**Study and characterization of novel ingredients recovered from plant material for application in novel foods, by advanced chromatographic technique and high-resolution mass spectrometry**

Lorenzo Del Vecchio (lorenzo.delvecchio@unipr.it)

Dept. Food and Drug, University of Parma, Viale Parco Area delle Scienze 27/A, 43124 Parma, Italy

Tutor: Prof. Martina Cirlini

This PhD thesis research project is aimed to study and chemical characterize plant materials potentially rich in bioactive and/or flavoring compounds, in order to develop new ingredients for food industry.

**Studio e caratterizzazione di ingredienti innovativi recuperati da materiale vegetale, da applicare in novel food, tramite tecniche cromatografiche avanzate e spettrometria di massa ad alta risoluzione**

Il presente progetto di tesi di dottorato sì pone l’obiettivo di studiare e caratterizzare chimicamente substrati vegetali potenzialmente ricchi in molecole bioattive o composti aromatici, con lo scopo di sviluppare dei nuovi ingredienti utilizzabili in diversi settori dell’industria alimentare.

# **1. State-of-the-Art**

In the last few years natural products, derived from several types of plant materials, are attracting the attention of an increasing number of companies interested in the application of plant-derived additives in the food sector (Essien *et al*., 2020). The demand for the application of bioactive food ingredients derived from plant materials has increased in recent years. Natural compounds derived from plants have the potential to be new sources of food components and environmentally beneficial food preservatives.

***Table*** *1 Some plant materials or biomasses as source of bioactive and aroma compounds.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Plant source** | **Aim** | **Compounds/**  **aroma** | **Results** | **Application** | **Reference** |
| Hemp microgreens/by-products | Chemical characterization of different varieties of hemp cultivated as microgreens. | Organic acids, amino acids, polyphenols and non-psychoactive phyto-cannabinoids | High amount of malic acid, citric acid, and tartaric acid; Highly content of total AA and EAAs in some cultivars; a good amount of phenolic acid and flavonoids; low level of psychoactive phyto-cannabinoid (Δ9-THC) and high concentration of cannaflavin A and B. | Potential use as an innovative functional food | Pannico et al., 2022 |
| Brassicaceae microgreens | Chemical analysis of Brassicaceae microgreens to understand the impact of crop factors involved in the production of phytochemicals of different species. | Phenolic compounds, vitamins (A, D, E, K) glucosinolates, anthocyanins, isothiocyanates and indoles | High amounts of bioactive compounds when the fiber is used as substrates; artificial lighting could increase the phytochemical accumulation; bioactive compound profiles could change in response to fertilization. | Potential use in the food industry as a source of bioactive compounds | Alloggia et al., 2023 |
| Kiwi fruit by-products | Chemical evaluation of new natural source of flavor and aroma metabolites | Volatiles organic compounds (phenyletyl alcohols, aldeydes, acids, chetons, furan) | The use of cheap substrates like kiwi fruit peel could be an interesting way to obtain 1-octen-3-ol (mushroom aroma), β-pinene (herbal, pine aroma), 3-octanol (mushroom, herbal aroma). | Natural flavors and aromas through low-cost substrates | Lindsay et al., 2022 |

Plant materials may represent excellent sources of nutrients and phytochemicals and for this reason, the potential use of them was evaluated as a more sustainable substitute of synthetic molecules (i.e., preservatives, antioxidant agents and flavoring). The use of plant materials or valorization of plant waste may play an important role in environmental sustainability for developing new ingredient products. Many plants material, as reported in Table 1, have been used to extract bioactive molecules or flavoring for a food application. In the past few years, microgreens have become more popular as new food with high concentrations of bioactive compounds, and it has been shown that modulation of growing conditions improves the phytochemical profile (Pannico *et al.,* 2022; Alloggia *et al.,* 2023). Likewise, also fruit by-products such as kiwi peel may be an interesting source of volatile organic compounds (Lindsay *et al.,* 2022). Currently, the widest extraction method used in the plant industry are not compatible with food industry. For these reasons, green technologies (i.e., pulsed electric field (PEF)) or supercritical fluid extraction are becoming increasingly preferred extraction methods to recover bioactive molecules in order to reduce the use of organic solvents. Thus, this PhD thesis will be aimed to select a plant material that can undergo extraction of bioactive compounds and subsequent chemical characterization, as to evaluate the re-use of the characterized by-product as potential ingredient in food preparations.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Preliminary assessment of plant materials** both in vitro culture and plant waste.

A2) **Extraction of bioactive compounds and VOCs** throughconventional and non-conventional techniques.

A3) **Preliminary assessment of bioactive compounds** applying several assays and evaluate the antimicrobial and cytotoxicity activity of more promising extracts. The most promising extracts will be used to fortify food products.

A4) **Advanced chemical characterization of bioactive compounds and VOCs**

A5) **Bio-accessibility of Phenolic Compounds**

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

**Table 2** Gantt diagram of PhD activity during next two years.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Preliminary assessment*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) *In-vitro* plant growth |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Recovery of plant or fruit material by-product |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Extraction of compounds*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Conventional extraction techniques |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Green techniques |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Preliminary assessment by assays*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Polyphenol content |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Antioxidant activity |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Antimicrobial activity |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 4) Cytotoxicity assay |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 5) Testing on food products |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Advanced chemical characterization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) UHPLC-MS/MS |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) HS-SPME/GC-MS |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Bioaccessibility*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) *In-vitro* digestion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Transepithelial trasposrt in Caco-2 cell |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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