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ORIGINAL ARTICLE

Physicochemical Properties of Thiaminase from Cassava Tubers (*Manihot esculenta*)

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ABSTRACT

Thiaminase (EC 2.5.1.2) is an antinutritional enzyme that is responsible for thiamine (vitamin B1) degradation, causing severe neurological and metabolic disorders in mammals. Cassava tubers were used for the extraction and purification of thiaminase using ammonium sulphate precipitation to 85 % and the CM-Sephadex C-25 chromatography. The purification process gave a specific activity of was 0.511 U/mg, 6.59 % yield and 7.61 fold-purification. The apparent Michaelis-Menten constant (K_m) values were 0.20 µM for thiamine and 0.23 µM for the co-substrate aniline, showing a much greater affinity for thiamine. The maximum velocities for both substrates were 0.0556 ± 0.01 µmol/min/mL and 0.107 ± 0.05 µmol/min/mL respectively. The optimum temperature and pH of the enzyme were 50 °C and 5.0 respectively. The enzyme activity was activated by low concentrations (0.1 mM and 1.0 mM) of Fe^{2+}, Hg^{2+}, Mn^{2+} and Na^{+} and also, a remarkable enhancement of the enzyme activity was exhibited with 10 mM concentration of all these cations indicating adaptation to the metallic profile of the soil. The enzyme was found to be stable at 40 °C to 60 °C. The findings of this study confirmed the presence of thiaminase in cassava tubers, suggesting that its activity may contribute to thiamine deficiency among populations that depend on cassava as a staple food, particularly when it is consumed raw or inadequately processed.

Keywords: Thiaminase; Purification; Enzyme kinetics; Antinutritional factors; Cassava.

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1.0 INTRODUCTION

Thiaminase is an enzyme that when ingested catalyzes the cleavage of thiamine (Vitamin B1) into its pyrimidine and thiazole moieties (Cheryl et al., 2013) effectively destroying its vitamin properties and reducing its availability in the body. Thiaminase has been classified into two types: Thiaminase I and Thiaminase II respectively. Thiaminase I enzyme is present in plants such as nardoo and bracken Fern and in marine animals such as salmon, silver carp, anchovy, freshwater prawn in certain bacteria and one eukaryote; *Naegleria gruberi* (Katie et al., 2023).Thiaminase II enzyme is present in specific bacteria and yeasts. A major cause of thiamine deficiency is an overreliance on diet items containing the enzyme thiaminase leading in deadly deficiency symptoms (Freya et al., 2023). Vitamin B1 is a vitamin necessary for proper cell function. It is important for energy production in the mitochondria, protein synthesis and plays a special role as coenzyme necessary for the metabolism of carbohydrates, fats and proteins. It is also needed to ensure the proper functioning of the central and peripheral nervous system thus, its deficiency leads to mitochondrial dysfunction, lactate and pyruvate accumulation, and consequently in neurodegenerative diseases including Alzheimer's disease which may manifest as Wernicke's encephalopathy or Wernicke-korsakoff syndrome. Its deficiency can also lead in neuropathy leading to ataxia, paralysis and confusion (Malgorzata et al., 2023). Once thiamine molecule is cleaved, the body is unable to restore it therefore, when significant amounts of thiaminase is ingested, thiamine deficiency may develop even when the concentration of dietary thiamine are adequate (Craig et al., 2024). Cassava (*Manihot esculenta)* belongs to one of the most essential and economically carbohydrate sources. It acts as a primary or supplementary dietary staple for more than 200 million people throughout sub-Saharan Africa alone (Banjoko et al., 2008). In spite of its high caloric benefits, cassava is naturally low in micronutrients, particularly thiamine. Given that processing methods vary and complete inactivation is not always achieved, understanding the enzymatic landscape of cassava tubers is highly important. Although thiaminase has been characterized in various plant and animal systems such as grapes, pawpaw, sweet orange Agboola & Okonji (2014), and giant freshwater prawns (Ehigie et al., 2015), exhaustive data on the purification and kinetic parameters of thiaminase isolated from *Manihot esculenta* remains limited. This research aims to clarify the biochemical profile of this antinutritional factor by evaluating its structural stability, substrate affinities, and behavior under the impact of environmental elements (temperature, pH, and heavy metals), to aid future food processing and public health interventions.

