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Purification and Characterization of Pear (Pyrus communis) Polyphenol Oxidase

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Abstract: Three isoenzymes of polyphenol oxidase (PPO) were isolated from a local pear variety (Ankara Armutu) through ammonium sulfate precipitation, dialysis and gel filtration. The sample obtained from dialysis after ammonium sulfate precipitation was used for characterization of the partially purified enzyme. Optimum pH values of PPO were 8.2 for pyrogallol, 7.2 for 4-methylcatechol, 7.0 for catechol, 5.6 for D-tyrosine, 5.0 for p-cresol and 4.8 for L-dopa. The optimum temperature of PPO was 35 °C with 4-methylcatechol. Catechol was oxidized more rapidly than the other substrates; however, 4-methylcatechol, chlorogenic acid and caffeic acid were also good substrates. The effect of 6 different inhibitors on the PPO activity was investigated in this work. L-ascorbic acid, L-cystein and sodium diethyldithiocarbamate were the most effective inhibitors. K_m and V_{max} values of the enzyme were estimated as 5.55 mM and 344.5 IU/mL min respectively for catechol substrate. Thermal inactivation data indicated an apparent activation energy of 0.19 cal/mol. Three isoenzymes of Ankara pear PPO were detected by activity analysis in nondenaturing-polyacrylamide gel electrophoresis. Their molecular weights were determined as 60, 40 and 28 kDa by sodium dodecyl sulfate-PAGE.

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