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# ORAL PRESENTATIONS



# **Environment as part of One Health**

# Unveiling potential antimicrobial peptides in dairy wastewater: enzymatic hydrolysis of milk proteins for the generation of bioactive peptides

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## **Abstract :**

The dairy industry generates substantial volumes of wastewater, comprising primarily white and cleaning wastewater derived from rinsing and cleaning-in-place procedures. The current study focuses on the hydrolysis of milk proteins from white wastewater, as an opportunity to valorize this dairy waste. Four different enzymes were tested (pepsin, trypsin, pronase E, and thermolysin) and the hydrolysis performances were analyzed. The degree of hydrolysis values, ranging from 2% to 13%, influenced the protein/peptide content in each hydrolysate. UPLC-MS/MS characterization identified differences in peptide sequences among hydrolysates produced by the four enzymes. The effect of hydrolysis duration was also explored with a comparison between these peptide populations with notable differences observed between 30 and 240 minutes of hydrolysis. Notably, 555 peptides were identified after 30 minutes, increasing to 693 after 240 minutes. After 240 minutes of hydrolysis, the number of peptide sequences identified was different according to the enzyme used: 181 peptides from thermolysin hydrolysis, 153 from pepsin, 126 from trypsin and 83 from pronase E. The main characteristics of these peptides were analyzed revealing 17 known antibacterial peptides prominently identified after 240 minutes of both pronase E and thermolysin hydrolyses. Despite their identification, these peptides were deemed insufficient in concentration to inhibit tested strains in antimicrobial assays, including *Clostridium tyrobutyricum* and *Pseudomonas aeruginosa*. Furthermore, peptides derived from Pronase E 30min, Pronase E 240 min, and Thermolysine 240 min. exhibited notable antifungal activity against *Mucor racemosus* at an MIC of 2.5 mg/ml of proteins, while no activity was observed against *Penicillium commune*. In future directions, electro dialysis with ultrafiltration membrane (EDUF) emerge as a prospective avenue to concentrate the potential antimicrobial peptides produced after 4 hours of hydrolysis with pronase E and thermolysin. Additionally, a parallel protocol is currently underway, focusing on the assessment of diverse bioactivities, including antioxidant and antihypertensive bioactivity, within the fractions generated through the four enzymatic conditions.

**Keywords:** Enzymatic hydrolysis; White wastewater; Antimicrobial peptides; Circular economy.

# Peptide migration and fraction antimicrobial activities are modulated by electric current conditions during electro dialysis with ultrafiltration membrane: a comprehensive machine learning-peptidomic study

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## **Abstract :**

Enzymatic hydrolysis of blood, by generating antimicrobial peptides, is a promising avenue to produce high added-value new products, in a circular economy context. However, activities of such complex solutions can be limited by the highly diverse peptide population. Electrodialysis with ultrafiltration membrane (EDUF), separating peptides based on their charge and molecular mass, is an eco-efficient strategy to obtain fractions with enhanced bioactivities and increased purity. While continuous electrical current (CC) is conventionally applied, alternative modes like pulsed electric field (PEF) and polarity reversal (PR) can modify mineral selectivity, mitigate fouling, and optimize energy consumption. To date, no studies investigated effects of different electrical current conditions on peptide migration. In this context, five conditions were explored - CC, PEF ratio 1 and 10, and PR ratio 1 and 10 - on a well-characterized discolored porcine cruor hydrolysate. Using UPLC-MS/MS, migration rates were calculated for 150 peptides and 45 peptide physicochemical characteristics were extracted using bioinformatic tools. Employing a machine learning approach, a regression tree model was applied for each electrical conditions to establish links between peptide migration rates, physicochemical characteristics, and specific electric current conditions. Results revealed distinct root nodes, with molecular mass identified as the major feature for PR 1, and pI for CC, PEF 10, and PR 10. PEF 1 exhibited an intermediate regression tree. Tree comparisons helped evaluate similarities among electrical current conditions and predict key physicochemical characteristics influencing the selective migration of peptides. This study proposes a tentative explanation of the phenomena involved, such as effects on the diffusion boundary layer and concentration polarisation. Antimicrobial activities were assessed post-separation and revealed how the current conditions, by modifying the peptide migration, modulated the antimicrobial activities towards antifungal or antibacterial. Moreover, under specific electrical conditions, EDUF showed the capacity to produce two valuable fractions simultaneously with different antimicrobial activities.

**Keywords :** Co-product; Peptides; Antimicrobials; Separation; Processes.

# **Integrated assessment of the toxicological and ecotoxicological effects of phytopharmaceuticals used during potato cultivation – the TEPoT French research project results**

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## **Abstract :**

Two laboratories, from ULille and JUNIA, have partnered with the Hauts-de-France Agriculture Regional Chamber to study the effects of the use of phytopharmaceutical products (PP) during a potato whole culture season. Multidisciplinary researches were carried out successively at three scales (field, experimental plots and lab), in order to establish an inventory of agricultural practices and to study the ecological, ecotoxicological and toxicological effects of the possible PP multi-residual contamination on soil functioning, the crop quality and different plant and animal models. The project first phase involved conventional (C) or organic (O) potato culture plots, all located in the region in two distinct contexts (large-scale or peri-urban fields). On each plot was carried out an analysis of cultural practices and uses, a soil recognition and sampling, a crop sanitary state characterization, a measurement of PP multi-residual contaminations, a study of plot biodiversity and an assessment of the impregnation of lichens transferred around plots. The second phase concerned potato crops (C or O) on experimental plots under better controlled conditions (soils, technical routes, PP natures and doses) and a same follow-up was conducted. Finally, the last phase used the soils and part of the harvested tubers of these experimental plots to conduct lab-exposures, to evaluate the ecotoxicological and toxicological responses of four biological models, namely earthworm, clover, cabbage and mouse. After a brief recall of the results obtained in the first phases (presented at the last OHID), we will discuss in more detail of the ecotoxicological project part. Results highlight the complementarity of our different test-organisms, and the interest of realistic exposures in microcosms. The toxicological project part is the topic of another presentation at the present OHID. We will conclude on the interest of our integrated assessment, which consider possible effects and interactions between PP residues for a whole culture season.

