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Determination of the thermodynamic binding constants of hydroxycinnamic acids with BSA using a flow-based approach with fluorimetric detection

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Flow analysis has started as an automation tool for wet chemical assays [1]. Its scope was further expanded to study chemical and biochemical processes. Due to its versatility and possibility for real-time monitoring, it has also become a powerful tool to study dynamic systems [2]. In this context, a flow based approach coupled to a fluorimetric detection system for the determination of the thermodynamic binding constants of hydroxycinnamic acids (HCA) (Fig.1.), namely p-coumaric acid (CA), caffeic acid (CFA) and trans-ferulic acid (FA), with bovine serum albumin (BSA) was used.

The fluorescence detection was chosen since molecular interactions can result in quenching of fluorescence when concerning biochemical systems [3]. These interactions can be described as dynamic quenching, that is a result of collisional encounters between the quencher and the fluorophore; or can be described as static quencher, as a result of binding between the fluorophore and the quencher [3]. Therefore, fluorescence quenching was used as tool to study the interaction between the protein and the hydroxycinnamic acids.

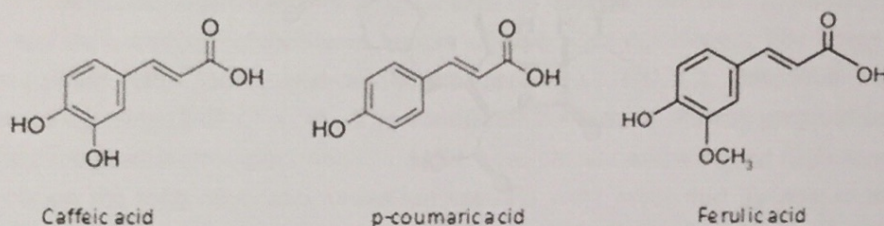


Fig. 1. Chemical structures of the tested hydroxycinnamic acids used.

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