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RESEARCH ARTICLE

CHARACTERIZATION OF PARTIALLY PURIFIED TYROSINASE ISOLATED FROM BITTER KOLA (*GARCINIA KOLA*)

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Abstract

Objective: Tyrosinase is a glycosylated, copper-containing oxidase that catalyzes the first two steps of mammalian melanogenesis as well as enzymatic browning events in damaged fruits during post-harvest handling and processing. Human skin hyperpigmentation and enzymatic browning in fruits are both undesirable. In this study, the properties and inhibitory effect of some compounds on bitter kola tyrosinase were investigated.
Methods: Bitter kola tyrosinase was isolated and characterized using standard protocols. Partial purification was carried out on Sephadex G-100 loaded column chromatography.
Results: Bitter kola tyrosinase was purified with a specific activity of 3.5 U/mg protein, purification fold of 2.4 and a yield of 34%. The optimum pH value was found to be 6.0 while the optimum temperature value for maximum enzyme activity was observed at 60°C. The enzyme was stable at 40°C for 20 minutes. Metals such as NaCl, KCl, $MgCl_2$ and $CaCl_2$ had inhibitory effect on the activity; though $MgCl_2$ and $CaCl_2$ had minimal effect. Also, EDTA, β-marcaptoethanol and glutathione greatly inhibited the enzyme activity at all the tested concentration.
Conclusion: The properties of bitter kola tyrosinase compare very well with the tyrosinase from other sources. Also, the study was able to establish the inhibitory effect of some compounds and this could be applied in food processing industries.
Keywords: bitter kola, inhibitors, melanin, specific activity, Tyrosinase.

INTRODUCTION

Tyrosinase (also called monophenol monooxygenase, EC 1.14.18.1) is a binuclear copper containing enzyme that catalyzes the conversion of monophenols (tyrosine) into o-diphenols followed by the oxidation of o-diphenol to the corresponding o-quinone derivative[1,2]. It is the only copper-containing enzyme known to catalyze both of these processes; the related catechol oxidases only catalyze the second reaction, which uses o-diphenols as a substrate[3,4]. For the first step, tyrosinases attract molecular oxygen as a co-substrate[5]. The phenolic ring incorporates one of the oxygen atoms, while the other is reduced to a water molecule. The first stage of this catalytic cycle necessitates the use of a reducing agent to provide two electrons. These two electrons originate from the second step of the reaction. The final product of the tyrosinase-catalyzed reaction is DOPA-quinone, which is a precursor for the synthesis of melanin[6]. Melanins are pigments that play important roles in the survival of the organisms ranging from bacteria, plants, to mammals[7-9]. In mammals, including humans, melanin is mostly found in the skin, where it functions in photo protection against UV radiation[10]. Plants employ o-quinones to modify and harden the protective exterior layer and as an agent against invasive organisms. Function also occurs in fruits and potato, and in the fruit bodies of fungi. Because of the importance of enzymatic browning in post-harvest physiology and food technology, numerous fruits and vegetables have had their plant tyrosinase extracted, purified, and characterized[11]. Numerous studies have been conducted on its role in the change of fruits and vegetables during processing and storage of processed foods. Although some tyrosinase inhibitors have been discovered and reviewed[12,13]; the need for more inhibitors cannot be overemphasized. Tyrosinases have been isolated and characterized from different sources. In this study, we focused on extracting tyrosinase from bitter kola and to

investigate the effect of pH, temperature and the inhibitory effect of some selected compounds on the enzyme.

MATERIALS AND METHODS

Bitter kola used in this study was obtained at Akinola market in Ipetumodu, Ile Ife, Osun State Nigeria. All used chemicals were provided by Sigma Chemical Company, St Louis, USA.

Preparation of bitter kola homogenate

The Bitter kola was peeled and 10 g of it was homogenized in 50 mM phosphate buffer at 4ºC. The 30% homogenate was centrifuged at 10,000 rpm for 30 min at 4ºC in order to obtain clear crude supernatants. The supernatant obtained was assayed for protein and tyrosinase activity.

Tyrosinase activity assay

L-3,4-dihydroxyphenylalanine (L-DOPA) was used as a substrate to assess tyrosinase activity in the partially purified sample. The modified Lerch and Etlinger[14] approach was used. In a spectrophotometer, the change in absorbance of the assay mixture was monitored. A final concentration of 50 mM sodium phosphate buffer pH 6.5, 1 mM L-DOPA, and an aliquot of enzyme concentration were contained in a 1 ml assay mixture in a cuvette. The blank was a complete assay mixture in which the enzyme was replaced with distilled water. For 2 minutes, absorbance was measured at 475 nm at 10-second intervals, and the initial rate of reaction was computed as the change in absorbance per minute. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 µmole of product (*o*-dopaquinone) per minute at 475 nm.

Determination of protein concentration

The protein concentrations in the crude homogenates, partially purified or purified tyrosinase were determined as described by Bradford[15] using bovine serum albumin (BSA) as the standard protein.

Effect of pH on activity tyrosinase

At pH levels ranging from 3 to 10, the effect of pH on the activities of tyrosinase from bitter kola was studied. At the pH ranges mentioned, the following buffer systems were used: 50 mM citrate buffer (pH 3.0–6.0); 50 mM phosphate buffer (pH 7–8); 50 M borate buffer (pH 9.0–10)

Effect of temperature on activity tyrosinase

The effect of temperature on activities of tyrosinase from bitter kola was carried out by incubating the reaction mixtures containing 50 mM phosphate buffer, pH 6.0 and 1.5 mM L-DOPA at temperatures ranging from 10 to 90ºC for 4 min. The residual tyrosinase activity was plotted against the different temperatures.