**Keywords :** Phytopharmaceuticals; Multiscale; Ecotoxicology; Crops; Multi-residue.





# **Plant as part of One Health**

# Microbial endophytes from *Isatis tinctoria* L. for the production of specialized metabolites

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## **Abstract :**

*Isatis tinctoria* L., commonly known as woad (*Brassicaceae*) has a long and well documented history as an indigo dye plant in temperate climates, but also as a medicinal herb. From the Middle Ages up to the 18th century, *Isatis tinctoria* was the most important indigo dye in Europe, but lost importance with the access to less expensive indigo sources. Woad was also widely utilizing for its medicinal properties, and was recommended for the treatment of wounds, ulcers, haemorrhoids, snake bites and various inflammatory ailments. With the declining importance as a dye and the disappearance of woad cultures, the plant also fell into oblivion as a medicinal herb. In recent years, renewed interest in natural dyes showed by the dyestuff industry and its potential use for medicinal and cosmetic products has encouraged growers to reintroduce *Isatis tinctoria* crops in the European agricultural system (Spataro, G. et al., 2008), but also very recently in the region Hauts de France. It is also well known that several genera and species of bacteria or fungi living in the internal tissues of plants as endophytic microorganisms, benefit plants by promoting their growth and increasing their resistance to abiotic and biotic stresses (Vandenkoornhuyse et al., 2015). In addition, there is no doubt that as essential components of the plant microecosystem, endophytes have a profound influence on the synthesis and accumulation of various specialized metabolites in the host plants (Li et al., 2023). However, many variables, including temperature, precipitations or soil characteristics, can affect the endophyte populations within the plant. Due to the therapeutic and cosmetic values of *I. tinctoria*, the plant-endophytes interaction approach represents a promising way to develop new effective extracts. In the present work, we explore the morphological, biochemical and genetic diversity of endophyte microorganisms isolated from roots of *Isatis tinctoria* grown on trace metal-contaminated soils or not. The role of the endophytes to improve plant growth, as well as to produce specific specialized metabolites (polyphenols, flavonoids) with antioxidant and/or antimicrobial properties is highlighted.

**Keywords :** *Isatis tinctoria*; Endophytes; PGPR; Heavy metals; Specialized metabolites.

# Exploring the bioactive effects and functional properties of peptides derived from brewer's spent grain proteins

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## **Abstract :**

To reduce pressure on climate change, using fewer fossil fuels and other natural resources, valorization of agri-food by-products in a circular economy context represents one of the most promising directions. Moreover, recent awareness of the connection between global population growth, health issues, and climate change, as well as food provision, has led to an increase in interest in alternative protein sources. Brewers' spent grain (BSG) is a source of plant protein, representing about 85% of all by-products generated in the brewing sector, accounting for 18–30% of proteins. Therefore, it is very interesting to explore their functional and bioactive potential by hydrolyzing these proteins to generate new and more promising means of valorization. Thus, this study proposes to produce a protein hydrolysate obtained from BSG protein concentrate and assess its functional and bioactive properties. The BSG protein concentrate was hydrolyzed with trypsin and chymotrypsin for 5h with a 3% protein solution. The degree of hydrolysis was then assessed by the O-phthalaldehyde (OPA) method, and RP-HPLC-MS/MS were used to identify the peptides produced. After hydrolysis, functional properties, including solubility, emulsifying capacity, foaming capacity, viscosity, color, and water retention power of the hydrolyzates at different hydrolysis stages were explored. Bioactive properties including antioxidant activity, antimicrobial potential and potential health benefits such as anti-diabetes and anti-hypertensive activity have also been evaluated. Additionally, the cytotoxicity tests were carried out on the obtained hydrolysates. These peptides can therefore have a high application potential as an ingredient in developing functional foods and nutraceuticals.

**Keywords :** Brewers spent grains; Proteins; Functional properties; Bioactive peptides.

# New fungicidal candidates: towards an innovative high-throughput screening approach for biological control

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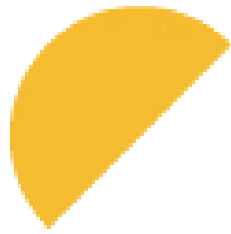
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## **Abstract :**

Plant diseases are a significant threat to global agriculture, causing substantial economic losses and affecting production yields [1,2]. Fungi contribute to approximately 70 to 80% of these diseases [1]. Synthetic pesticides have conventionally been employed to combat phytopathogens, resulting in the use of about 4 billion tons of pesticides in 2019 [1], but they cause health and environmental concerns [3]. Furthermore, the development of fungicide resistance has become a major problem in crop management [2,3]. In response, biological control methods using microorganisms and their metabolites have emerged as a sustainable alternative [2]. However, current biocontrol solutions have limitations, such as some plant disease stay currently without biocontrol solutions. To address this, our study aimed to isolate and characterize new fungicidal strains against specific phytopathogens. To this aim, we developed a new high-throughput screening workflow combining both colony picking, liquid handling and MALDI-TOF analysis, to perform fully automated microorganism identification and antibiograms against targeted phytopathogens in SBS-plate format. As a result, through our isolation strategy using 7 samples of rapeseed fields and 20 different culture media we recovered nearly 3,000 isolates. The first screening of about one-third of the bank using our workflow allowed to identify 226 isolates belonging to 11 genera and 15 species, and 227 showing activity against the targeted phytopathogen with different strengths. These findings demonstrate the promising of our high-throughput screening method in identifying potential biocontrol candidates. Further validation and investigation are in progress to confirm the efficacy and safety of these isolates for fields application. Our study highlights the potential of routinely integrating high-throughput screening methods with various isolation strategies to accelerate the selection of biocontrol agents. Our research holds promise for addressing the pressing need for sustainable solutions in global agriculture and plant pathology. By promoting biocontrol methods, we contribute to a comprehensive 'One Health' perspective, benefiting ecosystems, animals, and human well-being.

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**Keywords :** Antifungal testing; High-Throughput Screening; Microorganism; Isolation Strategy; Biocontrol.