2.0 MATERIALS AND METHODS

2.1 Materials

All chemicals, solvents and reagents used were of analytical grade.

2.1 Crude extract preparation
Cassava tubers were obtained from a cassava farm in Sekona, Osun state and carried to the laboratory in a sack. The tubers were washed in large quantities of water to wash off soil and debris and they were peeled and cut into small pieces. A 250 g portion was homogenized in 750 mL of 0.1 M phosphate buffer (pH 7.2). Homogenate was then centrifuged at 4,000 rpm for 30 minutes and the clear supernatant was considered the crude enzyme extract.

2.2 Protein concentration determination
Protein Concentration was determined using the Bradford method (Bradford, 1976). Using bovine serum albumin (bovine serum albumin) as a standard. A total of 1.0 mL of Bradford reagent and 100 µL enzyme was the assay mixture. Absorbance was determined at 595 nm, and the quantity of protein was determined.

2.3 Thiaminase activity assay
The thiaminase activity was determined using the modified method described by Nishimune et al. (2000). The reaction mixture consisted of 0.1M Tris-HCl buffer (pH 8.0), 10^{-5} M thiamine, 4×10^{-3} M aniline and 100 µL enzyme preparation (final volume 0.5 mL). The mixture was allowed to stand at 25°C for 30 minutes. Subsequently 0.5 mL of 200 g/L NaOH was added to convert the remaining thiamine to thiazole, which was then measured spectrophotometrically. The absorbance was measured at 411 nm. A unit of thiaminase activity is defined as the enzyme activity needed to produce 1 µmol of heteropyrithiamine in 30 minutes.

2.4 Ammonium sulphate precipitation
The crude enzyme extract obtained after centrifugation was subjected to ammonium sulphate precipitation by adjusting the solution to 85 % of the saturation level. This mixture was kept overnight at 4°C and then centrifuged at 4000 xg for 30 min. The precipitate was dissolved in 0.1M phosphate buffer (pH 7.2) and dialyzed against large volumes of the same buffer for 24 h.

2.5 Ion-Exchange chromatography using CM-Sephadex C-25
The dialyzed enzyme preparation was passed through a CM-Sephadex C-25 column pre-equilibrated with 0.1M phosphate buffer (pH 7.2). Unbound proteins were eluted with the equilibration buffer, and the bound proteins were eluted with 1M NaCl in the equilibration buffer. Samples of 3 mL were collected every 30 minutes. The protein analysis and the determination of thiaminase activity were done on each fraction. The active fractions were combined and dialyzed against 50% glycerol in 0.1M phosphate buffer (pH 7.2). The purified fractions were then tested for protein content and thiaminase activity. As a result, the purification yield and the fold purification were determined.

2.6 Determination of kinetic parameters
The purified enzyme's kinetic properties, such as the Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}), were measured by the modified method of Nishimune et al. (2000). Substrate concentrations of 10 to 100 mM thiamine and aniline were prepared in phosphate buffer (pH 8.0) to determine the K_m values for thiamine and aniline. The kinetic parameters were determined by Lineweaver-Burk plots (Lineweaver & Burk, 1934).

2.7 Effect of pH on thiaminase activity
The activity of thiaminase at various pH levels was studied with various buffer solutions. Citrate buffer (5 mM) was used for pH values ranging from 5.0 to 6.5, phosphate buffer (5 mM) for pH 6.5–8.0, and Tris-HCl buffer (5 mM) for pH 8.0–9.0. Ten microliters of enzyme solution, thiamine, aniline and 0.2 mL of the appropriate buffer were used to make a final reaction volume of 1 mL.

