Vol. 21(12), pp.553-558, December 2022
DOI: 10.5897/AJB2022.17524
Article Number: D9CAFB570139
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

*Full Length Research Paper*

# Some physicochemical properties of tyrosinase from sweet potato (*Ipomea batatas*)

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Received 17 September, 2022; Accepted 9 November, 2022

**Tyrosinase catalyzes formation of browning in plants, foods and vegetables. Sweet potato (*Ipomea batatas*) undergoes browning after harvesting or during post-harvest operations leading to spoilage and loss of economic value. The physico-chemical properties of purified tyrosinase from *I. batatas* are here described with a view to providing information on the suitability or otherwise of the enzyme for several industrial and biotechnological processes. The enzyme was purified using new approach resulting into final yield and purification fold of 76% and 7.1, respectively. The molecular weight (native) was 48.3 ± 2.5 kDa as estimated on Sephadex G-100. Highest tyrosinase activity was obtained at pH 6.5 while that of temperature was 50°C. Kinetic parameters studies resulted to 2.5 ± 1.2 mM and 451 ± 23.7 units/mg for Michaelis constant ($K_m$) and maximum velocity ($V_{max}$), respectively. This led to catalytic efficiency, $k_{cat}/K_m$ of 1.45 × $10^5$ $s^{-1}$ $M^{-1}$. It was concluded that, tyrosinase from *I. batatas* possesses remarkable properties that could be exploited for biotechnological processes.**

**Key words:** Polyphenols, Solanaceae, biotechnology, biocatalysis.

## INTRODUCTION

Tyrosinase (TYR) is a metalloenzyme with a highly conserved copper binding region and exists in fruits, fungi, vegetables, mammals, cuticle sclerosis and wound healing in insects (Song et al., 2022). They have been reported to be responsible for melanogenesis in humans and browining reactions in plants, fruits and vegetables (Halaouli et al., 2006). Tyrosinase has the ability to react with polyphenols; it has found various applications in biotechnology (Jus et al., 2009). Such functions include synthesis of important drugs (Ates et al., 2007), removal of phenols in wastewater reported by Martorell et al. (2012), grafting of silk proteins onto chitosan through tyrosinase reactions (Anghileri et al., 2007) and cross-linking abilities used in food processing (Ilesanmi et al., 2021). Tyrosinase has been reported in several organisms, but the most characterized is from micro-organism. Recently, higher plants have been exploited for the isolation tyrosinase.

Sweet potato (*Ipomea batatas*) is a dicotyledonous plant that belongs to the order Solanales and family Convolulaceae. It's large, starchy, sweet-tasting, and tuberous roots are used as a root vegetable. Sweet potato tubers undergo browning reactions when harvested and/or during processing. These could be associated with the presence of tyrosinase converting the released polyphenols into polymeric products. Tyrosinase

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has been enzyme of interest for biotechnologists, biochemists and industries because of its increasing use in several applications (Sabarre and Yagonia-Lobarbio, 2021). Hence, the need to exploit several sources for the enzyme to replace traditional source. The presence of tyrosinase in different yam species has been reported (Ilesanmi et al., 2014). In that work, the levels of tyrosinase in seven yam species were reported and four species (*Dioscorea praehensilis*, *Dioscorea rotundata*, *Dioscorea alata* and *Colocasia esculenta*) with highest activities were well characterized. Tyrosinase from *I. batatas* was not characterized. Whether the tyrosinase from *I. batatas* possesses even interesting and remarkable properties, in terms of stability and usability, is not known. The aim of this study, therefore was to investigate and document the physicochemical properties of tyrosinase from the sweet potato for it's suitability in biotechnological applications and industrial processes.

## MATERIALS AND METHODS

Fresh tuber of sweet potato was obtained from local farmers in Owo, Ondo State, Southwestern Nigeria.

### Chemicals

Anhydrous sodium phosphate monobasic ($NaHPO_4$), bovine serum albumin (BSA), L-dihydroxyphenyl-3,4-alanine (L-DOPA), blue dextran, tris base, trizma acid, sodium phosphate dibasic ($Na_2HPO_4$), glutathione, and Coomassie brilliant blue R-250 were obtained from Sigma, USA. Ammonium sulphate, *t*-butanol was obtained from Carl Roth, Karlsruhe, Germany. Sephadex G-100 was purchased from GE Healthcare Bio-sciences, Sweden. All other reagents were of analytical grade.