# **Animal as part of One Health**

# Probiotics and their derivative preparations as nature-based solutions for one health advancements

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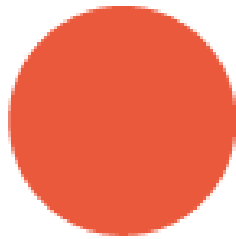
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## **Abstract :**

Face to recent global disease outbreak such as COVID-19, there has been a growing adoption of a One Health approach that considers how environmental changes influence the risks of infectious and chronic diseases for humans, animals, and plants. Therefore, designing adaptive, forward-looking and multidisciplinary solutions to these challenges is necessary with more preventive actions by addressing the root causes and drivers of infectious diseases, particularly at the animal-human-ecosystem interface. One health approaches, such as reducing synthetic agricultural inputs and increasing biodiversity, present a global strategic framework to reduce the risks of new emerging infectious diseases at the animal-human-ecosystem interface, which requires a collaborative effort of multiple disciplines-working locally, nationally, and globally. The exploration of probiotics and their internal and external molecular elements appears an interesting route in One Health initiatives. As benefit live microorganisms and preparations to the host, the probiotics and their derivatives act in promoting health in direct and indirect manners by preventing and treating many diseases for humans and animals, which are nowadays recognized throughout the world. Moreover, their roles in plant growth promoting and protection, soil fertilizing, as well as environment protection and depolluting have also been evidenced through many experiments conducted in labs and pilot with large scale, and currently in expansion. These bio-based preparations may exist in diverse forms, including mono- or multi-strain living microorganisms (living components), postbiotics as internal and external structural elements sourced from probiotics (non-living components), and synbiotic preparations containing a mixture of probiotics and substrates such as prebiotics. They can be incorporated into the formulations of either food and feed supplements or biopesticides and biofertilizers. Our contribution to this conference will be an overview and discussion of relevant functionalities and applications of probiotics and their derivative products as nature-based solutions for One Health advancements through various relevant examples.

**Keywords :** Probiotics; Prebiotics; Postbiotics; Synbiotics; Nature.



# **Human as part of One Health**

# Antifungal metabolites from *Streptomyces sp.* as potential biomolecules to combat multi-resistant *Candida sp.* responsible of vaginal candidiasis

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## **Abstract :**

Fungal infections are considered as a global public health issue. These affect more than a billion people with around 11 million cases per year, and responsible for significant morbidity and mortality worldwide, particularly in immunocompromised patients. *Candida sp.* is among the main fungi involved in hospital infections, and *Candida albicans* is especially the causative agent of candidiasis in humans. Even though this fungal species is part of the normal microbiota of the mucous membranes of the oral cavity, gastrointestinal tract, and vagina in healthy humans, it can cause superficial and systemic infections in immunocompromised situations in certain circumstances and in susceptible individuals. This is due to their high adaptability to different host niches. A recent increase in the number of *Candida albicans* strains resistant to currently used antifungal drugs has encouraged researchers and scientists throughout the world to search new sources of antifungal molecules to effectively treat mycotic infections. *Streptomyces* is a bacterial genus, gram positive, widely recognized for its capacity in producing multiple bioactive molecules such as numerous natural antibiotics. This microorganism appears therefore among the potential candidates for producing new natural drugs to combat the emergence of bacterial antimicrobial resistance (AMR). This paper will report on the antifungal activities of two efficient metabolites isolated by our team from a culture media of identified *Streptomyces sp.* for treating vaginal candidiasis caused by multidrug-resistant *Candida albicans*. The next step will be the chemical structure identification of these bioactive metabolites.

**Keywords :** Global public health; Fungal infections; Beneficial microbes; AMR; Secondary metabolites.



# Valorizing Belgian endive co-products for antioxidant ingredient innovation

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## **Abstract :**

Belgian endive (*Cichorium intybus* var. *foliosum*) is an *Asteraceae* mainly produced in the north of France with 124,400 tons per year, but this production generates a lot of co-products like roots after forcing or endive peelings (Anon s. d.). Belgian endive is rich in inulin, and in specialized metabolites like polyphenols and lactone sesquiterpenes. The latter are responsible for bitterness and have been reported to exert different activities such as anti-inflammatory or anti-tumoral activities. Polyphenols are the main specialized metabolites produced by Belgian endive, particularly hydroxycinnamic acids such as chicoric, chlorogenic, caffeic, or caftaric acid which are also presents in co-product. Their antioxidant capacity can be improved by bioconversion. In this context, the main goal of this study is to develop an antioxidant ingredient by bioconversion that exerts its activity after gastrointestinal digestion in the intestine and after crossing the intestinal barrier. To reach this objective, endive co-products were first bioconverted by selected acid bacteria. Then, the supernatants were concentrated and digested using the INFOGEST protocol, which mimics the three first steps of digestion (mouth, stomach, and intestine) (Brodkorb et al. 2019; Fouré et al. 2018). Digested samples were challenged with a cell co-culture (Caco-2/HT29-MTX) intestinal barrier model. The antioxidant capacity was followed in HepG2 hepatic cells at each step of the process (after bioconversion, digestion, and crossing the intestinal barrier). Results obtained evidenced that bioconversion enhanced the antioxidant activity of Belgian endive by-products which was maintained after digestion and after crossing the intestinal barrier. Therefore, this ingredient appears to be promising for nutrition, feed and pet-food, or even cosmetics applications.

**Keywords :** Belgian endive; Co-product; Valorization; Antioxidant activity.

# Antibiotic resistance in a One Health perspective: from animal to the plant

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Antibiotic use in livestock agriculture is high in many countries. When livestock animals receive antibiotics, a large fraction of these compounds is excreted unmetabolized in urine and feces. When manure is used to fertilize fields, antibiotic residues may end up in the soil. After fertilization of the fields, antibiotic residues may also spread to other ecological niches. They may leach to groundwater, which is dependent on the soil type and antibiotic properties. Due to runoff, residues may enter surface water and watercourses. Antibiotic residues may be taken up by crops growing in soils fertilized with manure or irrigated with water containing antibiotic residues. Antibiotic use in human and veterinary medicine is considered as the key driver for the antibiotic resistance selection. Correlation between antibiotic use and resistance has been demonstrated both in the human and the veterinary sector. Awareness is growing that very low antibiotic concentrations, up to several hundredfold below the Minimum Inhibitory Concentration (MIC), are also able to select for antibiotic resistance. Upon antibiotic use in animals, antibiotic-resistant bacteria may be selected in the gastrointestinal tract of the animals itself as well as in different environmental niches such as fertilized fields, groundwater, surface water and watercourses and possibly even on crops. Furthermore, in each ecological niche, these antibiotic-resistant bacteria may—when the antibiotic resistance gene is located on a mobile element—pass these resistant genes to susceptible bacteria. All ecological niches are connected according to the One Health Concept. Bacteria, including pathogens and antibiotic-resistant bacteria, and antibiotic residues may reach livestock or humans when raw manure is spread on arable lands. Eventually, this may lead to diseases difficult to treat for both animals and humans. At ILVO, through several projects, the fate of antibiotic residues and resistance genes was studied in manure, soil, water and leek.

