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Full Length Research Paper

Comparison of peroxidase activities from *Allium sativum*, *Ipomoea batatas*, *Raphanus sativus* and *Sorghum bicolor* grown in Burkina Faso

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Current applications of peroxidase in various areas of biotechnology and clinical biochemistry show the interest for further screening for peroxidase. Thus, peroxidase activities were screened in higher plants such as *Allium sativum*, *Ipomoea batatas*, *Raphanus sativus* and *Sorghum bicolor* grown in a tropical environment. The enzymes were investigated for their specific activities and best physico-chemical conditions for activity and stabilities. Optima conditions with respect to pH, temperature and their heat inactivation were determined by monitoring the hydrogen peroxide-dependant oxidation of guaiacol. Results revealed that peroxidase specific activities in *R. sativus* were higher than the other three plant species. Optimum pHs of all screened peroxidase activities were in the acidic range (pH 4.5 to 6.5). Optimum temperatures were ranging from 30 to 40°C. Peroxidase from *R. sativus* was the most thermostable enzyme among the four plants. This suggests that *R. sativus* is a good source of plant peroxidase, which could be used for various applications.

Key words: *Allium sativum*, *Ipomoea batatas*, *Raphanus sativus*, *Sorghum bicolor*, peroxidase.

INTRODUCTION

Peroxidases (POXs) (E.C.1.11.1.7) are among the most ubiquitous enzymes in plant species. POXs are also found in some animal tissues and microorganisms where they are assigned to play a role of protection against toxic peroxides (Welinder, 1992). In plants they participate in the lignification process (Wakamatsu and Takahama, 1993) and in the mechanism of defense in physically damaged or infected tissues (Biles and Martin, 1993).

POXs are heme-containing enzymes that use H_2O_2 to oxidize a large diversity of hydrogen donors such as phenolic compounds, aromatic amines, ascorbic acid, auxin and certain inorganic ions (Vernwal et al., 2006). The family of plant POXs comprises yeast cytochrome c POXs, plant ascorbate POXs, fungal POXs and classical plant secretory POXs. The group of mammalian POXs includes myeloPOX, lactoperoxidase, thyroid POX and prostaglandin H synthetase (Welinder, 1992). Reduction of hydrogen peroxides at the expense of electron dona-ting substrates makes POXs useful in a number of biotechnological applications (Regalado et al., 2004).

In the food industry for example, POXs have been widely used as an indicator of vegetables bleaching, due to their high thermal stability and wide distribution (Rodrigo et al., 1997). Plant POXs are used to produce dyes from natural phenolic compounds (Egorov, 1995). In analytical biochemistry, POXs are used as reagents in clinical diagnosis and in enzyme immunoassays (Agostini et al., 2002).

They can also be used for the treatment of containing phenols and aromatic amine (Kinsley and Nicell, 2000; Klibanov et al., 1980; Nakamoto and Machida, 1992; Diao et al., 2010). They found several applications in bio-bleaching processes, lignin degradation, fuel and chemical production from wood pulp, production of dimeric

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Abbreviations: BSA, Bovine serum albumin; **HRP,** horseradish peroxidase; **POX,** peroxidase.

alkaloids, biotransformation of organic compounds, etc. (Ryan et al., 1994). Although POXs are ubiquitous in the plant kingdom, until now the major source of commercially available POXs is from horseradish (*Armoracia rusticana*) and soybean (*Glycine max*). However, plants of other species may provide POXs whose characteristics are comparable or higher than those of horseradish (Dicko et al., 2006a, b).

The overall objective of this study is to find novel sources of plant POXs for biotechnological applications. Specifically, POXs from plants such as *Allium sativum, Ipomoea batatas, Raphanus sativus,* and *Sorghum bicolor,* grown in tropical climate may be predicted to display high thermostabilities and interesting catalytic properties.

MATERIALS AND METHODS

Chemicals and reagents

Guaiacol was purchased from Aldrich; bovine serum albumin was obtained from Sigma chemicals CO; hydrogen peroxide was purchased from Merck. Other chemicals and reagents employed were of analytical grade.

Plant materials

Enzymes were extracted from *A. sativum* bulbs, *I. batatas* tubers, *R. sativus* roots and *S. bicolor* germinated grains. To minimize stress related differences in POX biosynthesis, all the plant species were grown in the same farm and in the same natural environment in Ouagadougou (Burkina Faso), during the rainy-season 2007 to 2008.