RESULTS AND DISCUSSION

Enzyme purification

The partially purified enzyme had a specific activity of 3.50 micromole per mg of protein (U/mg), a purification fold of 2.4 and a percentage yield of 34.3%. Specific activity, purification fold and percentage yield of tyrosinase from different sources have been reported. The results obtained in this study is similar to the one reported by other researchers. The activity of bitter kola tyrosinase was found to be optimum at pH 6.0 while the optimum temperature for activity was 60ºC (Figure 1 and Figure 2).

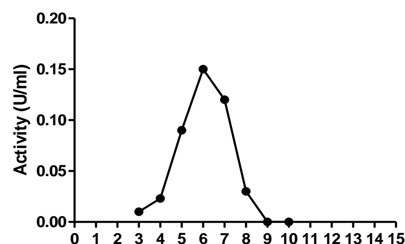

Figure 1: pH effect on bitter cola tyrosinase activity.

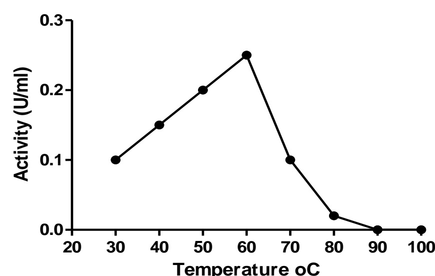

Figure 2: Effect of Temperature on bitter cola tyrosinase activity.

The result of heat stability study is shown in Figure 3. Research has shown tyrosinases with L-dopa as substrate to have maximum activity at different temperature. Balakrishnan and Kalirajan[16], reported an optimum temperature of 25ºC for tubers of *Amorphophallus paeoniifoliu* tyrosinase.

Also, Salah *et al*.,[17] reported an optimum temperature range of 37-40ºC for *Aspergillus terreus* tyrosinase. The result in this study corroborates the result obtained in literature. The activity of most plant tyrosinases peaks at or near neutral pH. The optimum pH for maximum tyrosinase activity in plants varies, depending on the extraction method, assay substrates, and enzyme localization in the plant cell. In this investigation, the optimal pH was found to be 6.0. The best pH for potato tyrosinase activity was 6.4, while the optimum pH for other fruits tyrosinase was 7.0.

Effect of salts on bitter kola tyrosinase activity

The results of the effect of salts show that the activity of the enzyme was inhibited by NaCl, KCl, $MgCl_2$ and $CaCl_2$ at high concentration; though the divalent metals had little effect on the enzyme (Figure 4).

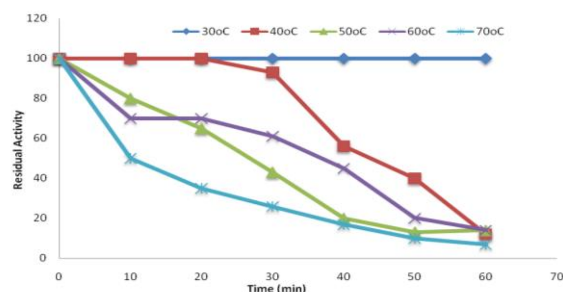

Figure 3: Heat stability study; graph of % residual activity against incubation time for the determination of heat stability of tyrosinase.

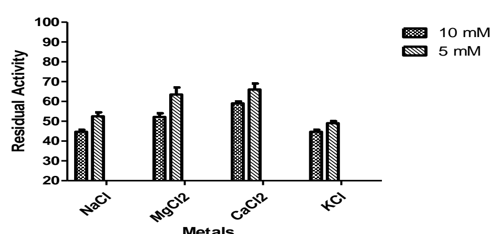

Figure 4: Effect of some selected metal ions on bitter cola tyrosinase activity.

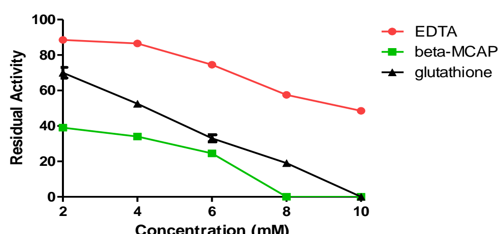

Figure 5: Inhibitory effect of some compounds on bitter cola tyrosinase activity.

Also, the result of inhibitory effect EDTA, Glutathione and β-mercaptoethanol is presented in Figure 5. A gradual loss of activity was noticed up to the highest concentration of EDTA (10 mM) that was used. β-Mercaptoethanol was found to be a potent inhibitor for the enzyme. At concentration of 8 mM, β-Mercaptoethanol abolish the activity of the enzyme. Concentrations of 10 mM glutathione also had great inhibitory effect on the enzyme. The result obtained in this study is in line with what was obtained in previous studies[12,13].

CONCLUSION

The properties of bitter kola tyrosinase compare very well with the tyrosinase from other sources. Also, the study was able to establish the inhibitory effect of some compounds and this could be of used in food processing industries.

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DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHOR'S CONTRIBUTIONS

Itakorode BO: writing original draft, literature survey. **Agboola OE:** methodology, conceptualization. **Adeboye MB:** formal analysis, review. **Benedict CC:** investigation, data interpretation. **Terkula KN:** critical review. **Aregbesola MF:** data curation, investigation. **Ajayi MO:** data curation, investigation. All authors revised the article and approved the final version.

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