**Keywords** : One health; Antibiotics; Residues; Resistance.

# Screening of mineral-chelating capacities of enzymatic milk protein hydrolysates using high throughput experimentation

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## **Abstract :**

Studies on the nutritional and health benefits of milk proteins have led researchers to question the impact of human gastrointestinal enzymes on the liberation of dairy bioactive peptides, particularly those with mineral binding activity. In this context, hydrolysis of caseins by trypsin has been widely shown to release peptides that enhance the bioavailability of essential biometal cations such as calcium, iron, and zinc through chelation mechanisms. And due to their ability to improve mineral bioavailability, by reducing mineral precipitation under gastrointestinal conditions and promoting their intestinal absorption, which is comparable or even superior to that of traditional organic salts with fewer side effects, the use of protein hydrolysates as ingredients capable of mitigating mineral deficiencies is now extensively considered. This study aimed to identify the combinations of milk protein enzymes that generate the highest metal chelating properties. Standardized experiments have been conducted at REALCAT high throughput robotic platform to reaffirm results commonly reported in the literature. Using a high reproducibility parallelized microbioreactor system, ingredients from the dairy industry (total milk proteins, micellar caseins and whey proteins) have been treated using five food-grade enzymatic products via one-step or two-step multienzyme hydrolysis processes, resulting in over one hundred different hydrolysates. These were then screened for their ability to bind calcium, magnesium, iron and zinc using a series of four automated colorimetric microplate assays. Subsequently, an automated microplate OPA assay was performed to determine their degree of hydrolysis. A factorial analysis of mixed data highlighted the differences in binding capacities between different protein sources, with ingredient rich in micellar caseins exhibiting superior properties compared to whey proteins. Interestingly, we observed that a high degree of hydrolysis did not necessarily correlate with improved iron binding capacity. These findings contribute to the understanding of how to optimize protein hydrolysates for enhanced mineral bioavailability in fortified dietary products.

**Keywords :** Mineral-binding; Milk; Protein; Hydrolysate; Screening.

# POSTERS



# **Plant as part of One Health**

## Taxonomic and functional characterization of the rhizosphere microbiome of chicory (*Chicorium intybus* var. *sativum*)

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### **Abstract :**

Industrial chicory (*Chicorium intybus* var. *sativum*) is a cultivated plant well known in the north of France for its food, fodder and medicinal importance. Many plant metabolites have been identified as essential to impart its functional food quality. Several agronomic and physiological parameters participate in maintaining the expected metabolic composition of this plant. And among them, the root microbiome which is known to play an important role for plant growth and health. We studied the structure and function of the rhizosphere microbiome of chicory plants using targeted sequencing and metagenomics analyses. We specifically aimed to characterize (i) the diversity of bacterial and fungal communities in soil microbiome associated with chicory plants and (ii) to elaborate relationships between microbial communities and functional traits. We observed an environmental site effect by cultivating the same chicory genotype on 6 different land plots at different locations in the department Haut de France. We demonstrated the importance of substrate type, soil composition and land use history in the composition of the root microbiome. We also demonstrated the role of heredity in the constitution of rhizosphere microbiome by cultivating several genotypes on the same land plot. Our study presented for the first time a comprehensive taxonomic and functional pattern of *Chicorium intybus* rhizosphere microbiome and highlighted the role of the environment and the role of the heredity of the plant for its constitution.

**Keywords :** Chicory; Rhizosphere; Microbiome; Metagenomics.

## Towards a robust stimulation of plant growth and health with PGPR

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### **Abstract :**

Soil and plant health are inextricably connected to agriculture, and root systems play a major role in the growth and health of cultivated plants. They are in constant interaction with microorganisms and are at the core of water and mineral nutrition. Also, agriculture needs to decrease the use of synthetic pesticides, and biostimulation and biocontrol agents could help pave the way towards a more sustainable agriculture. However, the efficacy of biostimulants and biopesticides varies depending on environmental conditions, and soil microbial communities represent a factor of variability. This context raises questions about the interactions between plant development, soil microbiota, and beneficial microorganisms. For this, the RootBoost project aims at addressing two issues related to the efficiency of Plant Growth-Promoting Rhizobacteria (PGPR) in agriculture. How do PGPR impact plant fitness and the related soil microbiota? The first part of the project aims to study the effects of a strain of *Bacillus velezensis* on the development of tomato plants. To address this question, we plan to conduct experiments to assess its bioactivity on root development, plant biomass, and the related microbial communities in a standardized substrate, and how it can be modulated with additives. How does the environment, such as the microbial communities in the soil, affect the efficiency of a PGPR? The second part focuses on the robustness of action of a PGPR on plants in contrasted soil conditions. In practice, several soils will be characterized and then used as growth substrates to evaluate how the bioactivity of *Bacillus velezensis* on wheat plants may change in the presence of contrasting soil microbial communities. The preliminary results related to (1) the selection of the *Bacillus velezensis* strain, (2) the first trials performed on tomato using the selected strain, and (3) the soil selection process for the experiment on wheat will be presented.

**Keywords :** PGPR; Soil; Microbiota; Plant; Root.

## Exploring the biological activities of *Pseudomonas*-derived biocontrol compounds on the wheat-*Zymoseptoria tritici* pathosystem

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### **Abstract :**

*Zymoseptoria tritici* is a hemibiotrophic fungal phytopathogen which is responsible for one of the most concerning foliar diseases on wheat, *Septoria tritici* blotch. This disease leads to disastrous yield reductions which can reach up to 50% during severe epidemics. The methods currently used to control this disease rely mainly on the use of chemical fungicides. However, because of the increasing concerns about the potential impacts of conventional phytosanitary products on human and environmental health, new agroecological protection tools have to be developed. One of the most promising alternatives is the use of microbials, and the large range of biomolecules they are able to synthesize, as biocontrol products. Here, we summarize some of the results displayed by crude extracts, metabolites (cyclic lipodepsipeptides (CLPs), rhamnolipids (RLs) and siderophores) as well as bioinspired molecules (rhamnolipids), obtained from *Pseudomonas sp.*, on the wheat-*Z. tritici* pathosystem. Firstly, the direct effect of all of these compounds against *Z. tritici* was assessed and the most active ones were selected for further experiments. Whereas CLPs and siderophores did not present significant antifungal activity in our conditions, they could display other benefits for wheat plants. Culture extracts of *Pseudomonas syringae* as well as some RLs exhibited significant fungal growth inhibition and had their protection efficacy evaluated in greenhouse conditions. In particular, the biological activities of 19 RLs on the wheat-*Z. tritici* were assessed. Among them, mono-RLs with either ether or ester links and a fatty acid tail composed of 12 carbons were the most promising ones. More specifically, the Rh-Est-C12 was found to be the most active compound, activating plant ROS detoxifying pathway and reducing disease symptoms by up to 79% in planta. This study provides new insights into the biocontrol activity of *Pseudomonas*-derived compounds against *Z. tritici*.

