) was funded by European Union's HEU research and innovation programme, the UK Research and Innovation fund under the UK government's HEU funding guarantee (GA n. 101081776) and The National Key Research and Development Funds of China. https://agriloop-project.eu/

Keywords: food loss, food waste, proteins, polyphenols, polyesters

Challenges in managing food loss and waste: the experience of StopMedWaste PRIMA Project

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Reduction of food loss and waste is an important part of several sustainable development goals (SDGs), and European Union adopted the halvin of food waste within 2030. PRIMA StopMedWaste project (www.stopmedwaste.net) aims to reduce the waste of fresh fruit, vegetable and aromatic plants through the application of physical means (e.g. ozone, electrolised water), natural compounds (e.g. chitosan, essential oils, other basic substances and potential basic substances) and biocontrol agents. Protocols setted up during the project should reduce the application of synthetic pesticides of around 20% and reduce waste from 30 to 15%. The network of scientists from UNIVPM, UNIBA, UNITO, CUT, IVIA, UE, INRAT is complemented by the involvement of SMEs (Decco, Ikachem), that will apply in large scale some of the established protocols. The project activities were collected in over 20 scientific papers and in international meetings held in Limassol, Rimini, Tunisi, Torino, Lyon, and planned in Valencia, Kusadasi and Ancona.

Project activities can be followed thorough social media listed on the website www.stopmedwaste.net.

Keywords: chitosan, edible coatings, food loss, food waste, ozone

BiOrangePack project

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Quality standard, health of the consumers and a long shelf-life are fundamental aspects affecting the competitiveness of citrus fruits produced by Mediterranean countries on markets. Rots caused by fungi are the main cause of post-harvest losses (about 30%) of fruits and may consistently reduce their shelf life. The project scope is to increase the efficiency, sustainability and competitiveness of organic citrus supply chain in the EU-Med area. Specific objectives include: 1. reduction up to 0.5% of post-harvest rots losses during storage and transportation, by treating the fruits with eco-friendly substances compatible with organic food (innovative green technology); 2. raising of the quality standards of fresh fruits and juice by using new diagnostics (innovative biotechnology) for the detection of pathogenic quarantine fungi (zero tolerance) and mycotoxins (under detectable levels) and excluding fruits not complying with EU and EPPO standards; 3. extension up to 70 days of fresh fruit shelf-life using biodegradable smart packaging; 4. application of smart technologies (ICT-based technologies and machine learning techniques) to increase up to 20% shipment efficiency to the markets and exclusion of complaints by GDO; 5. reducing up to 80% the waste of industrial fruit transformation by recycling the most of citrus pulp, the major by-product of citrus industry, by utilizing it as a raw material to produce a biodegradable and natural biocoating of fruit (green economy). Project includes 6 countries and 14 partners.

Keywords: Post-harvest diseases, eco-friendly substances, circular economy, packaging, smart technologies

Session 2.
Towards a plastic-free packaging

Renewable, biodegradable, and recyclable materials for functional packaging

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Renewability, compostability and recyclability of packaging are becoming more and more important because of the necessity of decreasing its burden on environment. The combination of these features can allow to contribute to reduced greenhouse gases emissions, circularity and as longer as possible storage of carbon in products before their composting, respectively. Bioplastics, Biocomposites (incorporating selected agro-food waste) and cellulosic packaging can be proposed as alternative substrates to fossil plastics, but the application of functional liquid or solid coatings can be necessary to obtain the requested gas barrier properties, anti-microbial properties or antioxidant behaviour to effectively replace fossil-based packaging. In the case of anti-microbial packaging chitin nanofibrils or chitosan obtained by crustaceans or fungi were used to obtain high-performance packaging based on bioplastic films or cellulosic board. Chitosan from insects, in particular from *Hermetia illucens*, was also investigated in terms of its chemical and morphology properties as an alternative abundant source of these anti-microbial biopolymers.

Keywords: biopolyesters, chitin, cellulose, recyclability, biobased

The chitosan "solution" to preserve the food quality

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Since ancient time solutions have been sought to extend the shelf life of fresh foods. Due to the high water content and the presence of substances prone to oxidation, fresh foods are in fact perishable by nature, and they can be subjected to degradation, loss of quality and microbial spoilage.