Preparation of POX extracts

Enzyme extracts were prepared by mixing 250 mg of plant ground material with 1.2 mL of 50 mM Tris-HCl buffer pH 7.3 containing 0.5 M $CaCl_2$ and 5 mM β-mercapto-ethanol, at 4°C for 1 h. The homogenate was centrifuged (14000 g, 4°C, 45 min) and the resulting supernatant was used as crude extract of POX. Protein assay was performed by the linearized method of Bradford (Zor and Selinger**,** 1996) using the ratio of A_{620}/A_{450} versus protein concentration. Bovine serum albumin was used as standard protein.

POX assay

POX activity was measured with a spectrophotometric assay by monitoring the H_2O_2-dependent oxidation of guaiacol, at 25°C. The reaction mixture consisted of 10 µL of 200-fold diluted crude enzyme extract, 20 µL of 100 mM guaiacol, 10 µL of 100 mM H_20_2 and 160 µL of 50 mM sodium acetate pH 5.0. Control assays in which the enzyme extracts or substrates were replaced by buffer were performed. The reaction was monitored at 450 nm. One unit of POX activity (U) is defined as the amount of enzyme releasing 1 µmol of guaiacol radical/min under the assay conditions.

Determination the effect of pH on enzyme activities

The optimum pH was determined at 25°C by measuring the activity of the enzyme in buffers of pH ranging from 3 to 8. The POXs activity was measured by performing the routine assay by changing the buffers at various pH values. The used buffers were those of McIlvaine, 50 mM citrate-phosphate.

Determination the effect of temperature on enzyme activities

The optimum temperature was determined at the optimum pH of each enzyme by measuring the activity of the enzyme in temperatures ranging from 30 to 90°C. Thermal stability was studied by preincubating enzyme extracts at temperatures ranging from 30 to 90°C for 10 min. After heating, samples were immediately cooled on ice during 10 min and the residual enzyme activity was then determined with the routine assay.

Statistical analysis

All spectrophotometric analyses were monitored with a MRX 96-well microplate reader on-line interfaced to a computer (Hewlett Packard). Kinetic data were determined in the linear phase of reaction traces using MRX revelation software version 1CXD-4239 (Dynex Technologies, Inc, USA). The reactions were monitored over 10 min. The initial slopes of the reaction traces caused by enzyme activities were corrected with the slopes of the blanks. All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Student t-test (P= 0.05, considered as signification) were used to determine statistically significant differences between enzyme assays.

RESULTS

Comparison of POX specific activities

The results of POX specific activities from *A. sativum* bulb, *I. batatas* tuber, *R. sativus* roots and grain of *S. bicolor* are summarized in Table 1. These four plants display different levels of POX activities using guaiacol and H_2O_2 as substrates. POX specific activities among these plants ranged from 22.1 to 294.6 U/mg. The highest specific activity was found in plant tissues from *R. sativus*, followed by *S. bicolor* and *I. batatas*. *A. sativum* showed significantly lower activity than the other three species. POX specific activity in *R. sativus* was 13 fold higher than that of *A. sativum* and 9 and 4 fold higher than those of *I. batatas* and *S. bicolor*, respectively.

Comparison of optimum pHs

The effect of pH on the activities of POXs from the four POX are shown in Figure 1. All POXs of these plants have their optima pH for activity in acidic buffers (pH < 6). POXs from *S. bicolor* were most active at pH 3.5 to 4 but POXs from *I. batatas* were strongly inactivated at this pH. However, *I. batatas* POXs were most active at pH 6.0. The optimum pH of activity for POXs from *A. sativum* bulbs was around pH 5.5 to 6.5 and POXs from *R. sativus* tubers presented an optimum activity at pH 5.

Table 1. Comparison of peroxidase activities* among four plants.

Plant	Organs	Total protein (mg)	Total POX activity (U)	POX specific activity (U/mg)
A. sativum	Bulbs	24.3 ± 7	537.03 ± 14	22.1 ± 2
I. batatas	Tubers	36.9 ± 3	1154.97 ± 6	31.3 ± 2
R. sativus	Roots	14.7 ± 4	4348.3 ± 52	294.6 ± 13
S. bicolor	Grains (germinated)	28.4 ± 9	1948.24 ± 54	68.6 ± 6

*Enzymes were extracted with 50 mM Tris-HCl buffer, pH 7.3; containing 0.5 M $CaCl_2$ and 5 mM β-mercapto-ethanol. Enzyme activities were determined by monitoring the H_2O_2-dependant oxidation of guaiacol.