**Keywords :** Wheat; *Zymoseptoria tritici*; Biocontrol; *Pseudomonas*; Rhamnolipids.





# **Animal as part of One Health**

A1

## Turkey Cruor as a source of bioactive peptides: How pH and peptic hydrolysis duration could affect the peptide population ?

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### **Abstract :**

The consumption of meat protein will be increasing by 14% and the poultry meat will constitute 41% of all the protein from meat sources in 2030 [1]. Consequently, global pressure is growing to minimize the environmental impact of slaughterhouses. One promising way to address this issue is to valorize by-products such as blood, by the production of bioactive peptides using enzymatic hydrolysis [2]. To the best of our knowledge, no research has been previously performed regarding the peptic hydrolysis of turkey cruor which contains, after blood centrifugation, hemoglobin. This project presents the impact of pH (2, 3, 4, and 5) and enzymatic hydrolysis duration (T30 min and T180min) of turkey cruor on the peptide population and their bioactivity (antimicrobial and antioxidant). Four bacterial strains *Listeria ivanovi* (HP B28), *Salmonella enterica ssp* Newport (ATCC 6962), *Campylobacter Coli* (ATCC 33559) and an *Escherichia coli* (MP 4100), two filamentous fungus strain, *Mucor racemosus* (LMA-722) and *Paecilomyces spp* (5332-9a), a yeast strain *Rhodotorula mucilaginosa* (27,173) were tested to evaluate the antimicrobial activities of hydrolysates at two different durations of hydrolysis (T30 min and T180min). Orac test was performed to evaluate the antioxidant activity. For the results, antifungal and antiyeast activity was observed: the lowest Minimum inhibitory concentration (MIC) for *Paecilomyces spp* was observed at pH2 T30min and pH3 T30min (0.63 mg/ml), for *Mucor racemosus* and *Rhodotorula mucilaginosa* the lowest MIC was observed at pH3 T30min (1.25 mg/ml and 0.63 mg/ml respectively). Furthermore, no antibacterial activity was detected against the four bacteria tested. For the antioxidant activity results, it was found that the concentration (0.313 mg /ml) of hydrolysates produced at pH 3 T30 min presents the highest antioxidant activity (199.67± 0.49 µM Trolox Eq). Based on these results, one can speculate that pH3 T30 min presents the most promising condition to produce antifungal, antiyeast and antioxidant peptides.

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References: 1-OECD-FAO Agricultural Outlook 2021-2030. (n.d.). 2-Bah, C.S.F., Bekhit, A.E.-D.A., Came, A., McConnell, M.A.: Slaughterhouse blood: an emerging source of bioactive compounds. Compr. Rev. Food Sci. Food Saf. 12, 314–331 (2013)

**Keywords :** Turkey cruor; Hemoglobin; Enzymatic hydrolysis; Antimicrobial and antioxidants peptide.

## Species diversity and antimicrobial resistance profile of *Clostridium* from cecal content of broiler chickens in Tunisia

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### **Abstract :**

**Background:** Many toxigenic *Clostridium* species could induce clinical infections in humans and animals. Hence the need to understand their ecology, route of transmission and levels of antimicrobial resistance (AMR).

**Objective:** This study determined the prevalence, AMR pattern, toxin-encoding genes and genetic diversity of *Clostridium spp.* isolates from caecal samples from industrial chickens in Tunisia.

**Materials and Methods:** A total of 102 cecal samples from industrial chickens were collected from different regions of Tunisia and processed for *Clostridium spp.* recovery. The samples were cultured (anaerobically) in Brain Heart Infusion (BHI) broth and plated on *Clostridium* selective media. All colonies presumptive of *Clostridium* were subcultured and identified by biochemical and 16S rRNA-PCR tests. The antimicrobial susceptibility phenotype for all identified isolates was investigated by disk diffusion method. Moreover, *C. perfringens* isolates were screened for the presence of genes encoding the alpha toxin (cpa), beta toxin (cpb), epsilon toxin (etx) and iota toxin (itx) by PCR.

**Results:** A total of 126 *Clostridium spp.* isolates were obtained from 77 of the 102 cecal samples tested (75.5%). The following species were identified among these isolates (n° isolates): *C. sporogenes* (54), *C. perfringens* (16), *C. tepidum* (14), *C. tertium* (12), *C. butyricum* (10), *Clostridium sp* (7), *C. cochlearium* (5), *C. sartagoforme* (5), *C. saccharobutylicum* (1), *C. niameyense* (1) and *Clostridiales bacterium* (1). The following antibiotic resistances were found among 104 tested *Clostridium* isolates: tetracycline (36.5%), ciprofloxacin (62.5%), chloramphenicol (23.1%), clindamycin (93.3%), rifampicin (27.9%), vancomycin (20.2%) and linezolid (20.2%). Multidrug resistance was found in all isolates. The following toxin genes were detected among the 16 *C. perfringens* isolates: cpa (11 isolates), cpb (9 isolates) but were negative for etx and iap genes. Moreover, type A (12.5%) and type C (56.25%) were the most prevailing types among tested *C. perfringens* isolates.

**Conclusion:** A high diversity of clostridiales species were detected in poultry caecum, being *C. sporogenes*, *C. perfringens* and *S. tepidum* the most frequent detected species. The high rate of antimicrobial resistance among these isolates, including very relevant antimicrobial agents what could pose a potential public health risk. Further surveillance of poultry and chicken meat should be performed in order to track and control potential dissemination along the food chain.