Coatings directly applied on the product surface, based on natural biodegradable biopolymers such as lipids, proteins, polysaccharides, are successful strategies to contrast food loss, particularly when the coating solution is formulated with the addition of functional compounds, able to improve the coating properties or to provide further advantages.

Chitosan is one of the most promising food-coating biopolymers, due to its film-forming activity and antioxidant and antimicrobial properties. Chitosan is primarily extracted from chitin present in the exoskeletons of crustaceans processed for human consumption, though in recent years its production from fungi and, even more, from insects has been increasing.

One of the objectives of Fedkito project was to demonstrate the effectiveness of chitosan solutions enriched with essential oils with a sensorial profile suitable for the coated food in preserving the quality of different food matrices. The results related to strawberries coated with chitosan + mandarine essential oil, and beaf meet patties coated with chitosan + laurel or chitosan + black pepper essential oils will be presented and discussed.

Keywords: chitosan, food quality, oxidation, strawberries, beef meat

Sustainable packaging based on chitosan and essential oils

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The quality and shelf life of Fresh Food are mainly affected by mechanical damage, microbiological spoilage as well as insect attacks.

The solution proposed with FEDKITO, for these problems, is the use of innovative smart active packaging based on chitosan (CHT) aromatized with essential oils (EOs), together with biosensor technologies to protect FF from insect and fungi attack in any conditions and to extend the shelf life of perishable Mediterranean fresh food products.

As part of the FEDKITO project, IPC has produced different types of biopackaging by using cast extrusion process to obtain films or thermoforming process to obtain trays.

In a second step, the performances of the developed bioactive packages were evaluated. The physical, mechanical as well as the barrier properties were analyzed. Bioactive effect of products (antibacterial, antifungal, and repellence properties against insects) were also considered.

Keywords: biopackaging, chitosan, essential oils, cast extrusion, thermoforming

Bioactive additives and new materials from agri-waste: a contribute to the circular economy

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The increasingly pressing demand of civil society to move towards the circular economy models drives the scientific community to explore new routes to fully valorise agri-wastes in order to achieve the zero-waste objective. From this perspective, agricultural waste and agri-industrial processing residues become a potential source of valuable products, that can be suitably exploited according to specific strategies based on the Green Chemistry principles.

Molecules characterized by antioxidant and antibacterial properties can be used to prepare polymeric formulations characterized by high performances for applications in active packaging or in agricultural fields. Strategies to protect the active molecules, extracted from agri-waste, can be developed in order to avoid degradation during the processing at high temperatures.

Novel research strategies, specifically designed to valorize the unique chemical structures of natural compounds, can be explored to create new macromolecular architectures with specific multifunctionalites.

The research work here described, mainly carried out in the framework of some H2020 European projects, aims to demonstrate how it is possible, according to the principles of the circular economy, to valorise the residues produced along a food value chain.

Keywords: agri-residues, ferulic acid, vanillic acid, itaconic acid, green chemistry

The economics of sustainable packaging

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Sustainable packaging is a hot topic, and its prominence in the scientific and academic debate is growing. Farm to Fork Strategy recognises the need to develop alternative solutions to reduce plastic disposal in the environment. While the literature has approached it focusing on technological changes with the availability or adoption of sustainable materials, other criteria such as economic viability and social impacts are often neglected. the existing literature remains limited to the evolution of new materials and consumers' attitudes towards sustainability. The result indicates an increase in low cost for the handleability and machinability, mainly for the spray, as it would be associated with an additional process along the existing industrial production. Conversely, the experts have indicated a very high and uncertain cost of machinability, and even not suitable for processor and transformation firms whether the innovative packaging solution will require changes to the production line.

The empirical analysis results have returned contrasting results on product conservation and safety as spay has some potential benefit and the trials for the film have returned unsatisfied results for example in meat. The empirical analysis and the interview have confirmed the potential interest in communicating further sustainability to consumers as the film's colour can be associated with a perception of a more "natural" material.