2.8 Effect of temperature on thiaminase activity
The enzyme activity and the optimum temperature were determined by carrying out the enzyme assay at different temperatures from 0 to 100°C. The pre-incubation time was 30 minutes at the specified temperature, and the reaction was started by adding the enzyme that had been equilibrated for the same time at the same temperature. The activity of enzymes was measured every 30 minutes. Further, to assess the thermal stability, 1 mL each of the enzyme preparation was incubated at 30, 40, 50 and 60°C for 1 h. The residual enzyme activity was determined after taking aliquots (0.1 mL) at 30 minutes intervals.

2.9 The effect of metal ions on thiaminase activity
The activity of thiaminase was studied in the presence of some metal ions, namely Fe^{2+}, Hg^{2+}, Mn^{2+} and Na^{+}. The enzyme was assayed under standard assay conditions with each metal ion at concentrations of 0.1 mM, 1.0 mM and 10 mM.

2.10 The effect of other compounds on thiaminase activity
The effect of different chemical compounds such as urea, β-mercaptoethanol and EDTA on the activity of the purified thiaminase was assessed. The various compounds were evaluated at 0.1, 0.5 and 1M in a standard thiaminase assay system. The compounds were prepared in distilled water with no additives and the activity measured without additives was regarded to represent 100% activity.

2.11 Thermal stability of thiaminase activity
The thermal stability of the purified rhodanese was determined between 30ºC to 100ºC. An aliquot of the enzyme was incubated at the set temperature for 1 h. At the 10 minutes interval, 0.1 mL of the enzyme was withdrawn and assayed for residual enzyme activity. The activity at each temperature was expressed as a percentage of the activity of the enzyme.

3.0 RESULTS

The results for the purification of thiaminase from cassava tuber (*Manihot esculenta*) are shown in Table 1. The specific activity obtained after CM-sephadex C-25 ion exchange chromatography for the enzyme was 0.51 µmol/min/mL, 6.59 % yield and 7.61 fold-purification. The elution profile shown in Figure 1 is obtained in the case of CEC on CM-sephadex C25.

Table **1.** Summary of the purification of thiaminase from cassava tuber

	Steps fold	Total Protein	Total activity	Specific activity	Yield
Crude	1	239.01	16.07	0.07	100
85% ammonium sulphate ppt	4.70	6.20	1.95	0.32	12.13
Ion-exchange chromatography	7.61	2.07	1.06	0.51	6.59

Table **2.** Effect of metals on thiaminase from *Manihot esculenta*

metals	Residual activity (%)		
	0.1mM	1mM	10mM
$FeCl_2$	38.81	57.46	92.70
$HgCl_2$	56.72	33.58	93.70
$MnCl_2$	27.61	99.40	95.70
$NaCl_2$	14.18	23.89	27.10

3.1 Effect of pH on the activity of thiaminase
The maximum activity of the thiaminase assay was obtained at pH 5.0.as shown in Figure 2

3.2 Effect of temperature on the activity of thiaminase
The maximum activity of the thiaminase assay was obtained at 50ºC as shown in Figure 3. In addition, the enzyme was also observed to be thermally stable when heated to range between 40°C and 60°C as shown in Figure 3.

3.3 Kinetic parameters of thiaminase
The K_m for aniline and thiamine were found to be 0.20 µM and 0.23 µM respectively. The V_{max} of aniline and thiamine respectively were 0.06 ± 0.01 µmol/min/mL and 0.11 ± 0.05 µmol/min/mL. The Lineweaver-Burk

plots for the fixed concentration of thiamine and aniline were shown in Figure 4 and 5, respectively.

3.4 Effect of metal ions on thiaminase activity

The results of the effect of metals showed that the enzyme was not inhibited by these metal at 0.1 mM, and 1 mM but it was enhanced by 10 mM of the metals. The result showed that for low concentration of the metallic salts, the rate of thiaminase activity in the cassava tuber was not affected, while at high concentration, it enhanced the activity. This could be as a result of high content of metals in the environment/soil. The results were shown in Table 2.

3.5 Effect of other compounds on thiaminase activity

The results showed that the enzyme was not affect by other compounds in any variation of concentrations (Figure 6).