### Preparation of sweet potato homogenate

Thirty grams of the fresh sweet potato tuber were rinsed with distilled water, peeled and homogenized using mortar and pestle in 70 mL of Na-phosphate buffer (0.05 M, pH 6.5) at 4°C to obtain 57 mL homogenate (30%). The homogenate prepared was subjected to centrifugation at 12,000 rpm for 30 min in a cold centrifuge (4°C) to obtain crude supernatants. The crude supernatant was assayed for tyrosinase activity using L-DOPA as substrate. The supernatants were separated and stored at -20°C.

### Tyrosinase activity assay

The activity of tyrosinase from *I. batatas* was determined according to Ilesanmi et al. (2014). It involved addition of 50 µL of enzyme, 750 µL homogenizing buffer and 200 µL (1 mM final concentration) of L-DOPA to initiate the reaction. The activity was measured kinetically in spectrophotometer.

### Determination of protein concentration

Protein concentration was determined according to the method by Bradford (1976) using bovine serum albumin as standard.

### Purification using three phase partitioning and gel filtration chromatography

Three phase partitioning (TPP) of the crude enzyme was carried out according to the method of Akardere et al. (2010). Briefly, ammonium sulfate was added to the crude tyrosinase to achieve 70% saturation. The mixture was stirred using vortex machine at 4°C. After, *t*-butanol was added at different ratios (1:0.5, 1:1 and 1:1.5 v/v). The mixture was allowed to stand for 1 h at room temperature and centrifuged at 4000 rpm for 10 min. The mixture phase was separated into three layers. The upper *t*-butanol layer was removed carefully. The interfacial precipitate (middle phase) and the lower aqueous layer (bottom phase) were collected separately. The middle phase precipitate was dissolved in 1 mL of sodium 0.2 M acetate buffer, pH 5.0. Thereafter, all three phases were dialyzed to remove ammonium sulfate and then analyzed for tyrosinase activity. The middle phase of all TPP systems gave the highest tyrosinase activity recovery.

The post TPP bottom rich was then further purified by layering on column packed with Sephadex G-100 previously washed and equilibrated with 0.05 M Na-phosphate buffer (pH 6.5). Fractions obtained were assayed for tyrosinase activity and protein profile measured at 280 nm. The active fractions were pooled together and concentrated using lyophilization. About 10 g of the lyophilisate was redissolved in phosphate buffer (50 mM, pH 6.5) for further use.

### Estimation of molecular weight for native tyrosinase

The molecular weight of the enzyme was determined on calibrated Sephadex G-100 column. The calibration curve for the estimation was obtained as plot of partition coefficient ($k_{av}$) of the standard proteins versus their respective molecular weights. Interpolation of the $k_{av}$ values on the standard curve was used to obtain the molecular weight of tyrosinase from sweet potato.

### Estimation of kinetic parameters

The kinetic parameters ($K_m$, $V_{max}$, $k_{cat}$ and $k_{cat}/K_m$) were determined from the plot of varied concentrations of L-DOPA against tyrosinase activities at these concentrations. The $K_m$ and $V_{max}$ were analysed using Graph pad prism 5 and the data obtained were used for estimation of $k_{cat}$ and $k_{cat}/K_m$.

### Effect of temperature on tyrosinase activity

This involved incubation of the substrate solution (1 mM L-DOPA in final mixture), 0.05 M phosphate buffer at pH 6.5 at the different temperature ranging between 10 and 80°C for 5 min. Enzyme was introduced immediately and read at 475 nm in the spectrophotometer. The activity was plotted against their respective temperature.

### Thermal stability of *I. batatas* tyrosinase

The tyrosinase from *I. batatas* was incubated differently at 20, 30, 40, 50, 60 and 70°C for 1 h. At 10 min interval, aliquot was taken for residual activity determination. The percentage residual activity obtained by comparing the activity at zero time was plotted against the time of incubation.