Keywords: costs and benefits, socio-economics, consumers, policy, farm to fork

Session 3.
Insects for food waste recovery in the circular economy

Making "gold" out of trash: Exploitation of agri-food waste streams for insect production

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The insect sector is continuously growing during the last decade, as the use of insects for both food and feed is associated with several benefits. Briefly, insects are highly nutritious, whereas they have low water needs and low land use requirements and their production has low environmental footprint (e.g., low GHG emissions). However, one of the most important elements of the sustainable profile of insect rearing is their ability to grow on organic side-streams and by-products. Apart from being a sustainable practice fully aligned with circular economy practices, the valorisation of largely untapped, locally produced organic side-streams of low or zero economic value can also substantially contribute to the reduction of the insect feed cost. However, one question that should always be addressed is which side-streams can be exploited as insect feed. For instance, in EU level, there are restrictions on the feeds which may be given to 'farmed animals' and these restrictions also apply to edible insects. Another issue that should be taken into consideration when thinking of potential side-streams that could be exploited as insect feed is their availability. The side-streams that could be used as insect feed should be available in sufficient quantities, so that the insect sector can rely on these quantities for insect production. So, when it comes to mass-production, it is not only a matter of suitability, but also a matter of availability. Along the same lines, the seasonality in the availability of specific side-streams during a year is a serious issue that should also be faced by the insect industry, e.g., by applying various storage strategies and techniques (like silage or fermentation) to increase their availability throughout the year.

Keywords: alternative proteins, circular economy, edible insects, insect as food and feed, side-stream valorisation

Insects for food and feed: current status and research priorities

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Current European agricultural production systems heavily depend on protein imports, to meet the nutritional needs of livestock animals and farmed fish. However, their dependency on imports of the main protein sources, renders them vulnerable to rapid disruptions of the supply chains. Therefore, EU is urgently in need of viable and locally produced alternative protein sources. Insect production has recently attracted a lot of research and business interest, since insects are considered as an efficient, alternative nutrient source that can be exploited for animal feed, as well as for human consumption and are associated with several advantages. Particularly, they have a high nutritional value, whereas they are highly efficient feed convertors. Moreover, their production is highly sustainable (reduced water and land requirements; low greenhouse gas emmissions), whereas for their rearing, insects can upcycle organic side-streams of low economic value. However, in order to fully expolit insects as an alternative source of nutrients, several hurdles and barriers have to be ovecome. The current high insect production cost could be partly alleviated through the automatization and the scale-up of the production processes, as well as the valorization of untapped, low-cost organic wastes and side-streams. Moreover, genetic breeding could help improve specific traits of the farmed insects and thus substantially contribute to the production yield increase. Finally, although several studies on the inclusion of insect meals to the diet of farmed animals (e.g. poultry, swine) and fish are already available, further evidences that show the suitablitily of insect meals as feed ingredients could further boost their use in animal nutrition.

Keywords: alternative proteins, circular economy, edible insects, insect as food and feed

The plastivorous activity of insects for waste recovery

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The world's production of plastics has increased dramatically in recent years (about 460 million tons today). The same phenomenon is evident in the production of plastic waste which has currently reached 353 million tons. Reducing plastic pollution has become an important environmental challenge that requires effective and integrated strategies. Recently scientific research is focusing on the identification of insect species which, thanks to their intestinal microbiota, can effectively degrade synthetic polymers. Although the larvae of some coleopterans and lepidopterans are the most studied organisms for the biodegradation of plastics, other species could have similar capabilities. Therefore, the present research has the aim of evaluating the "plastivorous" activity of *Hermetia illucens* (Diptera Stratiomyidae), reared on a diet contaminated by fluorescent polystyrene (PS) microparticles. Evaluation of larval performance did not reveal significant differences in development and mortality between the larval population reared on the control and treated diet. The examination of the larval intestinal tissues through the laser scanning confocal microscope, in bright and fluorescence fields, showed the abundant presence of fluorescent particles of PS in the foregut and midgut and minimally in the hindgut, with translocation of the fluorescent particles of PS within the cells of the midgut epithelium. Moreover, the formation of the PS biodegradation byproducts was investigated. This research, which should be completed with the analysis of the intestinal microbiota to identify the microorganisms involved in the degradation of PS, provides interesting results on the potential "plastivorous" capacity of *H. illucens*.

