Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (March, 2012), 20(1): 27-31
ISSN 0794-5698

Optimum Temperature and Thermal Stability of Crude Polyphenol Oxidase from some Common Fruits

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ABSTRACT: The effect of temperature on the activity and thermal stability of crude polyphenol oxidase (PPO) extracted from garden egg (*Solanum aethiopicum*), pawpaw (*Carica papaya*), pumpkin (*Cucurbita pepo*), guava (*Psidium guajava*) and bush mango (*Irvingia gabonnensis*) fruits were studied using 20 mM Catechol solution as a substrate. The optimum temperature was found to be 30^{0}C for the enzyme extracted from guava, 40^{0}C for that extracted from both pawpaw and pumpkin and 50^{0}C for that from both garden egg and bush mango. The PPO extracted from all the fruits is stable upon incubation for 10 to 120 minutes at temperature between 20- 70^{0}C. The activity of the crude enzyme from all the fruits used was found to decrease at various degrees after incubation for 10 to 120 minutes at temperature greater than 70^{0}C. Therefore increasing the incubation temperature above 70^{0}C would cause decrease in the activity of the enzyme and can be a good method of controlling undesirable changes caused by the enzyme in the products of these fruits.
Key words: Polyphenol oxidase, Common Fruits, Optimum Temperature, Thermal Stability

INTRODUCTION

Polyphenol oxidase, also known as tyrosinase (monophenol, *o*-diphenol: oxygen oxidoreductase EC 1.14.18.1), is a copper-containing enzyme which is widely distributed in plants (Vaughn and Duke, 1984) that catalyzes two different reactions, using molecular oxygen: the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) (Escribano *et al.,* 1997; Chazarra *et al.,* 1999; Orenes-Piñero *et al.,* 2005) and the oxidation of the *o*-diphenols to *o*-quinones (diphenolase activity) (Chazarra *et al.,* 1996; Chazarra *et al.,* 2001; Gandía-Herrero *et al.,* 2004; Núñez-Delicado *et al.,* 2005; Sellés-Marchat, *et al.,* 2006). This enzyme is a very important for food processing industries because during the processing of fruits and vegetables any wounding may cause cell disruption and lead to the formation of quinones, and their interaction with amino acids and proteins will enhance the brown colour produced (Valero *et al.,* 2003). Not only may the appearance of food and beverages be affected but also the taste and nutritional value, often decreasing the quality of the final product (Martinez and Whitaker, 1995) with considerable economic and nutritional loss. For these reasons, there are considerable losses in their market value and product quality (Gauillard and Richard-Forget, 1997). Heat treatment is the most widely utilized method for stabilizing foods because of its capacity to destroy microorganisms and to inactivate enzymes. Steam blanching is one of the most effectively applied methods of heat treatment for controlling enzymatic browning in canned or frozen fruits and vegetables (Vámos-Vigyázó, 1981). Steam blanching is not however feasible for the prevention of browning in fresh foods (Vámos-Vigyázó, 1981). Temperatures applied in steam blanching treatments vary in accordance with the thermostability of the enzyme to be inactivated as well as with the nature of the food product (Vámos-Vigyázó, 1981). Pasteurization is generally conducted at temperatures ranging between 60^{o}C and 85^{o}C, while blanching techniques are often operated at temperatures ranging between 70^{o}C and 105^{o}C or higher (Vámos-Vigyázó, 1981). Generally, exposing polyphenol oxidases to temperature range of 70-90^{o}C, will results in the destruction of their catalytic activity (Vámos-Vigyázó, 1981). Blanching in an automatic rotary hot water blancher of green beans at temperatures of 82^{o}C and above for 3.5 minutes, almost completely inactivated polyphenol oxidase activities (Lee *et al*., 1988). Thermal inactivation profiles of important enzymes such as peroxidase, polyphenol oxidase, and lipoxygenase in fruit and vegetable processing, follow first-order reaction kinetics (Svensson, 1977). This paper report on optimum temperature and temperature ranges of PPO extracted from garden egg (*Solanum aethiopicum*), pawpaw (*Carica papaya*), pumpkin (*Cucurbita pepo*), guava (*Psidium guajava*) and bush mango (*Irvingia gabonnensis*).

MATERIALS AND METHODS

Extraction of PPO

The fruits used in this research were purchased from Kofar wambai, Rimi and Yan Lemo market in Kano, Nigeria during raining season. The fruits were sliced

horizontally into halves with a sharp knife, seeds were removed and the fruit cavities were cleaned in case of pawpaw. Each half was cut into four equal slices, and the processed fruit samples were stored at 5^0 C until required.

