



Research Article

Physicochemical Properties of Rhodanese in Cashew (*Anacardium occidentale*) Mesocarp and Seed

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ABSTRACT

Cyanide is a lethal toxin that inhibits mitochondrial enzymes, thereby blocking oxygen utilisation and ATP synthesis, which leads to cellular death. Rhodanese, a mitochondrial enzyme, detoxifies cyanide by converting it to the less toxic thiocyanate. This study is aimed at investigating the physicochemical properties of rhodanese in *Anacardium occidentale* (cashew) mesocarp and seed as a potential detoxifier of cyanide. Enzymes were isolated and partially purified using 80% ammonium sulphate precipitation and dialysis. Rhodanese from the mesocarp had a specific activity of 0.442 $\mu\text{mol}/\text{min}/\text{mg}$, a purification fold of 10.524, and a 1.820% yield, while the seed enzyme showed 0.462 $\mu\text{mol}/\text{min}/\text{mg}$, a 0.774-fold, and a 21.664% yield. Kinetic studies revealed enzyme affinity for $\text{Na}_2\text{S}_2\text{O}_3$ ($K_m = 0.0422 \pm 0.0091$ mM, $V_{\text{max}} = 0.1611 \pm 0.0086$ $\mu\text{mol}/\text{min}/\text{mg}$ protein in mesocarp; $K_m = 0.0460 \pm 0.0194$ mM, $V_{\text{max}} = 0.4511 \pm 0.0492$ $\mu\text{mol}/\text{min}/\text{mg}$ protein in seed) and for KCN ($K_m = 0.2394 \pm 0.1091$ mM, $V_{\text{max}} = 0.1235 \pm 0.0325$ $\mu\text{mol}/\text{min}/\text{mg}$ protein in mesocarp; $K_m = 0.2373 \pm 0.2491$ mM, $V_{\text{max}} = 0.7116 \pm 0.4292$ $\mu\text{mol}/\text{min}/\text{mg}$ protein in seed). Optimum enzyme activities occurred at pH 5 and 7 for the mesocarp and seed, respectively, and at 50°C and 30°C for the mesocarp and seed, respectively. Metal ions and inhibitors variably affected enzyme activity: NaCl (10 mM) caused maximum inhibition, while SnCl_2 showed minimal inhibition. Urea, mercaptoethanol, and EDTA also affected activity to differing degrees. The detection of active rhodanese in *Anacardium occidentale* mesocarp and seed indicates their potential for cyanide detoxification and environmental protection. Further purification using advanced techniques is recommended to enhance understanding and application of this enzyme system.

Keywords: *Anacardium occidentale* mesocarp; *Anacardium occidentale* seed; Rhodanese; Cyanide detoxification

INTRODUCTION

Cyanide is a very deadly substance that is constantly taken in by cells that are alive and kills them by preventing oxygen utilisation by tissues (Ehigie *et al.*, 2019a; Ogbuagu *et al.*, 2019; Okonji *et al.*, 2015). Fires at homes and workplaces, as

well as industrial activities, release cyanides into the environment (Okonji *et al.*, 2015). Cyanides are obtained from a variety of sources (Kuyucak and Akcil, 2013), including processed foods and naturally occurring plants (Ndubuisi and Chidiebere, 2018) as well as natural sources including cyanogenic glycosides, “which can be found in apricot kernels, cassava roots and bamboo shoots” (Tewe and Iyayi, 2023; Onojah and Odin, 2015). Numerous organisms naturally create cyanides, “including bacteria,

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fungi, algae, insects and plants” (Ogbuagu *et al.*, 2019), which are compounds which contain the C≡N group in their structure, thereby posing great environmental concern (Gul *et al.*, 2024). Additionally, some fruits and seeds contain cyanides “including apple, mango, peach, and bitter almonds” (Bolarinwa *et al.*, 2016; Onojah and Odin, 2015). Cyanides are typically attached to molecules of sugar in plants as glycosides with cyanogenic properties, which aid in the plant's defence against herbivores (Tewe and Iyayi, 2023).