Keywords: plastic, biodegradation, black soldier fly, plastivorous larvae, microbiota

Extraction, characterization and yield of chitosan derived from *H. illucens* (Diptera Stratiomyidae) pupae reared with different organic waste-based diets

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Chitin, the second-most common polymer after cellulose, can be deacetylated to produce chitosan, a valuable biopolymer utilized in a variety of industries, including agriculture, the food technology, the textile and paper industry, the cosmetics and pharmaceutical industries, and many others. Currently at industrial level, chitin and chitosan are extracted from crustacean waste, a source that has some drawbacks largely related to seasonality and expensive transportation from coastal areas.

To achieve a steady and sustainable product, insects can be an alternative source. One of the most promising insect is *Hermetia illucens* (Diptera Stratiomyidae), which has a high capacity for bioconversion and can turn organic waste into useful biomass, serving as an excellent example of the circular economy.

Demineralization, deproteinization, and finally deacetylation are the three key processes needed to produce chitosan. Chitosan acetylation level, average molecular mass, and dispersity can all vary depending on the time, temperature, and solution strength, which can change the material's solubility and other characteristics.

There is little data on the impact of various diets on the chitin and chitosan yield derived from *H. illucens*, despite recent investigations on the extraction of chitin and the production of chitosan from insects generally, and from *H. illucens* specifically. For this reason, we investigated the physicochemical properties, through ATR-IR and SEM analysis, and yield of chitin and chitosan isolated from *H. illucens* pupae fed with various organic waste-based diets (vegetable, fruit, meat or mixed) and compared to those one reared with the standard diet. All the characteristic peaks were identified by ATR-IR analysis for both chitin and chitosan, meaning that the chemical process was efficient. No significant difference was found regarding the final yield of both by-products, while some modifications of the acetylation degree of chitin and chitosan indicated an influence of the diet factor.

Keywords: chitin extraction, chitosan, black soldier fly pupae, deacetylation, demineralization

Session 4.

Innovative approaches to improve and monitor food safety and quality

Paper-based optical biosensors for monitoring food quality and safety

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One of the current challenges, which is considered a priority by the United Nations, is to ensure food safety, security and sustainability to feed a population which is expected to reach 10 billion persons by 2050. About 420000 deaths per year are caused by unsafe food. Therefore, the monitoring of food safety and quality is an urgent priority.

In the last ten years, thanks to their numerous advantages, including low cost, low carbon footprint and greenness, paper biosensors have received a lot of attention from the scientific community. Paper is an inexpensive, biocompatible support with a network of capillaries enabling self-pumping of fluids without the need of external pumps. Both colorimetric and bio-chemiluminescence detections can be easily implemented in paper biosensors to obtain user-friendly analytical tools for monitoring food safety and quality. Here we report paper biosensors based on enzymes and plant-derived molecules which were integrated into smartphone-based devices for rapid detection of food contaminants and indicators of food spoilage. A chemiluminescence foldable paper-based biosensor was developed to detect organophosphorus pesticides in food matrices with a total assay time of 25 min. The biosensor uses three coupled enzymatic reactions catalyzed by horseradish peroxidase, choline oxidase, and acetylcholinesterase to produce hydrogen peroxide, which is then detected using a customized luminol substrate. The origami approach enables the successive reactions to be triggered sequentially. For smartphone detection, a small 3D-printed holder with a compact dark box was developed.

A colorimetric paper-based sensor that provides a very rapid analysis of biogenic amines produced in food was also developed by exploiting genipin's reaction with biogenic amines. Quantitative detection of putrescine was obtained with the paper sensor combined with the smartphone. Chicken meat samples were analysed to assess the suitability of the sensor to be included in smart packaging showing good potential for future commercialization as a smart label for food spoilage.

This work was in part supported by the PRIMA program, project Fedkito. The PRIMA program is supported by the European Union.

