PhD DISSERTATION PROJECTS

Protein and polyphenols interactions: functionality in food and biological systems

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This PhD research project aims at exploring the interactions between proteins/peptides and polyphenols. Food and beverage systems will be used as experimental model to explore the impact of the interactions on physicochemical characteristics of both proteins/peptides and polyphenols and on food quality. Moreover, the physiological effect of consuming the protein-polyphenol complexes will be explored by in vitro model systems and in vivo studies focusing on specific metabolites and markers of function of biological systems.

Interazioni tra proteine e polifenoli: funzionalità negli alimenti e sistemi biologici

Questo progetto di ricerca di dottorato mira a esplorare le interazioni tra proteine/peptidi e polifenoli. Sistemi di alimenti e bevande saranno utilizzati come modello sperimentale per esplorare l'impatto delle interazioni sulle caratteristiche fisico-chimiche di proteine/peptidi e polifenoli e sulla qualità degli alimenti. Inoltre, l'effetto fisiologico del consumo dei complessi proteine-polifenoli sarà valutato attraverso sistemi modello in vitro e studi *in vivo* incentrati su metaboliti specifici e marcatori di funzione dei sistemi biologici.

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

Dietary proteins are important food components providing beyond nutritional properties, both technological (solubility, foaming and emulsifying) and bioactive properties due to peptides can be originated over digestion. Polyphenols are a class of phytochemicals, recognized for mitigation of several chronic and degenerative diseases including obesity, cardiovascular disease, and neurodegeneration (Foegeding *et al*., 2017). These two nutrients co-exist in many foods and can easily form aggregates because of processing and after consumption when being co-ingested and interact in the gastrointestinal tract (GIT) (Zhang *et al*., 2021). The resulting interactions are classified into covalent interactions which are irreversible and stable and non-covalent interactions which are driven by hydrogen bonds, Van Der-Waals forces, hydrophobic and electrostatic interactions and are reversible. The generation as well as the strength of these interactions is modulated by intrinsic factors, depending on the type, the amount and the ratio of both molecules involved, and extrinsic variables such as pH, temperature, and the influence of other constituents. The effects of these interactions are still unclear due to inconsistent evidence. Such intermolecular associations induced changes in protein conformational structures leading up to modulate their solubility and other technological properties which are essential requirements for protein food applications. Moreover, the antioxidant activity of polyphenols can be masked by proteins due to the blockage of their reactive groups by intramolecular interactions (Ozdal *et al*., 2013). The changes caused by these complexes, result in different digestive behaviors of protein and polyphenols compared to parental free molecules. Protein digestion can be reduced by the formation of indigestible aggregates or by the possible inactivation of digestive enzymes (Zhang *et al.,* 2021). However, the presence of phenolics could also induce partial unfolding of protein structures, therefore increasing accessibility of the susceptible peptide bonds and digestibility (Jiang *et al*., 2018). Likewise, the bioaccessibility and the metabolic fate of complexed polyphenols is challenged by many factors. Polyphenols need to be released from the food matrices into the gastrointestinal tract and be absorbed, to exert their biological activities in peripheral organs (Ribas-Agustí *et al.,* 2018). Few in *vivo* and cell-based transportation studies reported either a deteriorative effect of proteins on the bioavailability of co-ingested polyphenols or an increased uptake due to an improvement in stability of bonded polyphenols which are protected by proteins from being degraded. Furthermore, during digestion, the interactions can be partially dissociated due to environment conditions and new associations might also occur between polyphenols and peptides leading to new potential bioactive complexes (Zhang *et al.,* 2021).

The objective of this PhD project is to study protein/peptide-polyphenol interactions to optimize functional properties in food and health-promoting effects in humans upon food consumption. To this purpose, food/beverage systems containing animal/plant proteins/peptides and polyphenols will be designed, developed and used to explore the interactions between proteins and polyphenols as well as the impact on food properties and protein/polyphenol bioaccessibilty, metabolism and functionality using both *in vitro* and *in vivo* approaches.

**2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be divided into the following activities according to the Gantt diagram in Table 1:

A1) **Design, development and characterization of food/beverage systems** containing protein/peptide-polyphenol complexes (focus on pulse-based preparations and plant-based beverages also using by-products coming from agro-food industry).

A2) **Study of potential functionality** of the protein/peptide-polyphenol complexes in food/beverage systems by using *in vitro* studies (focus on protein and polyphenol digestibility, metabolism and nutritional/functional properties through bioaccessibility of aminoacids, bioactive peptides, digestive enzyme inhibitory activity, antioxidant activity, etc).

A3) **Evaluation of physiological effects** of consuming food/beverage containing protein/peptide-polyphenol complexes (focus on specific dietary metabolites and physiological markers) in human studies.

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

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

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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) | ***Design, development and characterization of food/beverage systems*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Study of potential functionality of polyphenol-protein complexes in food/beverage systems in vitro*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Evaluation of physiological effects in vivo*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Thesis and Paper preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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