Title Characterization of polyphenoloxidase (PPO) and total phenolic contents in medlar (*Mespilus germanica* L.) fruit during ripening and over ripening

Author F.A. Ayaz, O. Demira, H. Torun, Y. Kolcuoglu and A. Colak

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Abstract

Characterization of polyphenoloxidase (PPO) enzyme and determination of total phenolic concentrations during fruit ripening and over ripening in medlar (*Mespilus germanica* L.) were determined. During ripening, PPO substrate specificity, optimum pH and temperature, optimum enzyme and substrate concentrations were determined. Among the five mono- and di-phenolic substrates examined ((*p*-hydroxyphenyl) propionic acid, l-3,4-dihydroxyphenylalanine, catechol, 4-methylcatechol and tyrosine), 4-methylcatechol was selected as the best substrate for all ripening stages. A range of pH 3.0–9.0 was also tested and the highest enzyme activity was at pH 7.0 throughout ripening. The optimum temperature for each ripening stage was determined by measuring the enzyme activity at various temperatures over the range of 10–70 °C with 10 °C increments. The optimum temperatures were found to be 30, 20 and 30 °C, respectively, for each ripening stage. Optimum enzyme and substrate concentrations were found to be 0.1 mg/ml and 40 mM, respectively. The V_{max} and K_m value of the reaction were determined during ripening and found to be 476 U/mg protein and 26 mM at 193 DAFB (days after full bloom) – stage 1, 256 U/mg protein and 12 mM at 207 DAFB – stage 2, 222 U/mg protein and 8 mM at 214 DAFB – stage 3. For all ripening stages sodium metabisulfite markedly inhibited PPO activity. For stage 1 of ripening, Cu^{2+}, Hg^{2+} and Al^{3+}, for stage 2, Cu^{2+} and Hg^{2+}, and for stage 3, Cu^{2+}, Hg^{2+}, Al^{3+} and Ca^{2+} strongly inhibited diphenolase activity. Accordingly, it can be concluded that as medlar fruit ripen there is no significant changes in the optimum values of polyphenoloxidases, although their kinetic parametres change. As the fruit ripening progressed through ripe to over-ripe, in contrary to polyphenoloxidase activity, there was an apparent gradual decrease in total fruit phenolic concentrations, as determined by using the aqueous solvents and water extractions.