Cyanides can be found in food, soil, exhaled air and water (Lachowicz *et al.*, 2024; Okunola *et al.*, 2022) and biological substances such as saliva, urine, and blood (Lachowicz *et al.*, 2024; Hendry-Hofer *et al.*, 2019). Likewise, cyanides can be carried down from farms and cities and contaminate water through manufacturing waste releases (Lachowicz *et al.*, 2024). Cyanogenic glycosides exist as natural toxins available in plants – a source of food consumed by humans due to breeding practices that strengthen these defence mechanisms and natural selection (Friday, 2019). About 90% of the larger class of plant-based pollutants known as cyanogens are composed of these cyanogenic glycosides, also known as cyanoglycosides (Tewe and Iyayi, 2023; Anjum *et al.*, 2022). The enzyme hydrolyses the non-lethal cyanogenic glycosides when water is available to generate cyanohydrins that rapidly break down into the poisonous hydrogen cyanide (Onojah and Odin, 2015).

As cyanide occurs in the three major states of matter, it can lead to human poisoning by various routes such as parenteral delivery, ingestion, inhalation, and cutaneous or conjunctival contact (Lachowicz *et al.*, 2024; Okunola *et al.*, 2022). Therefore, when organisms come in contact with substances that generate cyanide ions, cyanide poisoning happens (Goren *et al.*, 2023). Also, cyanide impacts cytotoxicity by blocking the activity of cytochrome oxidase (Ilesanmi *et al.*, 2020). According to Goren *et al.* (2023) and Devi (2021), cyanide poisoning is a major risk for miners, firefighters, and employees in the metallurgical, chemical, and galvanic industries. Thus, industrial activities and lifestyle allow the introduction of cyanide ions into the human body, thereby impacting human health. Similarly, the main human-caused source of cyanide ions that accumulate in the soil is the metallurgical and galvanic industries (Lachowicz *et al.*, 2024; Okunola *et al.*, 2022). Also, the beneath-ground coal gasification process also produces trash with significant cyanide contents (Devi, 2021). Moreover, resulting challenges from cyanide toxicity include loss of structural integrity and impairment of vital cellular functions (Akinmoladun *et al.*, 2021; Oyewopo *et al.*, 2021). Akinmoladun *et al.* (2021) and Oyewopo *et al.* (2021) reported the impact of cyanide poisoning to be impairment of oxidative phosphorylation, leading to reduced utilisation of oxygen for the production of adenosine triphosphate (ATP).

In addition, cyanide toxicity is deadly, as it affects the brain and heart by causing headache; anxiety; agitation; confusion; bradycardia, which is accompanied by reflex

tachycardia; pulmonary oedema; severe lung damage; hypotension; nausea; vomiting; and cherry-red skin (O'Connell *et al.*, 2022; Hui *et al.*, 2021). Thus, to combat the impact of cyanide toxicity, research has focused on the potential of enzymes that convert cyanide to less harmful metabolites. It is reported that one of such enzymes that metabolise cyanide into a less toxic metabolite (thiocyanate) is a hepatic enzyme named 'rhodanese' (Ehigie *et al.*, 2019b; Aladesanmi *et al.*, 2017; Okonji *et al.*, 2017).

Using thiosulphate as a donor substrate, the ubiquitous enzyme rhodanese biotransforms cyanide to thiocyanate, which is then eliminated in urine (Aladesanmi *et al.*, 2017; Okonji *et al.*, 2010). Also, Ehigie *et al.* (2019a) reported that rhodanese enables the interchange of atomic sulphur from appropriate donors to “nucleophilic” acceptors via a double-displacement approach. Rhodanese's active site is made up of nearby sulfhydryl and aromatic groups that interact with the cyanide molecule. Additionally, the cyanide moiety interacts with the rhodanese catalytic site, which is situated at the lower part of the gap created by the two regions of the enzyme (Ehigie *et al.*, 2019a). However, it is proposed that the loss of enzymatic activity by rhodanese sulfhydryl groups is crucial for catalysis when they react with aromatic nitro compounds, alkylating agents, or aliphatic mercaptans (Okunola *et al.*, 2022; Sanni *et al.*, 2018).

