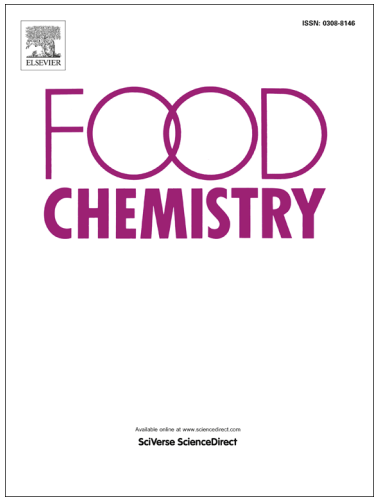

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Identifying an isoflavone from the root of *Pueraria lobata* as a potent tyrosinase inhibitor

Short title: Anti-tyrosinase activity of calycosin from *Pueraria lobata* root

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ABSTRACT

Traditionally, the roots of *Pueraria lobata* are widely used as a functional food. It was observed that a 70% ethanol extract showed a dose-dependent inhibition towards mushroom tyrosinase. Among the different isolated compounds, calycosin demonstrated potent inhibitory activity against substrates L-tyrosine and L-DOPA, with IC_{50} of 1.45 ± 0.03 and 7.02 ± 0.46 μM, respectively. Conversely, formononetin and daidzin exhibit weak inhibition. Moreover, kinetic studies revealed calycosin to be a competitive inhibitor for both substrates. Additionally, molecular docking simulation showed that the hydroxyl groups at C-3′ and C-7 positions interacted with the catalytic site and peripheral residues, demonstrating a higher affinity toward mushroom tyrosinase. Accordingly, our results suggest that, rather than a mono-substituted hydroxyl or methoxyl group, the presence of a hydroxyl group at C-3′ and a methoxyl group at C-4′ position of the isoflavone skeleton plays an essential role in the manifestation of anti-browning activity in food products.

Keywords: Mushroom tyrosinase, *Pueraria lobata*, isoflavones, calycosin

1. Introduction

It is undeniable that the nutritional and quality aspects of food products are a major concern for consumers. However, appearance is also important, as consumers perceive this foremost during food selection. Colour is the critical factor in the appearance of fruit, vegetables, and seafood products. Furthermore, browning usually damages the colour attributes and sensory properties. Browning occurs by two processes: enzymatic and non-enzymatic oxidation. Moreover, enzymatic browning is the outcome of the action of a group of enzymes, namely tyrosinases, distributed in nature. Enzymatic browning takes place in the presence of oxygen when tyrosinase and its polyphenolic substrates (L-tyrosine or L-3,4-dihydroxyphenylalanine (L-DOPA)) form a brown pigment, melanin, leading to the rupture of cell structure (Chang, Ding, Tai, & Wu, 2007; Loizzo, Tundis, & Menichini, 2012). The rate of enzymatic browning depends on the concentration of tyrosinase and phenolic substrates, oxygen availability, pH, temperature, and other factors (Zheng, Cheng, Chao, Wu, & Wang, 2008). As