### Effect of pH on *I. batatas* tyrosinase

The determination of pH influence on tyrosinase activity was carried

**Table 1.** Purification summary of tyrosinase from *I. batata*.

| Sample | Volume (ml) | Total activity (units) | Total protein (mg) | Specific activity (units/mg protein) | % Yield | Purification fold |
|---|---|---|---|---|---|---|
| Crude | 20 | 11000 | 30.0 | 367 | 100 | 1.0 |
| TTP | 12 | 13970 | 9.0 | 1541 | 127 | 4.2 |
| Sephadex G-100 | 30 | 8360 | 3.2 | 2606 | 76 | 7.1 |

TPP: Three phase partitioning.
Source: Author

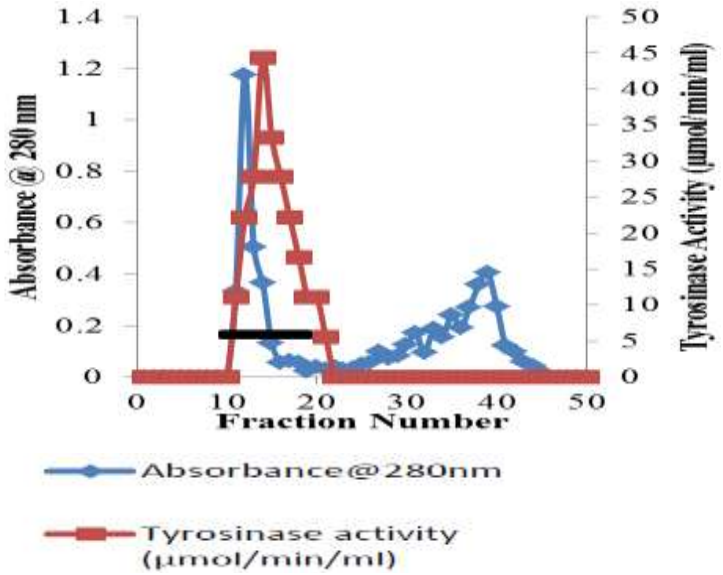


**Figure 1.** Elution profile of partially purified tyrosinase from *I. batata* on Sephadex G-100. The ATPP pool was then layered on Sephadex G-100 column (1.0 cm × 40.0 cm). The proteins were eluted with the equilibration buffer at a flow rate of 12 ml/h. Fractions of 1 ml each were collected and active fractions were pooled and concentrated.
Source: Author

out using pH range of 3.0 to 11.0. The buffer systems used were 0.05 M citrate buffer (pH 3.0 to 5.0), 0.05 M acetate buffer (pH 5.5 to 6.0), 0.05 M phosphate buffer (pH 6.5 to 8.5) and 0.05 M glycine-NaOH buffer (pH 9.0 to 11.0).

## RESULTS AND DISCUSSION

### Enzyme purification

Several traditional purification processes have been employed for tyrosinase from different sources. Most of these purification schemes are expensive and cumbersome and involved a number of steps (Ilesanmi and Adewale, 2020). In this work, a simple, more efficient and economical method we have been developed for separation and purification of target proteins. The three-phase partitioning (TPP) gave percentage yield and purification fold of 127% and 4.2, respectively (Table 1). After further purification using gel filtration chromatography on Sephadex G-100, a single peak of activity was obtained (Figure 1). The percentage recovery and purification fold of 76% and 7.1 were achieved, respectively. The summary of purification is shown in Table 1. ATPS purification method has continued to find application because of the advantage over the chromatographic techniques. The method combines both purification and concentration of the resulting enzyme. High yield obtained may be due to preferential partitioning of the enzyme from other unwanted materials.

### Molecular weight determination

The native enzyme had molecular weights of 48.3 ± 2.5 kDa as estimated on Sephadex G-100 column. This was estimated on calibrated column of Sephadex G-100. Shuster and Fishman (2009) reported molecular weight

**Table 2.** Kinetic Parameters of tyrosinase from *I. batata*.

| Species | $K_m$ (mM) | $V_{max}$ (units/mg protein) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ ($s^{-1}$ $M^{-1}$) |
|---|---|---|---|---|
| *I. batata* | 2.5 ± 1.2 | 451 ± 23.6 | 363.1 | $1.45 \times 10^5$ |

The data are the mean ± standard deviation (SD) of three independent determinations.
Source: Author