Rhodanese is active in bacteria yeast (Sanni *et al.*, 2018), fungi (Lawal *et al.*, 2023), plants and animals (Okunola *et al.*, 2022; Dokubo *et al.*, 2021; Wang *et al.*, 2020). As rhodanese is active in plants, cashew (*Anacardium occidentale*) is the focus of this current study. *Anacardium occidentale* is a well-known plant worldwide (Edet and Samuel, 2023); it contains significant concentrations of minerals, vitamins, carotenoids, amino acids, organic acids, phenolics, reducing sugars (fructose and glucose), and antioxidants (Zargarchi *et al.*, 2024; Cruz Reina, 2023; Sahie *et al.*, 2023). Likewise, the presence of carotenoids, ascorbic acids and antioxidants in *Anacardium occidentale* further supports its antibacterial, anti-scurvy and antidiarrheal properties (Shahrajabian and Sun, 2023). Thus, it is hypothesised by this study that the mesocarp and seed of *Anacardium occidentale* may contain rhodanese, thereby suggesting its role against cyanide detoxification. Hence, this current study is aimed at determining the physicochemical potential of rhodanese from the mesocarp and seed of *Anacardium occidentale*. Findings from this study will ascertain or debunk the need to isolate and purify more rhodanese from the mesocarp and seed of *Anacardium occidentale* as part of plans for domestic, industrial, and/or commercial detoxification of cyanide.

MATERIALS AND METHODS

Chemicals, reagents and apparatus

Ferric cyanide and potassium cyanide were purchased from Loba Chemie India. Ethylenediamine tetraacetic acid (EDTA), mercury chloride, and manganese chloride were purchased from Kermel Reagent Company Limited, Tianjin,

China. Ammonium sulphate, sodium chloride, ethanol, formaldehyde, and copper sulphate were procured from BDH Chemical Limited in Poole within England. CM-Sephadex C-2525 and Bovine Serum Albumin (BSA) were obtained from Pharmacia Fine Chemicals located in Uppsala within Sweden. Coomassie Brilliant Blue (G-250) was obtained from Sigma Chemical Company, St Louis, USA. Monosodium dihydrogen phosphate, disodium hydrogen phosphate, Tris-base, phosphoric acid, concentrated hydrochloric acid, and sodium thiosulphate were purchased from Guangdong, China. Sodium sulphite was purchased from Fisher Scientific Company, USA. Ammonium persulphate and boric acid were bought from Hopkins and Williams, Essex, England. Ammonium sulphate was purchased from Trust Chemical Limited. Mercaptoethanol, sodium borate decahydrate, nitric acid, citric acid, sodium citrate and other reagents utilised for the experiment were of analytical status and were purchased from credible outlets. Apparatuses used in this project include a digital analytical weighing balance (Atom), a visible spectrophotometer 23A (Gulfex Medical and Scientific, England), a tabletop microfield centrifuge (Helmreasin), a heating mantle and a compensated hot water bath.

Processing of *Anacardium occidentale* mesocarp and seed

Cashew fruits were bought from Mayfair, Ife, Osun State, and the nut was separated from the cashew apple. Cashew apples were weighed and stored in the refrigerator until they were needed. The weight was 268.67 g for mesocarp and 50 g for seed. After being removed and permitted to defrost at 25°C, the cashew fruits were blended in three volumes of the homogenising buffer (0.1 M Tris-HCl buffer pH 7.2). After filtering the mixture, the homogenate was obtained for determining Rhodanese activity and protein concentration.

Protein determination

BSA was used as the standard in Bradford's (1976) method of determining protein content. One millilitre of Bradford reagent and one hundred microlitres of the cashew homogenate made up the reaction mixture. The Bradford reagent was added concurrently with BSA concentrations from 0 µg/mL to 100 µg/mL for the standard graph, and absorbance was measured at 595 nm. On the standard plot, the absorbance of the unknown protein was used to interpolate for its protein concentration in µg/mL.

