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Journal of Food Engineering 68 (2005) 349–356
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Kinetics and inactivation of carrot peroxidase by heat treatment

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Received 5 March 2004; accepted 9 June 2004

Abstract

The activity and kinetics of carrot peroxidase were determined by using pyrogallol, guaiacol and *o*-dianisidine as hydrogen donors and peroxidase inactivation was studied by thermal and microwave heating. The kinetics of peroxidase showed characteristics which were dependent upon the identity and concentration of the hydrogen donor used. With pyrogallol and guaiacol, the true K_m was found to be 0.34 and 1.4 mM for hydrogen peroxide, respectively, whereas the apparent K_m with *o*-dianisidine was 7.7×10^{-3} mM. The lowest K_m and highest V_{max}/K_m with *o*-dianisidine exhibited the greater tendency of the enzyme toward hydrogen peroxide and the specificity of the competing substrate, *o*-dianisidine. Thermal treatment of carrot peroxidase was done in the range of 35–75 °C for 0.5–180 min. Inactivation kinetics of peroxidase showed a biphasic first-order model, while at 75 °C, peroxidase showed monophasic first-order behaviour. Kinetic parameters, k and E_a, were determined for heat labile and heat resistant fractions of peroxidase. Biphasic behaviour of enzyme inactivation was observed for the microwave treatment at 70 and 210 W, whereas at 350 and 700 W microwave powers enzyme inactivation was monophasic. Microwave heating was found to be more effective for inactivating the enzyme than thermal treatment and additionally vitamin C retention was higher in microwave treated samples compared to heat treatment.
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Keywords: Carrot; Peroxidase; Kinetics; Thermal heating; Microwave heating

1. Introduction

Peroxidase (POD) (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) catalyses the oxidation reactions using either peroxides or oxygen as hydrogen acceptor. The mechanism of peroxidases is based on the formation of enzyme–hydrogen donor complexes (Hemeda & Klein, 1991). The hydrogen donors may be phenols, amines or other organic compounds and the products that are formed during oxidation depend on the identity of the hydrogen donors.

The kinetics of POD is found to be altered by the concentration of donors, while it is hardly affected by the changes in the hydrogen peroxide concentration (Bergmeyer, 1974). Therefore the true K_m of hydrogen peroxide is that observed when the donor is saturating and the true V_{max} is observed when all substrates are present at saturating concentrations (Segel, 1975).

POD is recognized as being one of the most heat-stable enzyme and it is widely used as an index of blanching. Heat inactivation of POD generally occurs in biphasic stages due to the presence of isoenzymes with different thermal stabilities (Güneş & Bayındırlı, 1993; Ling & Lund, 1978; Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002; Naveh, Mizrahi, & Kopelman, 1982). Morales-Blancas et al. (2002) reported a biphasic first-order model for thermal inactivation curves of carrot peroxidase. Güneş and Bayındırlı (1993) assumed a first-order kinetic model to analyse the thermal inactivation of carrot peroxidase which consist of heat labile and heat resistant fractions. Anthon and Barrett (2002) also reported the presence of heat resistant and heat labile

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doi:10.1016/j.jfoodeng.2004.06.009

fractions of carrot peroxidase. Biphasic inactivation model was proposed to describe the inactivation kinetics of an enzyme system formed by a heat labile and a heat resistant fraction, both with first-order inactivation kinetics (Ling & Lund, 1978). The stability of different isoenzymes varies with the donor substrate supplied. Therefore, experimental results for POD assays are directly comparable only if they have been obtained with the same hydrogen donor. This is specially important if the enzyme is used as a thermal treatment index for fruits and vegetables (Rodrigo, Rodrigo, Alvarruiz, & Frigola, 1996).

Because the stability of isoenzymes varies with the donor substrate supplied, choice of the donor was important for the inactivation experiments. Although POD is widely distributed in nature and is used as an index for the adequacy of blanching, its structural and kinetic properties have rarely been studied. Therefore, the first aim of our study was to study the activity and kinetics of carrot POD with guaiacol, pyrogallol and *o*-dianisidine as hydrogen donors. The kinetic parameters obtained at saturating concentrations of hydrogen peroxide were compared and evaluated with respect to the tendency of enzyme toward the substrate at fixed amount of each hydrogen donor. The second aim of our study was to examine thermal inactivation kinetics of carrot POD and also to compare the effects of thermal and microwave treatments on the activity of POD.