**Keywords :** *Clostridium spp.*; Caecum; Chickens; Antibiotic resistance; Toxin gene.

## Probiotics for animal protection

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### **Abstract :**

To find innovative and sustainable solutions to problems linked to animal and human health, as well as the preservation of agricultural resources, a "One Health" approach is being adopted. This approach is supported by the Urbane programme funded by the European Commission, which includes my thesis project on the effectiveness of probiotics in protecting farm animals. The main aim of my research is to develop new probiotics for poultry applications. To achieve this, microbial strains are isolated from matrices native to Africa, then identified using 16S rRNA gene sequencing and confirmed by Maldi ToF-Mass Spectrometry identification. More than hundred strains (lactic acid bacteria, *Bacillus* and some yeasts) are identified and characterized based on criteria defined by the FAO. These criteria included the study their viability in acid conditions, their surface hydrophobicity, their self-aggregation capacity, their ability to express antagonistic activity towards certain pathogens and their ability to colonise the gastrointestinal tract. Five strains of lactic acid bacteria tested (*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Enterococcus durans* and *Lactobacillus casei*) inhibit the growth of pathogens such as *Bacillus cereus*, *Staphylococcus epidermidis* and *Escherichia coli*. These lactic acid bacteria strains produce a variety of compound such as emulsifying agents, short chain fatty acid and have a spectacular capacity for self-aggregation. These properties help the colonisation of intestinal mucosa by probiotic by preventing the attachment of pathogens, and hence competition for attachment sites. These strains need to be studied in greater depth in order to gain a better understanding of the beneficial effects observed in each strain and to develop in vivo applications, particularly in agriculture.

**Keywords :** Probiotics; One; Health; Poultry.

## Characterization of chicken cruor-derived hydrolysates produced through enzymatic hydrolysis and evaluation of the feasibility of their use as natural antimicrobials

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### **Abstract :**

Chicken blood is a rich protein source that can be upgraded to generate value-added products. Each year, 48 million liters of blood are produced during chicken slaughter in Canada. About 40% of the total volume of blood is composed of cruor, which mainly contains hemoglobin. Upon hydrolysis of cruor, bioactive peptides with antimicrobial and antioxidant activity may be obtained and can be used as an alternative to chemical preservatives. After collecting chicken blood from slaughterhouses and separating the cruor by centrifugation, 180 minutes of peptic hydrolysis were performed at pH 2, 3, 4, and 5. Then, peptide population, sequence, enzyme mechanism, and degree of hydrolysis (DH%) were determined to evaluate the hydrolysates generated during the process. The hydrolysates were then tested for their antifungal and antibacterial activity against three major pathogenic bacteria of poultry meat (*Listeria ivanovii* HP B28, *Campylobacter Coli* ATCC 33559, *Salmonella enterica* ssp Newport ATCC 6962), as well as one yeast (*Rhodotorula mucilaginosa* 27,173) and two molds (*Paecilomyces* spp. LMA-722 and *Mucor racemosus* LMA-722). Next, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of hydrolysates were determined. The highest DH% was recorded at pH 2, which was not significantly different from pH 3 ( $p < 0.05$ ). Inhibitory effects were observed for hydrolysates produced at pH 2 and 3 after 30 and 180 minutes of hydrolysis against *Paecilomyces*, *Rhodotorula mucilaginosa* and *M. racemosus*. Although all hydrolysates produced showed no antibacterial activity, some new potential antimicrobial peptides, according to bioinformatics tools, were identified which can show antibacterial activity after separation and purification. This project aimed to valorize blood, a by-product from slaughterhouses, into high-added value peptide fractions with effective antimicrobial properties in the context of a circular economy. The study examined the possibility of creating a natural preservative, in addition to reducing waste.

**Keywords :** Chicken cruor; Antifungal; Antimicrobial; Enzymatic hydrolysis; Bioactive peptide.

## Harnessing MALDI-TOF profiling to predict the behavior of *Carnobacterium* from food environments

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### **Abstract :**

Carnobacteria, which regularly prevail in the microbial communities found in meat and seafood products, are of particular interest to the food industry. However, their impact on food is controversial, as certain strains can contribute to product spoilage, while others can be of interest as bioprotective strains to inhibit undesirable organisms and pathogens. Since these traits vary between strains of the same species, taxonomic-based methods alone are not sufficient for efficient microbiota monitoring. Given that matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF) is already recognized as a useful method for taxonomic identification, we aimed to evaluate its suitability to predict several phenotypes in a *Carnobacterium* population. First, using an automated culture platform, bacteria were isolated in a selective medium and characterized in terms of growth rate, yield, motility, and antagonistic activity against *Listeria innocua* and *Listeria ivanovii*. The isolates were then identified using MALDI-TOF and the 163 *Carnobacterium maltaromaticum* profiles were probed to create machine learning (ML) and other predictive models. The comparison of the models revealed that the neural-boosted method outperformed other methods for four out of five phenotypes. The growth parameters predicted by the selected models were significantly correlated with the measured values ( $R^2 > 0.6$ , P-values).

**Keywords :** *Carnobacterium maltaromaticum*; Food microbiota; Phenotypic prediction; Machine learning.



# **Human as part of One Health**

H1

## New findings in the regulation of intestinal glucose absorption by dietary proteins

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### **Abstract :**

**Purpose:** Numerous studies have established the positive impact of high-protein diets on glucose homeostasis. However, the specific mechanisms responsible for this effect remain unclear. Preliminary investigations indicate that different digested proteins enhance glucose tolerance while reducing glucose absorption and the activity of the glucose transporter GLUT21. We hypothesise that a “cross-talk” between the oligopeptide carrier PepT1 and glucose transporters GLUT2 and SGLT1 is at the origin of this regulation. This work thus investigated the cellular and molecular mechanisms regulating glucose absorption induced by digested proteins from various food sources.

**Method:** Fish gelatin, caseins and pea protein preparations were subjected to digestion using the INFOGEST static gastrointestinal digestion protocol. Digested proteins were subsequently incubated with glucose (25 mM) in an intestinal barrier model (Caco-2/HT29-MTX coculture) with or without a PepT1 inhibitor. RT-qPCR, western blot and immunofluorescence evaluated the mRNA and protein expression of SGLT1 and GLUT2.