Prepared fruit sample (50g) was homogenised using pestle and mortar for 1 minute in 400 cm^3 of cold acetone. The homogenates were filtered quickly under vacuum of a Buchner funnel. The filterate was suspended in 150cm^3 of 0.1M sodium phosphate buffer pH (6.5) and stirred for 30 minutes at 0^0C. The suspension was centrifuged at 10,000rpm for 30 minutes at 4^0C. The homogenate contained the extracted PPO (Ying and Zhang, 2008).

PPO Assay:

Polyphenol oxidase activity was determined by measuring the increase in absorbance at 410 nm with a spectrophotometer. The sample cuvette contained 2.0 cm^3 of catechol (10-80mM), 0.9 cm^3 of 0.2 M sodium acetate buffer pH 4.0 and 0.1 cm^3 of fruit extract. Each sample was assayed in triplicate. Reference cuvette (blank) contained 2.0 cm^3 of the same substrate solution and 1.0 cm^3 of 0.2 M sodium acetate buffer (Ying and Zhang, 2008).

Effect of Temperature on PPO Activity and Stability

The PPO activity was determined at various temperatures created in a thermostat controlled by a water-bath (DS Lab Thermostatic bath, model DSB-1000 made in Taiwan). The mixtures of 0.9 cm^3 of 0.2 M sodium acetate buffer (pH 4.0) and 2.0 cm^3 of 20mM catechol solution were incubated for 5 mins at various temperatures over the range of 10– 90^0C, prior to the addition of 0.1 cm^3 of fruit extract. The relative activity of PPO at a specific temperature was determined spectrophotometrically by addition of fruit extract to the mixture as rapidly as possible (Marcos *et al*., 2008). Residual PPO activity was determined in the form of percent residual PPO activity at the optimum temperature (Marcos *et al*., 2008).

To determine the effect of temperature on PPO stability, 0.2 cm^3 of crude enzyme solution were injected into Eppendorff tube, and incubated at various temperatures (20, 30, 40, 50, 60, 70 and 80^0C) for different time (10-120 minutes) in a water-bath, and the tubes were cooled in an ice bath immediately. The sample cuvette contained 2.0 cm^3 of 20 mM catechol, 0.9 cm^3 of 0.2 M sodium acetate buffer, pH 4.0 and 0.1 cm^3 of heated enzyme solution. The percentage residual PPO activity was calculated by comparison with unheated enzyme (Marcos *et al*., 2008).

RESULTS

The effect of temperature on the activity of crude PPO from the common fruits at pH4.0 and using catechol as substrate is shown in Figures 1-5. The optimum temperature for the crude enzymes ranges from 30^0C for PPO from both guava and bush mango to 50^0C for that from garden egg.

The effect of temperature on the stability of PPO from garden egg, pawpaw, pumpkin, guava and bush mango is shown in Figures 6-10. It reveals that, the crude enzymes from all the fruits used are stable upon incubation for 10 to 120 minutes at 20- 70^0C. Decrease in activity was observed at temperature greater than 70^0C after 10 to 120 minutes of incubation which varies in degrees with source of the enzyme (0% activity was detected for the enzyme extracted from 700C for garden egg after 80minutes, five minutes for pawpaw, 70minutes for pumpkin, 10minutes for guava and 90munutes for bush mango respectively).

