1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Mechanisms of formation, stability, and in vitro bioavailability of anthocyanin and flavonoid complexes with selected milk proteins

1.1. Project goals

- Elucidation of the molecular mechanisms underlying the formation of non-covalent and covalent complexes between anthocyanins/flavonoids and milk proteins through the application of advanced spectroscopic and chromatographic techniques (e.g., FTIR, fluorescence spectroscopy, HPLC).
- Evaluation of the physicochemical stability of the complexes under various environmental and processing conditions, including pH, temperature, ionic strength, and exposure to light or oxygen. Investigation of the protective role of milk proteins on the chemical stability of phenolic compounds with particular focus on thermal degradation and oxidative stress during food-relevant processes.
- Determination of the *in vitro* bioaccessibility and bioavailability of the complexes – using a standardized gastrointestinal digestion model to simulate the fate of these compounds during digestion.
- Correlation of structural features of protein–phenolic complexes with their biofunctional potential including antioxidant activity and potential enhancement of intestinal absorption.
- Contribution to the scientific foundation for the development of functional food ingredients – by advancing knowledge of protein–polyphenol interactions that can improve the delivery and stability of bioactive compounds.

1.2. Outline

This project aims to investigate the mechanisms of formation, stability, and in vitro bioavailability of complexes formed between anthocyanins/flavonoids and milk proteins. These natural phenolic compounds are known for their health-promoting properties but suffer from poor stability and low gastrointestinal bioavailability. By complexing them with milk proteins, the project seeks to enhance their resistance to environmental and digestive degradation and improve their intestinal absorption. The study will combine advanced spectroscopic and chromatographic techniques, *in vitro* digestion models, and transport assays using Caco-2/HT29-MTX intestinal epithelial and mucus cells to comprehensively evaluate the structural, functional, and bioavailability aspects of these complexes. The results will provide fundamental insights into protein–polyphenol interactions and inform the development of more effective functional food ingredients and dietary strategies.

1.3. Work plan

WP1: Selection and Characterization of Compounds

- Selection of model polyphenols (e.g., cyanidin-3-glucoside, quercetin) and milk proteins
- Purity assessment and baseline spectral characteristics

- Initial screening of cytotoxicity of selected polyphenols and complexes on Caco-2 cells (MTT assay)

WP2: Complex Formation and Optimization

- Experimental formation of complexes under controlled pH, temperature, and molar ratios
- Analysis of binding efficiency and interaction mechanisms using FTIR, fluorescence spectroscopy, and UV-Vis
- Preliminary evaluation of complex stability in cell culture medium (DMEM) to ensure compatibility with *in vitro* intestinal model

WP3: In Vitro Digestion and Bioavailability

- Application of standardized digestion models (e.g. INFOGEST protocol)
- Quantification of phenolic content and antioxidant activity in digested fractions (HPLC-MS, ABTS/DPPH)
- Exposure of digested samples to a three-cell intestinal model is an *in vitro* co-culture system that mimics the human intestinal barrier (Caco-2 cells as absorptive enterocytes, HT29-MTX cells as mucus-secreting goblet cells) growing on Transwell inserts

WP4: Structure–Function Relationship

- Correlation between complex structural features (binding type, size, morphology) and biological properties (stability, antioxidant activity, absorption)
- Comparison of bioavailability outcomes from digestion models and Caco-2 cell transport studies to identify optimal complexation conditions
- Identification of structural and physicochemical parameters that maximize intestinal delivery of polyphenols

1.4. **Literature** *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

- Brodkorb, A., Egger, L., Alminger, M. et al. Nat Protoc 14, 991–1014 (2019). https://doi.org/10.1038/s41596-018-0119-1
- Chen, Xiumin & Elisia, Ingrid & Kitts, David. (2010). J. Pharmacol. Toxicol. Methods 61. 334-42. 10.1016/j.vascn.2010.02.004.
- Ren S, Jiménez-Flores R, Giusti MM. Compr Rev Food Sci Food Saf. 2021 20(6):5992-6011. doi: 10.1111/1541-4337.12854.
- Kong F, Kang S, Zhang J, Jiang L, Liu Y, Yang M, Cao X, Zheng Y, Shao J, Yue X. Food Chem. 2022 15;394:133455. doi: 10.1016/j.foodchem.2022.133455.

1.5. Required initial knowledge and skills of the PhD candidate

The candidate should hold a Master's degree in Chemistry and possess basic experience in instrumental analysis, including UV-Vis, HPLC, and FTIR. They should be able to plan and perform chemical analyses, interpret data, and document results. Good organizational skills, independence, and willingness to work in an interdisciplinary team are essential.

1.6. Expected development of the PhD candidate's knowledge and skills

The candidate will expand their expertise in advanced analytical techniques and interdisciplinary research, particularly in the analysis of bioactive compounds in food systems. They will gain skills in method development, data interpretation, and scientific communication.