**Results:** Digested proteins reduced the expression of GLUT2 mRNA and SGLT1 protein expression at the apical side of enterocytes. These findings further support the notion that peptides and amino acids derived from digested proteins play a crucial role in regulating glucose homeostasis, emphasising their significance in intestinal glucose absorption.

References : Dugardin C, et al., *Front Nutr.* 2022 Jan 19;8:769773.

**Keywords** : Dietary proteins; Intestinal glucose absorption; PepT1; SGLT1; GLUT2.



## **Bacteriocins: natural weapons for fighting *Clostridium* in silage**

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### **Abstract :**

Silage is a key component of dairy cattle diets and an important source of nutrients, particularly energy and digestible fibre. Silage quality is greatly dependent on the microbial community and its metabolites present throughout the ensiling process. Poorly maintained silage with high levels of butyrate and ammonia has been associated with clostridial activity which may impact not only the quality and nutritional value of silage but also of raw milk and milk products. The aim of this study was to evaluate the inhibition activity of nisin against *Clostridium* during silage fermentation. It also aimed to evaluate the impact of nisin on the overall quality of silage as well as on the composition and metabolic activity of the endogenous microbiota. In this respect, the silage was prepared and inoculated by a cocktail of 5 isolates most often encountered in silage then sprayed with 3 different concentrations of nisin. They were divided into 1.5 kg samples in PVC mini-silos, pressed at 1200 kPa, covered with a cap fitted with a valve allowing expulsion of gas, then kept at room temperature. After 28 days of fermentation, samples were collected and submitted to several physicochemical analyses and bacterial count. Our results indicate that Nisin is effective in eliminating the clostridial growth (5 log reduction) in silage at a final concentration of 60 µg/ml. However, at this concentration, endogenous LAB were also affected leading to high pH compared to the control. At lower concentration of 30 µg/ml, Nisin not only eliminated the growth of *Clostridium* but also didn't have a negative effect on LAB leading to a final pH close to that of the control. These results indicated that Nisin can be considered as a natural and efficient way to control *Clostridium* contamination of silage.

**Keywords :** Silage; Dairy Products; *Clostridium*; Bacteriocin; Microbiota.

H3

## **Methodological strategy to in vitro analysis of protein preparation digestibility**

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### **Abstract :**

Dairy proteins have been the subject of intensive research for several decades, notably due to their nutritional qualities and interesting physicochemical properties. The aim of this study was to determine in vitro the digestibility and nutritional properties of casein preparations that had undergone different processes leading to different degrees of demineralization. Protein preparations were digested using the INFOGEST static digestion model coupled to a Caco-2/HT29-MTX intestinal barrier model. The extent of hydrolysis and the peptidomes were studied in the different gastrointestinal compartments and after the intestinal barrier challenge using size exclusion chromatography, OPA assay, and peptidomic analysis. Intestinal calcium transport was also studied, and the digestible indispensable amino acid score (DIAAS) was evaluated in vitro for each casein preparation. Overall, the results revealed significant differences highlighted by statistical PCA analysis in terms of digestibility depending on the demineralization and industrial processes of the casein preparations. Focus will be done on each compartment of the gastrointestinal tract. This work implemented an innovative in vitro method to characterize and compare the digestibility and the peptidomes of different protein matrices, representing a good alternative to in vivo and clinical studies. This work took place in the ProteinoPepS project (<https://www.proteinopeps.com/>) funded by the French National Research Agency and Ingredia.

**Keywords :** Caseins; Demineralization; in vitro gastrointestinal digestion; Peptidomics; DIAAS.

## Highlighting the antioxidant and hypocholesterolemic properties of peptides from spirulina (*Arthrospira platensis*)

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### **Abstract :**

Hypercholesterolemia, a pivotal public health concern, propels the collaborative international project CASH (Chromophores Actifs en Santé Humaine) to explore natural alternatives to statins for blood cholesterol level reduction, aiming to prevent cardiovascular risks. This project, cofounded by Walloon Region through the WAGRALIM Competitiveness Cluster, is a partnerships between the University of Liège, the University of Louvain-La-Neuve, the University of Lille, Tilman sa and Biores sa (two Wallonia-based compagnies). Spirulina, a blue-green alga of the *Cyanophyceae* family, is celebrated for its exceptional nutritional properties and well-established antioxidant, antidiabetic, and anti-inflammatory activities [1,2]. Spirulina contains the phycocyanin, a chromoprotein used as natural blue pigment in food. The CASH project unfolds through diverse tasks ranging from: (i) assessing the bioactive potential of peptides from spirulina proteins through an in silico approach, (ii) developing bioactive spirulina protein hydrolysates, (iii) evaluating the hypocholesterolemic and antioxidant activities before and after simulated in vitro static INFOGEST gastrointestinal digestion [3,4], and (iv) identifying peptides and chromopeptides responsible for the studied bioactivities through a peptidomic approach. The project led to the identification of fractions obtained by gel filtration of phycocyanins hydrolysates with high antioxidant activity. This innovative study promises to redefine preventive approaches against cardiovascular diseases by harnessing the bioactive potential of spirulina peptides, paving the way for natural and sustainable solutions to enhance cardiovascular human health.

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**Keywords :** Spirulina; Hydrolysis; Antioxidant; Hypocholesterolemiant; Peptidomics.

## Human milk treated by high pressure processing improves linear growth of growth-restricted mouse pups

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### **Abstract :**

**Introduction:** When a mother's milk is either unavailable or insufficient to meet the nutritional needs of her preterm infant, human milk banks (HMBs) offer donor milk (DM) as an alternative. DM is sterilized using the conventional holder pasteurization (HoP) process, which involves heating DM to 62.5°C for 30 minutes, to guarantee its microbiological safety. High hydrostatic pressure (HHP) processing, has been suggested recently as an alternative to HoP, since it retains many of the bioactive components of DM that HoP breaks down, however no in vivo data are available. Our aim was to determine if the intestinal effects of HoP- or HHP-DM, when orally administered daily, differ in postnatally growth-restricted (PNGR) pups.

**Methods:** A pool of DM from 8 donors (Lille HMB, CHU Lille) was divided in two batches, one processing by HoP and the other by HHP (4 cycles of 5 minutes, 350 MPa, 38°C). PNGR was induced by increasing litter size from 8 to 15 pups per mother at postnatal day (PND) 4. Oral gavages with either HoP- or HHP-DM (5 mL/kg) were administered daily to pups between PND8 and PND20. Body weight was measured until weaning. Intestinal maturation was evaluated by histological and molecular approaches and the microbiota by metagenomic sequencing at PND21.