3.6 Thermal stability

The enzyme was found to be stable at 40 °C to 60 °C.

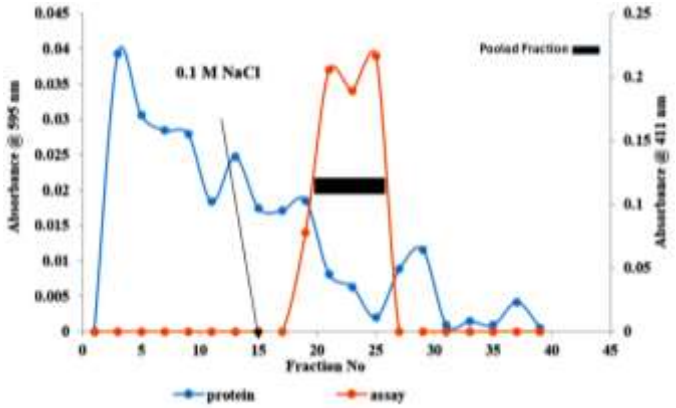

Figure **1.** Elution profile after Ion Exchange Chromatography.

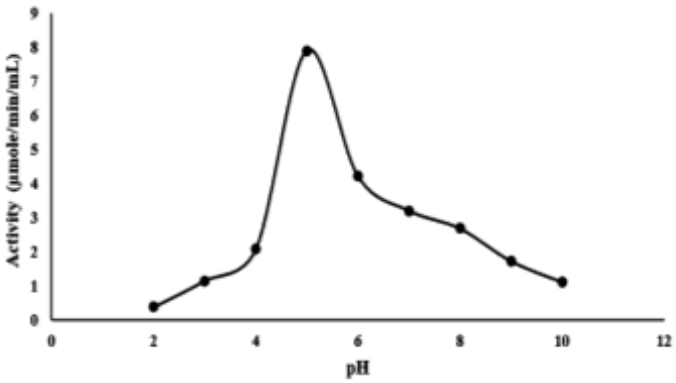

Figure **2.** Effect of pH on cassava tuber thiaminase activity.

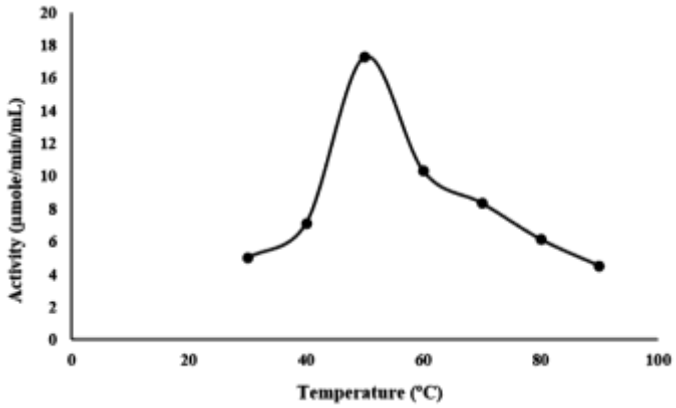

Figure **3.** Effect of temperature on thiaminase activity from cassava tuber.

