**Interaction between gallic acid in red pitaya fruit and porcine pancreatic α-amylase: molecular biochemistry, enzymatic assays, spectroscopy and *in-silico* simulations**

*Yanyi Huang, Samantha Richardson, Charles Brennan, Stefan Kasapis*

*School of Science, RMIT University, Bundoora West Campus, Melbourne, VIC, 3083, Australia*

α-Amylase is a digestive enzyme that catalyses the breakdown of starch into reducing sugars in the digestive tract. Existing literature has reported that phenolic compounds derived from plants are effective therapeutic agents with a strong inhibitory effect on digestive enzymes. This is due to binding interactions impeding enzymatic activity, thus decreasing caloric uptake in the digestive system. The binding interactions generally occur *via* hydrogen bonding and/or hydrophobic forces between the enzyme and the phenolic compound. Our previous study revealed that the binding of p-coumaric acid to the active site of α-amylase was mainly *via* hydrogen bonds. The α-amylase-p-coumaric acid complex contained less α-helical and β-sheet components, suggesting diminishing thermal stability of the enzyme. To identify the most efficient inhibitors present in red pitaya fruit, the study of molecular mechanism between α-amylase and gallic acid are presently carried out[1,2].

Receptor-ligand interactions in food systems have been studied by Lineweaver-Burk kinetic analysis, UV-vis spectroscopy, circular dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), fluorescence spectroscopy, differential scanning calorimetry (DSC) and molecular modelling[3,4]. This combined analysis reveals structural changes and interaction forces resulting from complexation between protein and ligand, with binding sites and residues responsible for ligand stabilisation being proposed by molecular modelling. Understanding of the α-amylase-gallic acid interacting mechanism along these lines would provide a theoretical basis for the design of novel plant-based functional foods for the prevention and treatment of diabetic patients.

The present work has found a significant increase in the UV-vis absorption of α-amylase upon complexation with gallic acid, with addition of gallic acid also appearing to quench the intrinsic fluorescence of α-amylase with a 1:1 stoichiometry, leading to the decrease in fluorescence intensity. This outcome is likely a result of the formation of hydrogen bonds between hydrogen acceptor sites of protein and hydroxyl groups of the phenolic acid, which expands the π electron cloud density in the vicinity of aromatic amino acid residues and the phenolic ring of the ligand. CD and FTIR analysis showed an increase in α-helical and β-sheet components of α-amylase upon complexation with gallic acid, likely hampering the channelling of the substrate into the catalytic sites. Kinetic analysis, DSC and molecular modelling have also been conducted to support and further identify the binding pocket between gallic acid and α-amylase.

**References**

1. Ferreira, P.S.; Victorelli, F.D.; Fonseca-Santos, B.; Chorilli, M. A review of analytical methods for p-coumaric acid in plant-based products, beverages, and biological matrices. *Critical Reviews in Analytical Chemistry* **2019**, *49*, 21-31.

2. Sun, L.; Warren, F.J.; Gidley, M.J. Natural products for glycaemic control: Polyphenols as inhibitors of alpha-amylase. *Trends in Food Science & Technology* **2019**, *91*, 262-273.

3. Condict, L.; Hung, A.; Ashton, J.; Kasapis, S. High-temperature binding parameters and molecular dynamics of 4-hydroxybenzoic acid and β-casein complexes, determined via the method of continuous variation and fluorescence spectroscopy. *Food Hydrocolloids* **2021**, *114*, 106567.

4. Abdollahi, K.; Ince, C.; Condict, L.; Hung, A.; Kasapis, S. Combined spectroscopic and molecular docking study on the pH dependence of molecular interactions between β-lactoglobulin and ferulic acid. *Food Hydrocolloids* **2020**, *101*, 105461.