2. Materials and methods

2.1. Materials

Carrot (variety: Chantenay) was purchased from local market. *o*-Dianisidine, guaiacol, pyrogallol and hydrogen peroxide were obtained from Sigma Chemical Company, USA. The stock solution of *o*-dianisidine was prepared freshly in 100% methanol and solutions of other reagents, guaiacol, pyrogallol and hydrogen peroxide were prepared using distilled water. The common reagents used were all reagent grade (Merck or Riedel de Haen).

2.2. Methods

2.2.1. Composition of carrot

Total sugar, lipid, moisture, ash, protein and vitamin C contents of carrot were determined at least in triplicate runs. Sugar content was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Lipid content was determined by extracting the oil through hexane by Soxhalet method. Moisture content was determined by heating at 105 °C for 3.5 h in the oven and by using infrared dryer at 105 °C for 30 min. Ash content was determined in Nüve MF 120 type oven by igniting at 550 °C for 4 h. Protein content was determined by Kjeldahl method (AOAC, 1990). Vitamin C content of the crude and treated extracts were determined by the usual indephenol titration method (Egan, Kirk, & Sawyer, 1981). Vitamin C content was expressed as mg ascorbic acid per 100 g carrot.

2.2.2. Preparation of crude extract

Carrots were washed and cut into small pieces and blended in Waring blender at high speed with 74% (w/v) water addition at 4 °C. The slurry was filtered through two layers of cheese cloth and the filtrate was centrifuged for 15 min at 12000 rpm in an Eppendorf model 5810 R centrifuge at 4 °C (Hemeda & Klein, 1990). The supernatant was kept frozen approximately at −20 °C and used as the enzyme source.

2.2.3. POD assay and kinetics

All of the activity measurements were done in potassium phosphate (KP) buffer at pH 6.0 and 21 °C. The carrot extract contained 1.7 mg protein/mL. A Beckman model 24 double-beam spectrophotometer with a Beckman recorder model 24-25 AC was used for activity measurements and the temperature of cell compartment was maintained with Haake circulator KT 33. The initial rates of reactions were taken from the slopes of linear progress curves generated on the recorder. One enzyme unit is the change in absorbance per minute under assay conditions. Lineweaver–Burk plot was prepared to determine K_m for H_2O_2 and V_{max} for the enzyme.

2.2.3.1. POD assay and kinetics by pyrogallol. POD activity of carrot extract was determined spectrophotometrically at 430 nm. The assay mixture contained 0.025 M KP buffer (pH 6.0), 4 mM pyrogallol as hydrogen donor, 1.2 mM hydrogen peroxide (H_2O_2) as substrate and 0.1 mL enzyme extract. Pyrogallol and H_2O_2 were prepared as mixing together with defined concentrations in 0.05 M KP buffer (Weng, Hendrickx, Maesmans, Gebruers, & Tobback, 1991). For determination of K_m and V_{max}, H_2O_2 concentrations from 0.0375 to 1.5 mM were used at 4 mM pyrogallol concentration in 0.025 M KP buffer and at fixed enzyme concentration (0.085 mg protein/mL). Molar extinction coefficient, 2800 $M^{-1}cm^{-1}$ was used to convert absorbance into molarity (Keyhani, Verssizadeh, & Keyhani, 2000).

2.2.3.2. POD assay and kinetics by guaiacol. POD activity was determined spectrophotometrically as the change in absorbance at 470 nm. The reaction mixture contained 2.15 mL of 0.01 M KP buffer (pH 6.0) containing 1.0% (v/v) guaiacol; 0.25 mL of 0.1% (v/v) H_2O_2 and 0.1 mL enzyme extract (Flurkey & Jen, 1978). For determination of K_m, H_2O_2 concentrations from 0.326 to 26

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