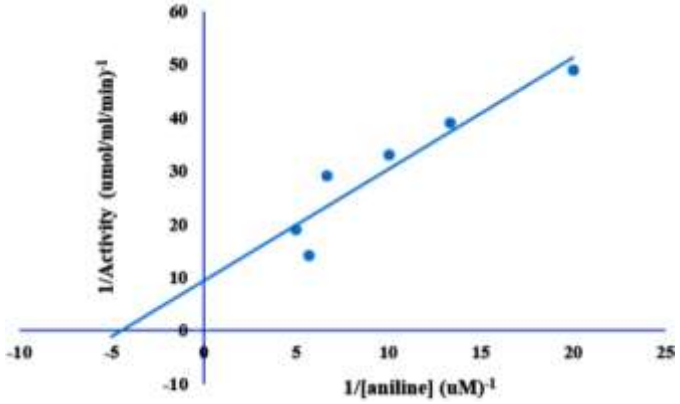

Figure **4.** Lineweaver-Burk plot for varying concentration of thiamine

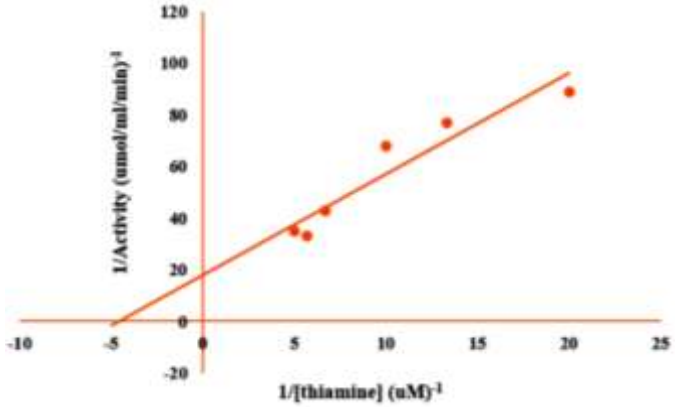

Figure **5.** Lineweaver-Burk plot for varying concentration of aniline

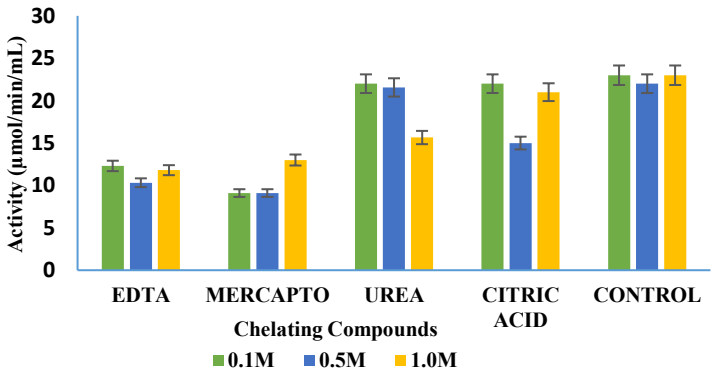

Figure **6.** Effect of other compounds on thiaminase activity from cassava tuber

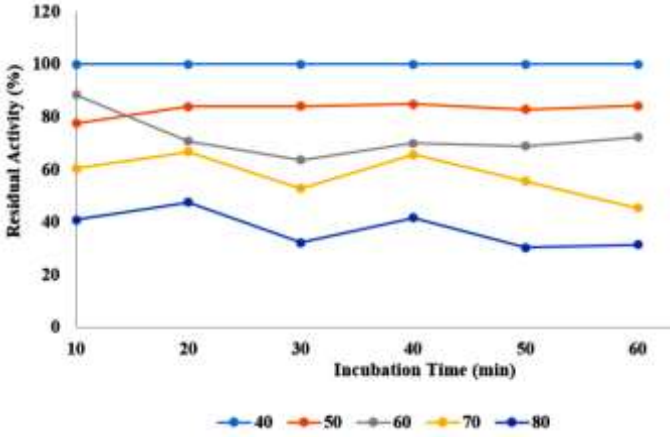

Figure **7.** Effect of thermal stability on thiaminase activity from cassava tuber**.**