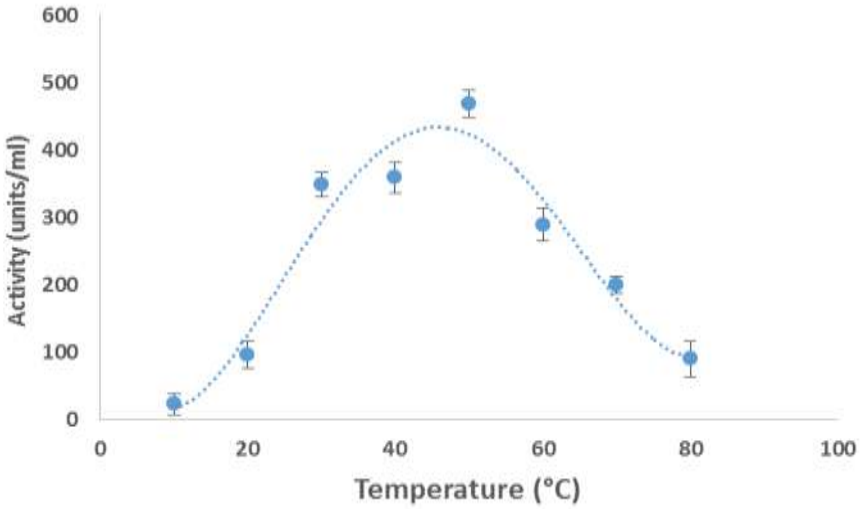


**Figure 2.** Effect of temperature on the activity of tyrosinase from *I. batata*. Activity at each temperature was determined under the standard reaction conditions. The activity was plotted against temperature. From the plot, the optimum was estimated to be 50°C for the tyrosinase.
Source: Author

of approximately 31 kDa for native *Bacillus megaterium* tyrosinase. The native molecular weight of tyrosinase from different sources is usually in the range between 35 and 55 kDa (Ilesanmi et al., 2014; Zekiri et al., 2014). The *Thermomicrobium roseum* tyrosinase was different from the enzymes of *Agaricus bisporous* (Strothkamp et al., 1976) which is composed of two H subunits (43 kDa) and two L subunits (13 kDa), and of *Neurospora crassa* (Lerch, 1983) and *Streptomyces glaucescens* (Huber and Lerch, 1985) which are monomers of 46 and 31 kDa, respectively.

## Kinetic parameters of Tyrosinase from *I. batatas*

The Michaelis constant, $K_m$ of the tyrosinase from *I. batatas* for L-DOPA was 2.5 ± 1.2 mM while that of the maximum velocity, $V_{max}$ was 451 ± 23.6 units/mg proteins (Table 2). This led to catalytic efficiency, $k_{cat}/K_m$ value of $1.45 \times 10^5$ $s^{-1}$ $M^{-1}$. The kinetic properties obtained in this study are similar to that obtained for tyrosinase from yam species (Ilesanmi et al., 2014). Dolashki et al. (2009) reported a $K_m$ of 7.8 mM for L-DOPA for *Streptomyces albus* tyrosinase. The low *K*m value obtained for the tyrosinase from *I*. *batatas* revealed that the enzyme has good affinity for L-DOPA as substrate. However, it was not tightly bound to the active site of the enzyme leading to rapid release of the product. The catalytic efficiency of the enzyme could be an advantage in its application for biotechnological processes.

## Effect of temperature on tyrosinase from *I. batatas*

Figure 2 shows the influence of temperature on tyrosinase from *I. batatas*. The optimal temperature obtained was 50°C. The enzyme was not fully stable at temperatures below 50°C. The enzyme was fully stable at temperatures of ≥50°C, but rapidly lost its activity above 70°C. Thus, the enzyme is stable at higher temperatures when compared with tyrosinase from other plant. The data obtained in this work is also comparable with tyrosinase from *Rhizobium etli* and *B. megaterium* displaying optimum activity at 50°C (Cabrera-Valladares et al., 2006; Shuster and Fishman, 2009), but higher than those obtained from other microorganism sources such as *Pseudomonas putida* F6 and *Trichoderma reesei* (30°C) (McMahon et al., 2007). In thermal stability,

