Development of new approaches for the evaluation of the metabolism of bioactive compounds of nutritional interest

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This PhD thesis research project aims to apply desorption electrospray ionization (DESI) mass spectrometry imaging (MSI) to evaluate (poly)phenol distribution in tissues. This innovative analytical technique will allow us to shed light on the transport mechanisms and cellular absorption involved in the uptake of phenolic compounds. The objective is therefore to verify the relationship between ingested (poly)phenols, metabolite and colonic catabolite production and their ability to reach target organs in which they can exert their functionality.

Sviluppo di nuovi approcci per la valutazione del metabolismo di composti bioattivi di interesse nutrizionale

Questo progetto di ricerca di tesi di dottorato si propone di applicare la spettrometria di massa *imaging* (MSI) a desorbimento per ionizzazione elettrospray (DESI) per valutare la distribuzione dei (poli)fenoli nei tessuti. Questa tecnica analitica innovativa ci permetterà di far luce sui meccanismi di trasporto e assorbimento cellulare coinvolti nell'*uptake* dei composti fenolici. L'obiettivo è quindi verificare la relazione tra i (poli)fenoli ingeriti, la produzione di specifici metaboliti e cataboliti colonici e la loro abilità a raggiunge gli organi *target* nei quali possono svolgere la loro potenziale attività funzionale.

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

(Poly)phenols are organic compounds produced by plants as secondary metabolites and are abundantly present in fruits, vegetables, nuts, herbs, and other plant-derived products (Del Rio *et al*., 2013). Various studies have demonstrated the potential positive impacts of these compounds on human health (Rana *et al*., 2022). Nonetheless, the comprehension of the absorption and distribution mechanisms of phenolic compounds within the human body is of great importance for precisely identifying their bioactive form in target organs or tissues and, consequently, for understanding their biological effects.

Mass spectrometry imaging, or MSI, is a highly effective analytical method that provides a visual *in situ* representation of the molecular distribution within complex samples and biological tissues. This technique allows to create a map of various molecules in a tissue sample, providing spatial information that cannot be obtained through traditional analytical techniques (Chughtai and Heeren, 2010). Desorption electrospray ionization (DESI) is an imaging source that, thanks to a charged solvent, ionizes the compounds directly on the tissue at atmospheric pressure and is ideal for the detection of small molecules that range from 50 to 2000 Da (Monge *et al*., 2013). The available literature shows that the application of MSI for the study of phenolic compounds is yet to get a foothold but is expected to increase as researchers seek to advance their comprehension of the health-promoting attributes of (poly)phenols and their metabolites. DESI MSI represents a viable approach for exploring the absorption and distribution of bioactive compounds in tissues, useful to enhance the comprehension of the pharmacokinetics of (poly)phenols and their metabolites, along with their possible site of action and transport mechanisms implicated in their distribution.

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

During the 1st year of the PhD, the activities were focused on literature search to write one review on the applications of MSI so far published for evaluating (poly)phenol distribution in animal tissues, and a second review on the absorption and transport mechanisms involved in the distribution of (poly)phenols in target organs and tissues.

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 1:

A1) **Training** **with DESI MSI** to develop the method (A1.1), including verification of sensitivity, reproducibility, and functionality of the instrument, and preliminary **trials** (A1.2).

A2) ***In vivo* study** with 36 C57BL6/J male mice, giving the standard compound deuterated D5-(−)-epicatechin via oral gavage. The mice will be sacrificed in groups of 4 at different timepoints, and all the organs will be collected, together with plasma, urine, and feces.

A3) **Analysis of collected samples**, including i) plasma, urine, and feces to check the nutrikinetics of the administered compound (A3.1); ii) microscopy evaluation of cross-sectional tissue/organ slices to acquire the histological image (A3.2); iii) study of the images obtained from DESI MSI for the *in situ* visualization of D5-(−)-epicatechin metabolites and catabolites to evaluate the *in vivo* distribution within animal’s tissues and organs, with a focus first on the gastrointestinal tract, and subsequentially on the other organs (A3.3 and A3.4).

A4) **Writing and editing** the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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) | ***Training with DESI MSI***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Method development |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Preliminary trials |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***In vivo study***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
| A3) | ***Analysis of collected samples*** |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Plasma, urine, and feces |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) Histological evaluation  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
|  | 3) Gastrointestinal tract DESI images  |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 4) Other organs DESI images |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

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