

## ***In Silico and In Vitro* Analysis of Inhibition of Rice Bran Lipase to Extend the Shelf Life**

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Rice bran is a byproduct of the rice (*Oryza sativa*) milling process. The hard, outer layer removed from the starchy endosperm of the rice grains is known as rice bran. Rice bran is a rich source of nutrients. Rice bran is used to produce rice bran oil and as animal feed. Oxidation of fatty acids in lipids is one of the main causes for the spoilage of rice bran during storage. Lipase enzymes catalyze the hydrolysis of ester bond in triglycerides (lipids) releasing free fatty acids, which are more prone to oxidation than fatty acids in triglycerides. Therefore, inhibition of lipases can be a possible solution to restrict the lipid oxidation. The active site of lipases contains a characteristic GXSXG pentapeptide sequence (where X = any amino acid) which plays a major role in the lipase enzymatic activity. Orlistat is a lactone known to act as a potent inhibitor of human lipase. Therefore, in the present study, we evaluated the lipase inhibitory potential of lactones that are present in *Psidium guineense*. Homology-modelling of rice bran lipase and molecular docking studies (SWISSDOCK) were carried out to identify compounds with high affinity at the binding site. The top 20 docking poses with the lowest estimated Gibbs free energy values ( $\Delta G$ ) were considered from the molecular docking study. Low  $\Delta G$  values of lactones show preferable binding at the binding site of *Oryza sativa* lipase. Close proximity of electrophilic carbon of lactones to the nucleophilic oxygen of the serine residue indicates the possibility of a nucleophilic attack by the oxygen of the serine residue to the electrophilic carbon of lactones leading to a covalent bond formation inhibiting the lipase enzyme. This suggests that lactones present in guava may be capable of inhibiting the lipases present in *Oryza sativa*. The in silico data were validated using lipase purified from rice bran. Rice bran lipase was purified by ion exchange chromatography followed by size exclusion chromatography. Inhibition of lipase activity was assessed using phenyl acetate assay. Percentage inhibition of lipase activity by guava leaf extract and Orlistat were 74.1% and 58.8% respectively. This indicates that guava extract contains compounds with inhibitory action towards lipase enzyme and they are more effective in inhibiting lipase than Orlistat. Even though the rice bran is one of the richest and cheapest sources of antioxidants, easy oxidative spoilage makes the shelf life of rice bran short. However, when the antioxidants stripped from rice bran re mixed with rice bran, a concentration dependent inhibition of the formation of oxidation products in rice bran was observed suggesting that bioavailability of the antioxidants present in the rice bran is low. While the docking studies provide evidence of inhibition of rice bran lipase activity, empirical evidence require analysis using purified lactones from guava on the inhibition of rice bran lipase. Our findings suggest antioxidants and lactones inhibit rancidity of rice bran during storage by inhibiting oxidation of lipids and inhibiting the lipase activity.

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