Rhodanese assay

Rhodanese activity was assessed using Agboola and Okonji (2004) following slight modification of Lee et al. (1995). The reaction mixture totalling 1.0 mL included 0.2 mL of KCN (250 mM), 0.5 mL of borate buffer (50 mM, pH 9.4), 0.2 mL of Na₂S₂O₃ (250 mM), and enzyme solution (0.1 mL). After one minute of incubation at (37°C), formaldehyde (0.5 mL) and Sorbo reagent (1.5 mL) were added to stop the reaction. At 460 nm, the absorbance was measured. The absorbance

at 460 nm was measured and used to determine the amount of thiocyanate formed and, consequently, rhodanese activity under the assay conditions. As regards the selection of 250 mM for KCN and thiosulphate, preliminary optimization trials guided substrate selection. The amount of enzyme required to change one micromole of cyanide into thiocyanide in a minute is known as one Rhodanese Unit (RU).

Ammonium sulphate precipitation

To the homogenate obtained, 80% (w/v) ammonium sulphate (156 g/L) was added with occasional stirring for about 1 hour. This was done to precipitate out the protein present in the sample. This was left for 18 hours for optimum precipitation in the refrigerator. Centrifugation was used to gather the precipitate after 30 minutes at 3500 rpm. Following a 30-minute centrifugation at 3500 rpm, the leftover sediment was immersed in Tris-HCl buffer (0.01 M; pH 7.2), and enzyme dialysis was done. The supernatant was then discarded.

Desalting

Dialysis was used to separate molecules in the homogenate according to their sizes through a semi-permeable membrane. The pores of the membrane allowed smaller molecules such as solvents, salts and small metabolites to diffuse across them but hindered larger molecules (macromolecules) from passing through. For 30 minutes, the dialysis bag was treated and activated by washing in distilled water and boiling in 500 mL solution containing 1.0 mM EDTA. The bag was afterwards rinsed with distilled water and stored in the buffer solution to prevent weakening of the bag until it was required. The dialysate was centrifuged at about 3500 rpm at room temperature; thereafter, protein concentration and rhodanese activity were measured in the supernatant after insoluble materials were eliminated.

Determination of kinetic parameters of rhodanese in *Anacardium occidentale* mesocarp and seed

At a given concentration of Na₂S₂O₃ (250 mM), K_m and V_{max} of rhodanese were calculated by changing the KCN concentration between 10 mM and 200 mM. Similarly, at a given concentration of KCN (250 mM), Na₂S₂O₃ concentration was also changed between 10 mM and 200 mM. The nonlinear regression (Michaelis-Menten model) was used to calculate the kinetic parameters (K_m and V_{max}) using GraphPad Prism (version 8.0). Initial reaction velocity (V_o) was plotted against different substrate concentrations. The goodness of fit was evaluated using the coefficient of determination (R²).

Effects of pH on rhodanese activity in *Anacardium occidentale* mesocarp and seed

Enzymes, being proteins, are pH sensitive. Every enzyme has a pH at which it exhibits its highest activity, called its optimum pH. Once the enzyme is placed in an environment

whose pH is further apart from the optimum, the enzyme loses its native conformation and activity. Using the techniques outlined by Agboola and Okonji (2004), the impact of pH on the activity of rhodanese was investigated. A variety of buffer systems, including 50 mM phosphate buffer (pH 6-7), 50 mM citrate buffer (pH 3-5), 50 mM borate buffer (pH 9-10) and 50 mM Tris HCl buffer (pH 8), were used to test the rhodanese enzyme. As previously stated, rhodanese activity was measured.

Effect of temperature on rhodanese activity in *Anacardium occidentale*

Rhodanese activity was measured between 30°C and 80°C to ascertain its ideal "most active" temperature. After one minute of incubation at the specified temperature, the assay mixture was stopped. Rhodanese activity was calculated by measuring absorbance at 460 nm.

Effects of metal ions on rhodanese activity in *Anacardium occidentale* mesocarp and seed

The effects of different salts on Rhodanese activity from cashew mesocarp and cashew seed were investigated using the Lee *et al.* (1995) technique. Three different concentrations – "0.1 mM, 1 mM, 10 mM" – of NaCl, MnCl₂, HgCl₂, ZnCl₂ and SnCl₂ were used to investigate their effects on rhodanese activity. The reaction mixture without added salt was used as the control and its activity was designated as 100%.