**Results:** Compared to HoP-DM pups, pups treated with HHP-DM had a higher body weight from PND12 to PND21. However, both forms of milk supplementation did not significantly change the microbiota, the intestinal morphology and permeability, or the amount of short-chain fatty acids in the pups' caecum. Discussion: We demonstrated that HHP-DM enhances the body growth during the lactation period. This effect may implicate the preservation by HHP of DM bioactive compounds. Finally, HHP-DM is well tolerated and exerts similar intestinal effects than HoP-DM. HHP might be an innovative technique for pasteurizing DM in HMBs.

**Keywords :** Human milk; High hydrostatic pressure; Holder pasteurization; Body growth; Mice intestine.

## Effect of pesticide use and sprouting inhibitor on potato crops on gut microbiota and gene expression in mice

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### **Abstract :**

**Purpose:** The widespread consumption of potatoes is attributed to their easy availability and affordability. While potatoes provide numerous benefits, concerns arise due to their high carbohydrate content, often associated with health issues like obesity and diabetes. Nevertheless, they are rich in health-promoting compounds such as ascorbic acid, vitamins (C, B3, B6), proteins, minerals, and dietary fibers. Resistant starch, found in potatoes, acts as a beneficial nutrient for the gut microbiome. However, pesticides are commonly used during potato crop cultivation to combat diseases like mildew. Additionally, sprouting inhibitor treatments are employed for extended potato storage. Despite the known health hazards associated with pesticides, limited research has examined their effects within food matrices, particularly the combined impact of pesticide mixtures within such matrices. This study aims to explore the influence of pesticide treatments during crop cultivation and sprouting inhibitor treatments during storage on the gut microbiota population and hepatic gene expression.

**Methods:** Lyophilized bio or conventional potato with or without sprouting treatment or water was administered through orogastric gavage in mice over a 3-week period. Then, mice were euthanized to collect organs like the liver. Feces were collected before the initial gavage and after 3 weeks of gavage. Nutrigenomic analysis was conducted on liver RNA and intestine RNA, and metagenomic analysis was performed on feces.

**Results:** Findings from this study demonstrated notable modifications in gut microbiota, liver and intestine gene expression in mice following the consumption of potato bio or conventional, with or without storage (sprouting inhibitor treatment).

**Conclusion:** This study highlights the effect of pesticide treatment and storage of potato on both gut microbiota population and gene expression modulation in the liver and the intestine.

**Keywords :** Potato; Gut microbiota; Gene expression; Health; Pesticide mixtures.

## **In vitro identification of intestinal hormones regulatory peptides derived from digested protein of different origins**

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### **Abstract :**

**Purpose:** Besides their nutritional function, proteins are known to intervene in short- and long-term energy homeostasis regulation. Nevertheless, studies on the food intake regulation effects of proteins regarding their quality and origin are limited and often incomparable from one study to another. Moreover, and in parallel, digested dietary proteins are known to exert a satiating effect by stimulating intestinal hormone secretion (CCK, GLP-1). A preliminary study was conducted to compare the potential of various digested proteins to enhance GLP-1 and CCK secretion, with pea and gluten proteins emerging as the most effective. The present study aims to identify peptides from these two different proteins (pea and gluten proteins) that could be involved in intestinal hormone secretion.

**Methods:** First of all, gastrointestinal digestion of pea and gluten proteins will be mimicked thanks to the static INFOGEST protocol. Digested proteins will then be fractionated by two chromatographic methods. First, ion exchange chromatography will be used to separate fractions based on cationic affinity with a cation column. The potential of each fraction to increase intestinal hormone secretion will be tested using the enteroendocrine STC-1 cell line. For the fraction showing the best potential, separation based on physicochemical properties will be performed using C18-RP-HPLC. Each collected subfraction will be tested again to identify which one shows the best potential to increase CCK and GLP-1 secretion in STC-1 cells. The peptides constituting the selected subfraction will then be identified using mass spectrometry (LC-MS/MS).

**Perspectives:** The perspectives of this project include synthesizing some of the peptides identified by mass spectrometry to determine which ones could be involved in intestinal hormone secretion. Additionally, receptor inhibitors could be used to try to determine the pathway(s) through which these peptides enhance CCK and GLP-1 secretion.

**Keywords :** Food intake regulation; Proteins digestion; Bioactive peptides; Intestinal hormones.

## Application of the One Health concept to the development of a natural, biodegradable dry cosmetic support

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### **Abstract :**

In recent years, there has been a worldwide growing interest in cosmetic skincare products. These products include moisturisers, sunscreen creams, serums and cosmetic masks. Among cosmetic masks, the 'tissue mask' category, which can be applied directly to the face, is hardly developing. This type of product consists mainly of an inert film, biodegradable or not, which acts as support for active ingredients supplied in liquid form, usually in excess. This type of product, which consumers will, causes major environmental issues: (1) excess active ingredients and chemical products to be treated in waste water; (2) treatment of the inert support after use (poor selective sorting, recycling, destruction); (3) probable microplastic pollution, posing a risk to humans and the environment. Thinking about cosmetic formulation in terms of One Health strategy to improve the health of the Environment, and consequently of Humans and Animals, is therefore becoming a priority.

It is thus necessary to find more eco-responsible alternatives to this type of cosmetic products. The study presented here shows the feasibility of producing an inert support from biotechnological plant and/or fungal cultures. The advantages of these cultures are that they require little energy, can be scaled up for industrial production, and are both repeatable and reproducible. The main objective is to limit as far as possible the mechanical and/or chemical treatments applied to the biomass produced before the support formulation stage - to limit the ecological impact of its manufacture in addition to the ecological impact of its use.

With the aim of eliminating the liquid aspect of current 'tissue mask' products, the created supports are tested for their ability to retain and preserve active ingredients and their biological activity; to release them on contact with the skin; and to resist consumer's use. Their compostability also need to be assessed. This type of formulation will allow applying the right amount of active ingredients to the skin without risk to impact biodiversity through waste water contamination. Preliminary results of this study are presented here.

**Keywords :** Cosmetics; Antioxidant; Skincare; Biobased material; Resveratrol.