Keywords: enzymes, plant-derived molecules, smartphone detection, chemiluminescence, colorimetric

Electrochemical biosensors for food contaminants detection

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Ensuring food safety directly impacts quality of life, necessitating efficient food contaminant detection devices. Electrochemical biosensors offer advantages of miniaturization, affordability, high sensitivity, selectivity, rapidity, and low sample usage, enabling on-site food analysis. This communication surveys recent electrochemical biosensors for detection of food contaminants with focus on citrinin (CIT), maleic hydrazide (MH), bisphenol-A (BPA) and cadmium ion (Cd++).

A rapid electrochemical method for CIT detection is introduced, employing water-dispersed graphene nanoflakes as a sensing layer on screen-printed electrodes. This approach enables CIT determination below the permissible limit (LOD = 5 µg L−1) in various foods. Molecularly imprinted polymer (MIP) facilitated selective CIT extraction from samples (e.g., rice, blueberry), exhibiting reproducible results and strong correlation with liquid chromatography/tandem mass spectrometry (LC-MS/MS) quantification.

A rapid method to detect harmful MH residues in food is proposed. Synthesis of MIP using ultrasound, combined with an electrochemical sensing, achieved sensitive MH detection (40 ppb LOD) in vegetables. The approach exhibited good reusability, fouling resistance, and accuracy, making it a promising tool for ensuring food safety.

A novel MIP was designed targeting BPA. Theoretical results highlighted methacrylic acid and acrylamide as favorable monomers and acetone as an optimal porogen solvent. A magnetic MIP (magMIP) was synthesized using ultrasound, characterized via multiple techniques, and displayed selective BPA adsorption. The magMIP facilitated BPA detection with a low limit of detection (66 nM) and strong recovery in tap water samples.

An enzymic membrane method was developed for sensitive Cd++detection in water. Horseradish peroxidase was rapidly immobilized onto a membrane for easy Cd++ determination, eliminating pretreatment. An electrochemical biosensor combined the membrane with screen-printed electrodes, providing a linear range of 0.02–100 ppb and a 50 ppt detection limit. The method successfully countered interference from copper ions through a medium exchange procedure.

Keywords: food, citrinin, maleic hydrazide, bisphenol-A, cadmium ion

Development of new technological solutions to extend the shelf life of citrus fruits

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The coating of citrus fruits is a common practice for prolonging their storage lifetime. It aims at preventing physiological and microbiological decays, which are the two major causes of losses along the citrus postharvest chain. Commercial coatings are typically microemulsions containing resins and/or waxes often amended with active chemicals with antifungal action. Due to the growing concerns for health of consumers and food safety as well as for reducing environmental pollution, the research is increasingly approaching the development of new natural, biodegradable, edible coating formulations with safe antifungal properties. Within the framework of the PRIMA project BiOrangePack an innovative and safe bio-active coating, which was prepared through a chemical immobilization of biodegradable chitosan to green processed citrus wastes (peels), has been developed. Laboratory tests have shown that the new coating formulation has a marked antifungal activity, strongly inhibiting both *in vitro* and *in vivo* the viability of fungal and oomycete pathogens of the post-harvest supply chain of citrus fruits, including *Penicillium*, *Alternaria*, and *Phytophthora* species. The active biocoating of BiOrangePack made from citrus wastes is a virtuous example of a circular green economy.

Keywords: coating, citrus fruits, bioactive formulation, antifungal, *Penicillium*

New eco-friendly antifungal products for managing plant pathogens in the citrus fruit supply chain

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Citrus fruits are threatened by several post-harvest diseases, among which those caused by fungi and oomycetes stand out for determining the major product losses. The management of these disease comprises several post-harvest practices, which often include the treatment of fruits with synthetic chemicals. However, due to the increasing concerns for health hazards and environmental pollution, the use of synthetic actives is undergoing increasing limitation, which make necessary the adoption of alternative strategies to control post-harvest citrus diseases. Within the framework of the PRIMA project BiOrangePack, new eco-friendly products for the safe management of citrus post-harvest diseases caused by fungi and oomycetes have been developed. Specifically, an extract obtained from the minimal processing of shrimp wastes provided a significant *in vitro* inhibition of fungal and oomycete plant pathogens; in *in vivo* tests it also strongly reduced the severity of molds of citrus fruits caused by *Penicillium* species. Additionally, a green formula of seaweed extract and plant derivatives mixed with a reduced dose of active chemical, triggered the natural defense response of citrus fruits, drastically reducing the severity of infection by *Penicillium digitatum*. The eco-friendly products developed in BiorangePack project could represent a promising alternative to traditional post-harvest chemical treatments of citrus fruits.