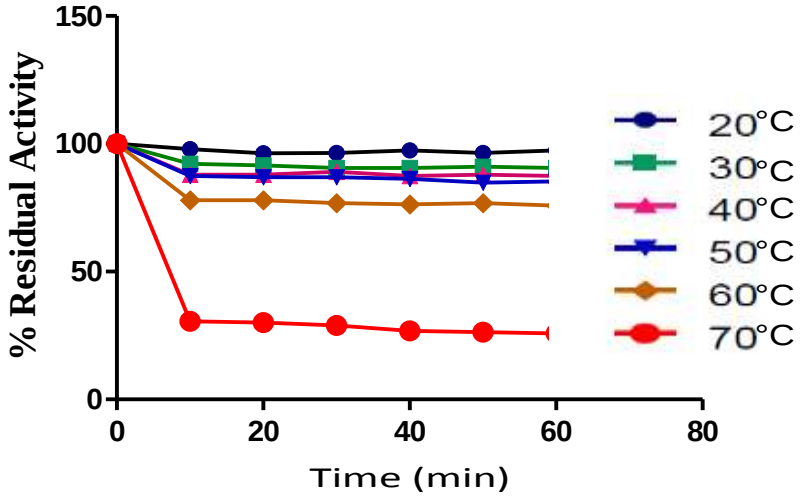


**Figure 3.** Thermal Stability of Tyrosinase from *I. batata.* The enzyme was incubated at different temperatures (20 – 70°C) for 1 h. An aliquot was taken at 10 min interval and assayed for tyrosinase activity and the residual activity was determined under the standard reaction conditions. The activity at zero time was taken as 100%. The residual activity was plotted against the time of incubation.
Source: Author

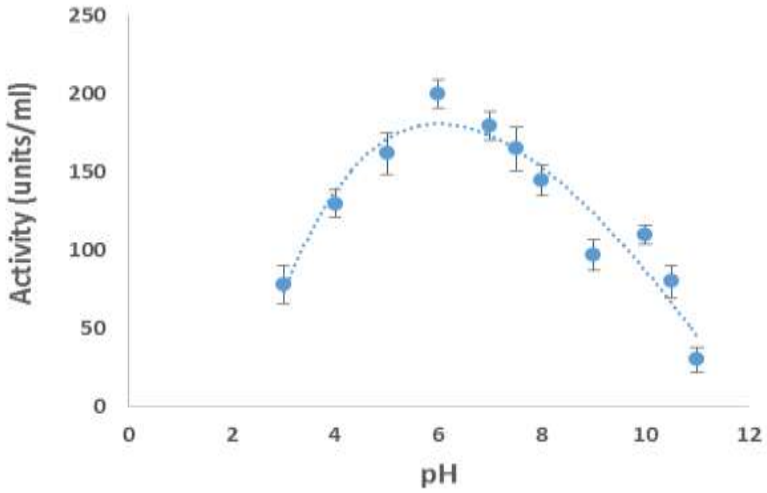


**Figure 4.** Effect of pH on the Activity of Tyrosinase from *I. batata.* The enzyme was assayed for activity at the indicated pH values. The highest activity was obtained at pH of 6.5.
Source: Author

residual activity was plotted against the time of incubation (Figure 3). About 70% activity was retained at 60°C for the enzyme after 1 h of incubation. About 35% activity was retained even at up to 70°C. The activity and stability of the enzyme was retained at temperatures between 30 and 60°C.

Thermostable enzymes are advantageous as several industrial processes are usually carried out at high temperature. The heat stability of *I. batatas* tyrosinase would be an advantage for its application in these processes especially in wastewater treatment.

### Effect of pH on tyrosinase from *I. batatas*

When the activities of enzyme were plotted against the pH, (Figure 4), maximum activity was obtained at pH 6.5. The value is in agreement with that obtained for

tyrosinase from *Dioscorea bulbifera*, *Pseudomonas putida* F6 (pH 7.0), *Streptomyces* species (pH 6.8) (McMahon et al., 2007); *Vibrio tyrosinaticus* (pH 6.6-7.8) (Pomerantz and Murthy, 1974), portabella mushrooms with optimum pH of 7.0 (Fan and Flurkey, 2004); hamster (pH 6.8) (Hearing, 1987); *B. megaterium* of optimum pH 7.0 (Shuster and Fishman, 2009); and fungal tyrosinase from *Pycnoporus* strains (Halaouli et al., 2005). Most results confirm that tyrosinase is not significantly active under basic conditions. The pH dependence of tyrosinase activity showed that the enzyme was more active in acidic buffers than basic buffers.

## Conclusion

This study has been able to establish fast purification protocol for tyrosinase from *I. batatas*. It was further established that the enzyme possess several interesting properties that could be exploited in several technical and biotechnological processes.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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