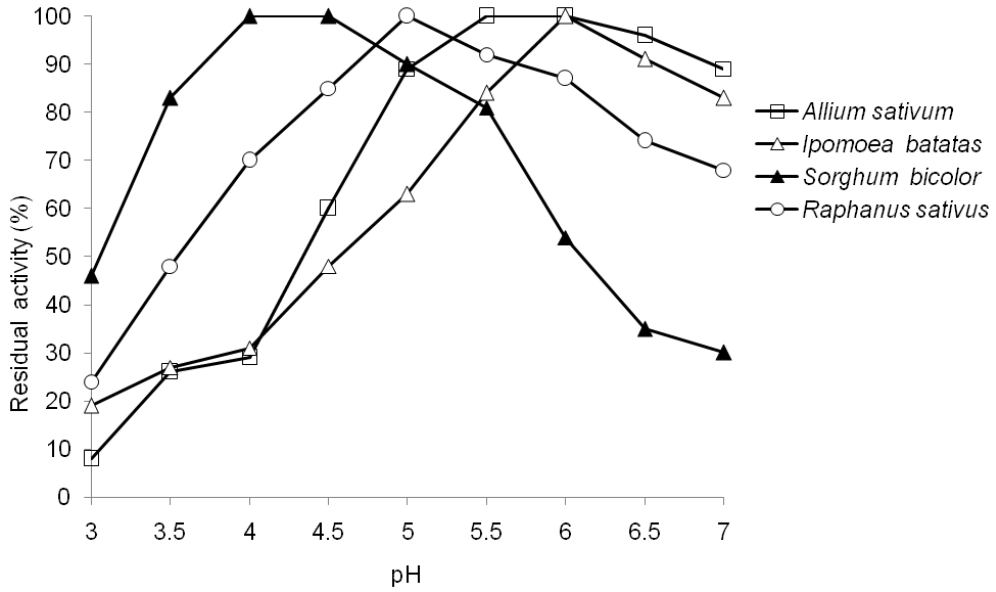

Figure 1. Comparison of the effect of pH on POX activities**.** Enzyme activities were determined by monitoring the H_2O_2-dependant oxidation of guaiacol in citrate-phosphate buffers (pH 3 to 7).

Comparison of temperature effect

The effect of temperature on the activities and stabilities of POXs from the four plant species was examined (Figures 2 and 3). POXs from sorghum were most active at 40°C, but they were completely inactive at 80°C. However, at this temperature, POXs of *I. batatas* and *R. sativus* conserved more than 30% of their activities and were then later completely inactivated at 100°C. These POXs also retained above half of their activities after 10 min of incubation at 70°C. POXs from *I. batatas* and *R. sativus were* most active at 30°C. However, POXs from *A. sativum* are most active at 40°C and were completely inactivated at 90°C.

DISCUSSION

In this contribution, POXs from four plants were compared for their specific activities, and catalytic properties. Based on their hydrogen peroxide-dependent oxidation of guaiacol, all the four plants had significant differences ($P < 0.05$) in their content in POX activities. Among the four plant species, the highest POX activities were detected in *R. sativus*. However, these activities (294.6 ± 13 U/mg) were lower than those (413.5 U/mg) reported by Wang et al. (2004) on the black varieties of the same plant. POXs from *A. sativum* showed significantly lower activities than the other three species. POX specific activities in *I. batatas* (31 U/mg) are higher than data (15 U/mg) from the same plant reported by Santos et al. (2004). The environment may play a significant impact in these differences. POXs from these four plants had optimum pHs ranging from 4.0 to 6.5 with guaiacol as substrate.

Indeed using guaiacol as hydrogen donor, acidic optimum pHs have been reported for many plant POXs (Agostini et al., 1999; Mika and Lüthje, 2003). POXs from *A. sativum* and *S. bicolor* had a great range of pH activities. Other authors (Bhunia et al., 2002; Dicko et al., 2006a) showed that POX activities usually increased with decreasing pH.

Sakharov et al. (2003), using anionic POXs purified from African oil palm tree as biocatalysts, showed that