Keywords: citrus, post-harvest diseases, biostimulants, resistance-inducers, natural extracts

Sensory analysis as a screening tool for the feasibility of essential oils combined with chitosan as food storage protectants

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Insect food spoilage contribute to the global issue of food loss and waste, which annually involves over 30% of food, negatively affecting both people and the planet.

To reduce insect food spoilage, the use of plant essential oils (EOs) is spreading, since EOs are characterized by being bioactive, biodegradable and proven effective. However, EOs application is limited by their high volatility, their composition variability and above all by their strong smell.

The research aim was to identify EOs with a suitable sensory profile to be added to chitosan (CH) edible coating and to evaluate the EOs-enriched CH effectiveness in preserving fruits against spotted *Drosophila Suzukii* (spotted drosophila), a small fly responsible for damaging many fruit crops, and meat products against *Calliphora vomitoria* (blowfly), which lays its eggs in meat causing its deterioration.

A specific sensory analysis method was developed for the EOs selection, testing the samples as pure ethanol solutions, in combination with food and with food + CH.

The effect of CH, EOs, and EOs-enriched CH solutions on food against drosophyla or blowfly as well as against water loss, lipid peroxidation, and colour changes were also evaluated.

Our results suggest that the application of EOs as fruit and meat protectants is possible, on condition that the EOs have been chosen considering both their repelling action against the insects and their sensory acceptability.

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Keywords: *Laurus nobilis*, *Piper nigrum*, *Melaleuca alternifolia*, *Citrus reticulata*, sensory acceptance

Application of chitosan and essential oils to protect fresh fruits from *Penicillium* spp. infections during storage

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Consumers have high consideration of their health and the environment. The use of natural antifungal agents and biodegradable materials has become more popular to prevent food spoilage. Chitosan (CHT) and the addition of essential oils have been shown to be valid strategies to improve the preservation of fresh fruits and reduce their degradation in the post-harvest. The aim of this study was to investigate the effects of CHT (from crab shells and *Pleurotus ostreatus*) combined with the red mandarin essential oil (MEO, extracted from *Citrus reticulata*) on the growth of *Penicillium* spp. (causal agents of apple and citrus rot). A collection of *Penicillium* spp. isolated from apple, grape and citrus fruits were selected on the basis of their pathogenicity on apple and citrus. Then, *P. expansum* Ba-4/M1 and To-05/M1, *P. digitatum* An-4/M1 and RC/M1, and *P. italicum* RC/M1 were further tested in vitro for antifungal activities of CHT and MEO. The combination of 1.0% CHT, added with 1.0% MEO resulted as the best to control both mycelial growth and spore germination as well as infection of fresh fruits (around 30% and 70% of infection reduction on apple and citrus, respectively). According to results here obtained, CHT incorporating MEO could be used, as an alternative to chemical treatments, for fruit packaging.

Keywords: chitosan, red mandarin oil, fresh fruit, post-harvest, *Penicillium*

Have chitosan associated to essential oil a protective effect on tomatoes against the pest *Spodoptera littoralis*?

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In this project, we tested the effects of chitosan associated with essential oils after application on tomato fruits against the polyphagous pest *Spodoptera littoralis*. We thus established the toxicity curves of essential oils and then tested the effects on the post-embryonic development of the insect by feeding food contaminated with oils. We also studied the behavioral responses of the larvae to essential oils and chitosan in order to know if the odors had a repulsive or attractive power over time.

