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Development of chemometric-assisted supercritical fluid extraction of effective and natural tyrosinase inhibitor from *Syzygium aqueum* leaves

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ABSTRACT

Tyrosinase is a key enzyme in enzymatic browning, causing quality losses in food through the oxidation process. Thus, the discovery of an effective and natural tyrosinase inhibitor via green technology is of great interest to the global food market due to food security and climate change issues. In this study, *Syzygium aqueum* (*S. aqueum*) leaves, which are known to be rich in phenolic compounds (PC), were chosen as a natural source of tyrosinase inhibitor, and the effect of the sustainable, supercritical fluid extraction (SFE) process was evaluated. Response surface methodology-assisted supercritical fluid extraction (RSM-assisted SFE) was utilized to optimize the PCs extracted from *S. aqueum*. The highest amount of PC was obtained at the optimum conditions (55 °C, 3350 psi, and 70 min). The IC_{50} (661.815 μg/mL) of the optimized extract was evaluated, and its antioxidant activity (96.8 %) was determined. Gas chromatography-mass spectrometry (GC–MS) results reveal that 2′,6′-dihydroxy-4′-methoxychalcone (2,6-D4MC) (82.65 %) was the major PC in *S. aqueum*. Chemometric analysis indicated that 2,6-D4MC has similar chemical properties to the tyrosinase inhibitor control (kaempferol). The toxicity and physiochemical properties of the novel 2,6-D4MC from *S. aqueum* revealed that the 2,6-D4MC is safer than kaempferol as predicted via absorption, distribution, metabolism, and excretion (ADME) evaluation. Enzyme kinetic analysis shows that the type of inhibition of the optimized extract is non-competitive inhibition with Km = 1.55 mM and Vmax = 0.017 μM/s. High-performance liquid chromatography (HPLC) analysis shows the effectiveness of *S. aqueum* as a tyrosinase inhibitor. The mechanistic insight of the tyrosinase inhibition using 2,6-D4MC was successfully calculated using density functional theory (DFT) and molecular docking approaches. The findings could have a significant impact on food security development by devising a sustainable and effective tyrosinase inhibitor from waste by-products that is aligned with the United Nation's SDG 2, zero hunger.

1. Introduction

Consumers are concerned about the nutritional aspects and quality of food products. The appearance and color of foods are also critical factors during food selection. Therefore, maintaining the shelf life and color of food is a significant issue for food industries and food chemists. One challenge is the phenomenon of food browning, or enzymatic browning, which is the main reason for the color changes that occur in fruits and vegetables. Enzymatic browning is a chemical process involving oxidation reactions in food caused by the enzyme polyphenol oxidase (PPO), also known as tyrosinase, which results in the occurrence of brown pigments [1,2].

Tyrosinase is a multifunctional copper-containing enzyme that catalyzes the production of melanin and other pigments via the oxidation of tyrosine; these pigments are widespread in plants, mammals and bacteria [3]. In mammals, melanin is responsible for the pigmentation of the

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