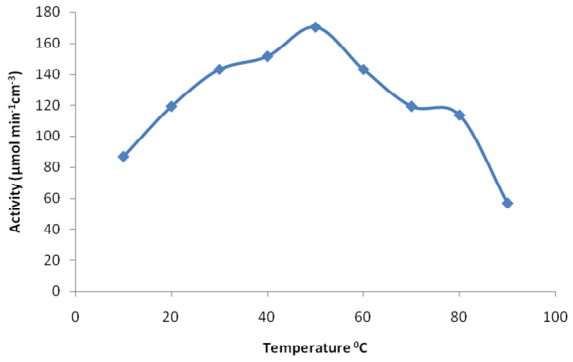

Figure 1: Activity- temperature profile of crude PPO from garden egg

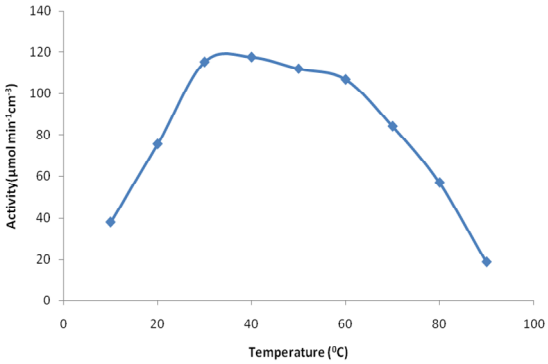

Figure 2: Activity- temperature profile of crude PPO from pawpaw

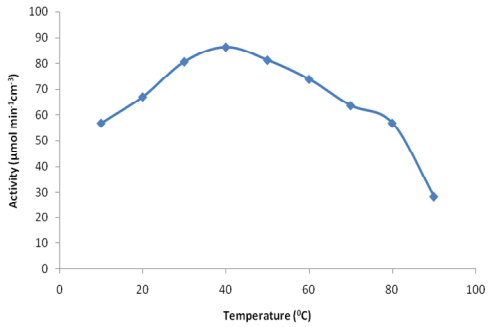

Figure 3: Activity- temperature profile of crude PPO from pumpkin

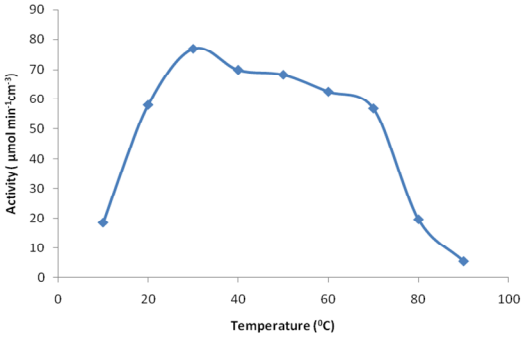

Figure 4: Activity- temperature profile of crude PPO from guava

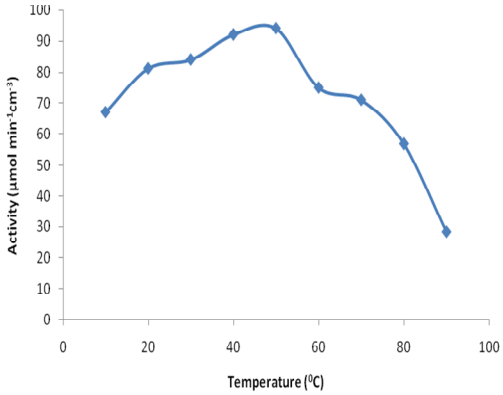

Figure 5: Activity- temperature profile of crude PPO from bush mango

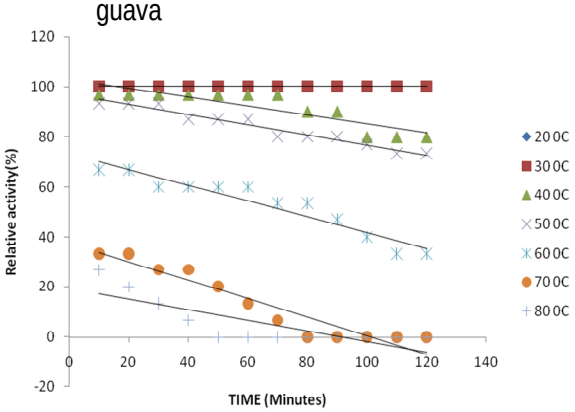

Figure 6: Plots of Relative activity (%) against time of incubation for PPO extracted from garden egg at different temperature.

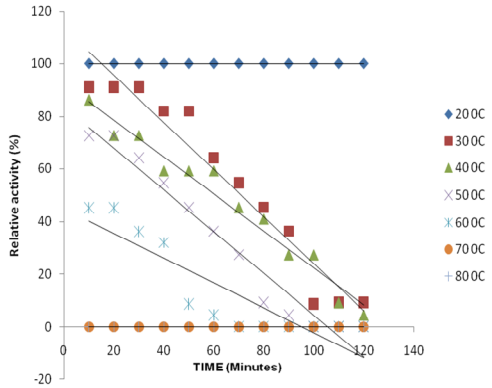

Figure 7: Plots of Relative activity (%) against time of incubation for PPO extracted from pawpaw at different temperature.