Keywords: chitosan, essential oils, insect pest, tomatoes

Liquid chitosan with essential oils to protect fresh foodstuffs from the oviposition of dipteran pests

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One of the FEDKITO project goals is the protection of fresh foodstuffs from the threat posed by target insect pests during post-harvest and post-production stages to try to reduce losses and wastes. Work package 3 focused on the evaluation and comparison of the protection given by liquid chitosan (CH) formulations enriched with specific essential oils (EOs) against the oviposition of four Diptera species: *Calliphora vomitoria* (Calliphoridae), *Piophila casei* (Piophilidae), *Drosophila suzukii* (Drosophilidae), and *Ceratitis capitata* (Tephritidae). Our deterrence tests were carried out on representative food matrices such as, respectively, raw beef meat, cheese and ham in curing, blueberries, and kumquat fruits. The EOs involved were, first, selected by trained sensory analysts and, then, characterised through Gas-Chromatography coupled with Mass-Spectrometry. We used *Laurus nobilis* and *Piper nigrum* EOs to match with meat products and cheese and *Cinnamomum verum* and *Citrus reticulata* EOs on fruit.

The necessity to enhance and prolong the efficacy of EOs, limited by their volatile nature that causes rapid changes in their composition, was achieved by adding them to CH as a carrier matrix. All the proposed treatments produced a significant oviposition reduction compared to the untreated control samples, and we ascertained prolonged protection over time (up to 72-96 hours) when using the EOs-enriched CH solutions. The results of the trials will be discussed based on the different biology and oviposition behaviour of the four dipteran pests and the suggested practical applications of the liquid CH formulations enriched with EOs in food protection.

Keywords: oviposition deterrence, *Calliphora vomitoria*, *Ceratitis capitata*, *Drosophila suzukii*, *Piophila casei*

Effect of chitosan coating solutions enriched with essential oils on fresh food quality during storage

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Biopolymers include a wide range of molecules, such as proteins, nucleic acids, polysaccharides and other complex organic compounds, were extracted from different bio-wastes. Among these polymers we can cite, Chitosan (CHT) which is an edible and biodegradable polymer derived from the deacetylation of chitin, with antimicrobial and antifungal activity and low gas permeability (CO2 and O2). Coating solutions and films based on chitosan and natural extracts have shown promise due to their notable effectiveness in extending the shelf life of food products. The conducted studies aimed to evaluate the effect of Chitosan-cinnamon, laurel, and clove essential oils (EO), as a natural packaging material, on apple, tomato and meat quality during cold storage. The Chitosan was obtained from shrimp exoskeletons. Cinnamon, laurel, and clove essential were added to form a liquid packaging material. The fresh foods treated were soaked in Chitosan-extracts emulsion for 1 min and left to dry, and then stored at 4°C for 10 to 30 days.

The results obtained by the infrared spectroscopy method and the spectrophotometric assay showed that the degree of deacetylation of the extracted chitosan was greater than 80% when compared with commercial chitosan. Regarding the results, apples treated with CHT and cinnamon essential oil had the lowest weight loss, whereas the highest was recorded for untreated apples. Titratable acidity decreased during storage for all samples, while pH values remained relatively stable. Phenol and flavonoid contents and free radical scavenging capacity of apples treated with CHT and CHT-Cinnamon essential oil were higher as compared to control fruits. Regarding the treatment of tomato CHT in combination with laurel essential oil increased significantly the phenol, flavonoid, and lycopene content of treated tomatoes compared to untreated tomatoes. The treatment of tomatoes with CHT-laurel essential oil showed significant antioxidant activity compared with chitosan alone. Moreover, tomatoes treated with chitosan and chitosan-LEO inhibit the growth of spoilage bacteria. On the other hand, the treatment of the meat with a coating solution based on CHT and clove EO allowed to preserve the meat quality during storage. In fact, the meat treated with the coating solution enriched with EO had the highest protein and lipid content compared to the untreated one.

In summary, our studies showed that CHT and the combination of CHT-EO used as packing material could improve the preservation of fresh food quality during cold storage. These results suggest that this biopolymer may be an interesting and promising compound for a natural packaging alternative to maintain the quality of fresh foods and extend their post-harvest shelf life.

Keywords: chitosan, extraction, essential oils, coating solution, storage

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