Title	Rapid assay of polyphenol oxidase using fluorescence oxygen probe
Author	M. Miyawaki
Citation	Book of Abstracts, 2004 IFT (Institute of Food Technologists) Annual Meeting and Food Expo, 13-16 July 2004, Las Vegas, Nevada, USA. 321 pages.
Keyword	polyphenol oxidase; browning

Abstract

Polyphenol oxidase (PPO) catalyze the oxidation of phenolic compounds into o-quinones, causing discoloration of fruits and vegetables upon cutting/ blending. The most common assay of PPO uses increase in absorbance at 420 nm. However, this method requires cleanup to obtain optically clear enzyme solutions and takes ~15 min per sample. A rapid alternative for PPO activity determination is measurement of dissolved oxygen by PPO-catalyzed reaction. This approach has the advantage over spectrophotometric method in that crude homogenates can be used as PPO source. Our objectives were to develop a prototype instrument for rapid measurement of PPO activity and to compare its performance to the conventional spectrophotometric method. Our prototype consisted of a tubular reaction cell, in which the oxygen was determined by fluorescence oxygen probe, and two syringes for simultaneous injection and on-line mixing of PPO solution and buffer/ substrate mixture. A standard comparison between our prototype and spectrophotometric methods to determine PPO activity used purified tyrosinase at selected concentrations and catechol as substrate. In addition, PPO activity in apple homogenates using our prototype method was compared to that in the extract determined by spectrophotometric assay. Using the prototype, oxygen depletion was linear for 300 s using 2.86 units/ mL of tyrosinase, while spectrophotometric assay with equivalent tyrosinase resulted in linear absorbance increase at 420 nm for 45 s. Both oxygen consumption and change of absorbance at 420 nm were correlated to tyrosinase concentration with regression coefficients of 0.995 and 0.993, respectively. Similar activities of apple PPO, 14.2 units/g and 13.8 units/g based on tyrosinase activity, were obtained from two different assays. While our prototype experiments were carried out for 5 min, assay time can be reduced to 2 min without loss of precision. Compared to the conventional spectrophotometric method, our instrument reduced assay time by 70 to 80%, providing accurate PPO activity.