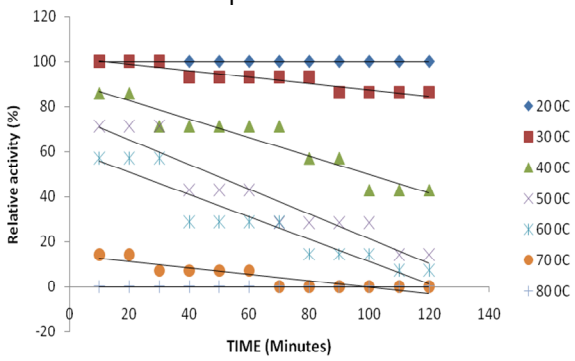

Figure 8: Plots of Relative activity (%) against time of incubation for PPO extracted from pumpkin at different temperature.

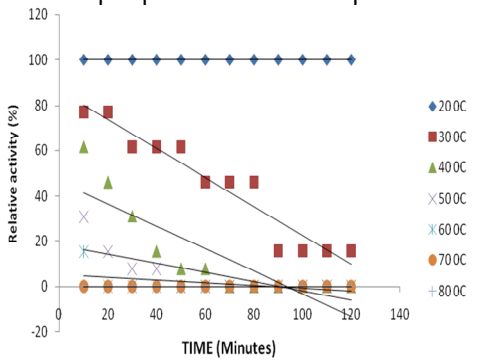

Figure 9: Plots of Relative activity (%) against time of incubation for PPO extracted from guava at different temperatures.

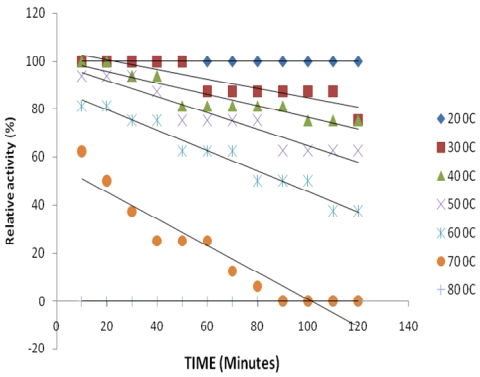

Figure 10: Plots of Relative activity (%) against time of incubation for PPO extracted from bush mango at different temperatures.

DISCUSSION

Temperature is one of the factors that affect the rate of an enzyme catalyzed reaction (Martin, 2006). Optimum temperature is the temperature at which an enzyme shows its highest catalytic activity, a decrease or increase in the temperature below or above the optimum temperature results in decrease in activity. Figures 1 to 5 show that, the optimum temperature for crude PPO from garden egg and bush mango was 50^{0}C which is less than that from melon (60^{0}C) reported previously (Marcos *et al*., 2008) and higher than that from lily (40^{0}C) (Ying and Zhang, 2008). The optimum temperature of PPO from pawpaw and pumpkin was found to be 40^{0}C, which is similar to that from lily(Ying and Zhang, 2008). PPO from guava fruit was found to have the least optimum temperature (30^{0}C) but higher than that from plum (25^{0}C) reported previously (Siddiq *et al*., 1992).

The effect of temperature on the stability of PPO from garden egg, pawpaw, pumpkin, guava and bush mango reveals that; the enzyme from garden egg, pumpkin and bush mango shows no significant decrease in activity upon incubation at 20 to 30^{0}C for 10 to120 minutes (100% for the enzyme from garden egg; 100 and 86% for that from pumpkin and 100 and 75% for that from bush mango) as depicted in Figures 6-10 respectively. PPO from garden egg, pumpkin and bush mango shows more stability at high temperature because activity was detected even after incubation for 120 minutes at 70^{0}C. On the other hand, PPO from pawpaw and guava also showed 100% relative activity upon incubation at 20^{0}C but it showed decrease in activity (91 to 4.5 %) at 30 to 60^{0}C for 10 to 120 minutes and no activity was detected at 70 to 80^{0}C. Therefore PPO from pawpaw and guava are less thermally stable than those from garden egg, pumpkin and bush mango. This result is in line with work of Ying and Zhang (2008), in which PPO extracted from lily showed increased inactivation with increase in temperature (>30^{0}C) and that of Marcos *et al*. (2008) in which PPOs from melon varieties (*Amarillo* and *Charentias*) were nearly completely inactivated after 30 minutes of incubation at 60^{0}C (94% loss of activity).This could be described by a first order decay process.

In conclusion, since the stability of the enzyme upon incubation at a particular temperature for a given time is source dependent. The crude enzymes from all the sources used were found to be inactivated upon incubation at 70^{0}C for 10 to 120 minutes. Therefore Products formed from such fruits be prevented from the unwanted changes cause by enzyme through incubation at 70^{0}C for 10 to 120 minutes respectively.

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