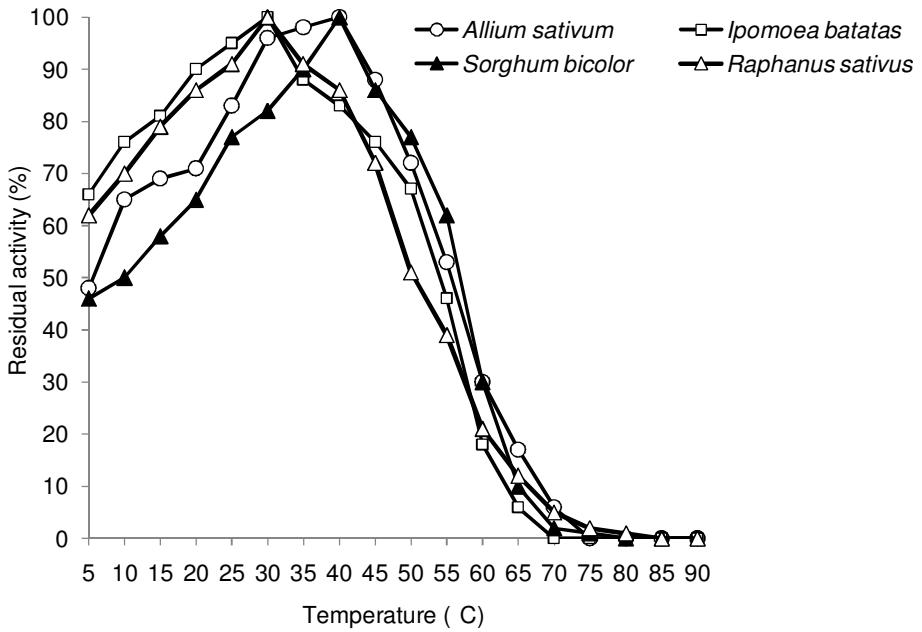

Figure 2. Comparison of the effect of temperature on POX activities. Reactions were performed in 50 mM sodium acetate buffer pH 5, with incubation temperatures ranging from 5 to 90°C. Enzyme activities were determined by monitoring the H_2O_2-dependant oxidation of guaiacol.

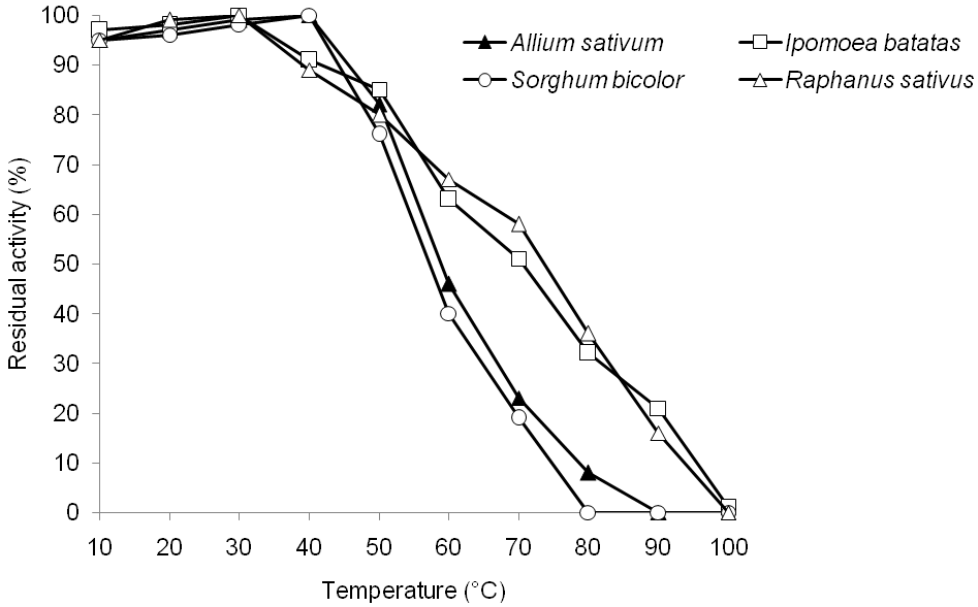

Figure 3. Comparison of thermal stability of POXs. Enzyme activities were determined by monitoring the H_2O_2-dependant oxidation of guaiacol.

polymerization of aniline was optimum at pH 3.5. Polyaniline is one of the most extensively investigated conducting polymers because of its high environmental stability and promising electronic properties. POX from *S. bicolor* displaying similar activities at the same range of pH may be efficient for the same applications. Optima temperatures of activities were at 30°C for POXs from *I. batatas*, *R. sativus* and 40°C for POX from *A. sativum* and *S. bicolor*. El Ichi et al. (2008) reported an optimum temperature of 30°C for POX from *A. sativum* bulbs

cultivated in Tunisia similar to those of *I. batatas*, and *R. sativus.* Among the four plants, POXs from *R. sativus* are the most heat stable followed by POXs from *I. batatas*, *A. sativum* and *S. bicolor*.

Conclusion

It appeared that *A. sativum* bulbs, *I. batatas* tubers, *R. sativus* roots and *S. bicolor* grains have different level of peroxidase activities. Peroxidases from *R. sativus* exhibited the highest specific activities. The present contribution shows that *R. sativus* cultivated in tropical climate such as Burkina Faso may be an alternative source to horseradish for peroxidases. It may also display interesting catalytic properties as well as thermal resistance.

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