4.0 DISCUSSION

Thiamine deficiency has been widely documented in mammals, including humans, consuming diets containing thiaminase (Nishimune et al., 2000), and thiaminase-induced deficiency symptoms have also been reported in fish fed diets containing the enzyme (Honeyfield et al., 2005). More recent work has reframed thiaminase not only as an anti-nutritional factor but also as an enzyme of possible therapeutic interest, particularly in cancer research (Adeoye et al., 2024). Cassava (*Manihot esculenta*) is a low-cost, readily available source of carbohydrates and a staple food for millions of people in developing countries, particularly those with extensive cassava cultivation. It is a food crop for more than 200 million Africans and is increasingly used as feed for monogastric animals (Banjoko et al., 2008). Cassava has, however, been reported to contain relatively low thiamine content, so nutritional inadequacy is a concern when it forms a major component of the diet. In the present study, thiaminase was extracted and purified from cassava tubers (*Manihot esculenta*) by ammonium sulphate precipitation and ion-exchange chromatography on CM-Sephadex C-25, yielding a 761-fold purification. The purified enzyme had a specific activity of 0.511 U/mg and a 6.59 % yield. Figures 2 and 3 indicated that cassava tuber thiaminase preferred thiamine as substrate, with a Km value of 0.20 µM compared to 0.23 µM for aniline, while the maximum velocities for both substrates were 0.06 ± 0.01 µmol/min/mL and 0.11 ± 0.05 µmol/min/mL, respectively. The enzyme was determined to be heat-stable between 40°C and 60°C. Activity was assayed across a temperature range of 30°C to 100°C, and the best temperature for the cassava enzyme was found to be 50°C. Thiaminase from various sources has different optimal pH requirements and is differentially affected by activators and cofactors; the pH optimum for cassava tuber thiaminase was 5.0. Different biological sources of thiaminase show marked variation in specific activity. Agboola & Okonji (2014), for instance, reported specific activities of 1.38 ± 0.90 U/mg in grape, 0.45 ± 0.26 U/mg in pawpaw, and 0.13 ± 0.01 U/mg in sweet orange; such differences can be attributed to variation in enzyme content, extraction methods, and enzyme source. The optimum temperature of thiaminase in the buffer extract of *Anaphe* pupae was 70°C (Nishimune et al., 2000), this thiaminase is a relatively heat-resistant, stable enzyme previously implicated as a cause of seasonal ataxia and loss of consciousness in Nigerians consuming diets containing it (Nishimune et al., 2000). Ehigie et al. (2015) reported an optimum temperature of 50°C for *Macrobrachium rosenbergii* thiaminase, which preferred thiamine as substrate with a Km of 0.5 mM compared to 1 mM for aniline (Edwin et al., 1982), with an apparent thiamine Km of 176 µM. An apparent Km for aniline of 319 mM was recorded when thiamine concentration was optimal (Boyd, 1985). The extracellular thiaminase I of *Bacillus thiaminolyticus* exhibited an optimum pH over a wide range of 5.8–6.8 and an optimum temperature of 37°C (Wittliff & Airth, 1968). More recent biochemical characterization of thiaminase I from carp liver similarly confirms this enzyme's activity across a defined pH and temperature range consistent with earlier reports (Wolfe et al., 2023), and mechanistic work on thiaminase I in *Burkholderia thailandensis* has further clarified how such enzymes salvage thiamine-derived precursors from the environment (Edwards et al., 2023) supporting the broader picture that thiaminase activity, substrate preference, and thermal behavior are conserved features across microbial, plant, and animal sources. For *Marsilea drummondii* (nardoo fern), McCleary & Chick (1977) reported a pH optimum of 8–9 and stability across a pH range of 3 to 12. The temperature for 50% denaturation was found to be 60–65°C, and the enzyme remained stable at 55°C, underscoring consistent with newer fluorescence-based kinetic assays of thiaminase in fish tissue (Porter & Pinger, 2025) that thiaminase across taxa is a comparatively heat-resistant, stable enzyme.

Conclusion

This study confirms the presence of thiaminase in tubers of *Manihot esculenta*. Understanding its physicochemical properties can advance our knowledge of its impact on human health, particularly in relation to thiamine deficiency, which has been linked to cognitive impairments and various metabolic disorders.

Conflict of interest

There is no conflict to declare.

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This study was not funded

Author contributions

Okonji Raphael Emuebie: Conceptualization; Okanlawon Kehinde Peter, Olaitan Aanuoluwa Feyisayo: Data curation; Okanlawon Kehinde Peter, Fadunsin Moromoke Ajisope, Okonji Raphael Emuebie: Methodology; Fadunsin Moromoke Ajisope, Okanlawon Kehinde Peter, Writing– review and editing and Okonji Raphael Emuebie Supervision, Writing– review and editing.

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