Substrate specificity of rhodanese from *Anacardium occidentale* mesocarp and seed

A range of sulphur-containing compounds, including sodium metabisulphate, sodium sulphate, ammonium persulphate and sodium sulphite, were used to investigate the substrate specificity of rhodanese. Sodium thiosulphate was used as the reference sulphur donor, and the activity of rhodanese in the presence of the tested compounds was

expressed relative to the activity obtained with sodium thiosulphate

Effect of inhibitors (Urea, EDTA, and Mercaptoethanol) on rhodanese activity in *Anacardium occidentale* mesocarp and seed

This assay was conducted to determine the influence of Urea, EDTA and Mercaptoethanol at different concentration of 5.0 mM, 2.5 mM, 1.0 mM, and 0.5 mM on rhodanese in *Anacardium occidentale* mesocarp and seed.

Statistical analysis

Data were analysed using GraphPad Prism (version 8.0). Results were presented as mean ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA). Statistical significance was considered at $p < 0.05$.

RESULTS

Partial purification of rhodanese

The summary of rhodanese purification from *Anacardium occidentale* mesocarp and seed is shown in Table 1 and Table 2. Rhodanese produced a specific activity of 0.442 $\mu\text{mol}/\text{min}/\text{mg}$, a purification fold of 10.524, and a percentage yield of 1.820% were recorded for the mesocarp. Also, the specific activity of 0.462 $\mu\text{mol}/\text{min}/\text{mg}$, purification fold of 0.774 and yield of 21.664% were observed for the seed.

However, ammonium sulphate fractionation did not result in purification of rhodanese from the seed fraction, as evidenced by the decrease in specific activity (0.597 to 0.462 $\mu\text{mol}/\text{min}/\text{mg}$) and purification fold (<1). In addition, the identity of the enzyme as rhodanese was inferred based on activity assays; however, confirmatory techniques such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE or molecular weight determination) were not performed.

Table 1: Summary of Purification of Rhodanese in *Anacardium Occidentale* Mesocarp

Steps for Purification	Total Protein (mg)	Total Activity (U/mL)	Specific Activity ($\mu\text{mol}/\text{min}/\text{mg}$)	Purification Fold	Yield (%)
Crude Sample	5.39	126.91	0.042	1	100
80% Ammonium Sulphate Precipitation	1.02	2.31	0.442	10.524	1.82

Table 2: Summary of Purification of Rhodanese in *Anacardium Occidentale* Seed

Steps for Purification	Total Protein (mg)	Total Activity (U/mL)	Specific Activity ($\mu\text{mol}/\text{min}/\text{mg}$)	Purification Fold	Yield (%)
Crude Sample	60.3	100.95	0.597	1	100
80% Ammonium Sulphate Precipitation	10.1	21.85	0.462	0.774	21.644

Kinetic parameters

Rhodanese K_m and V_{max} from cashew seeds and mesocarp were represented in the nonlinear regression (Michaelis-Menten model) (depicted by figure 1). At varying concentration of KCN, the K_m and V_{max} were discovered as 0.2394 ± 0.1091 mM and 0.1235 ± 0.0325 $\mu\text{mol}/\text{min}/\text{mg}$ protein, respectively, with a high goodness of fit ($R^2 = 0.9513$)

for mesocarp. At varying concentration of $\text{Na}_2\text{S}_2\text{O}_3$, K_m and V_{max} was discovered as 0.0422 ± 0.0091 mM and 0.1611 ± 0.0086 $\mu\text{mol}/\text{min}/\text{mg}$ protein, respectively, with a high goodness of fit ($R^2 = 0.9499$) for mesocarp. In addition, at varying concentration of $\text{Na}_2\text{S}_2\text{O}_3$, K_m and V_{max} were discovered as 0.0460 ± 0.0194 mM and 0.4511 ± 0.0492 $\mu\text{mol}/\text{min}/\text{mg}$ protein, respectively, with a high goodness of

fit ($R^2 = 0.8185$) for seed. At varying concentration of KCN, K_m and V_{max} were discovered as 0.2373 ± 0.2491 mM and 0.7116 ± 0.4292 $\mu\text{mol}/\text{min}/\text{mg}$ protein, respectively, with a high goodness of fit ($R^2 = 0.7353$) for seed. The summary of values of kinetic parameters (K_m and V_{max}) is presented in Table 3 and Table 4 for rhodanese in *Anacardium occidentale* mesocarp and seed, respectively.

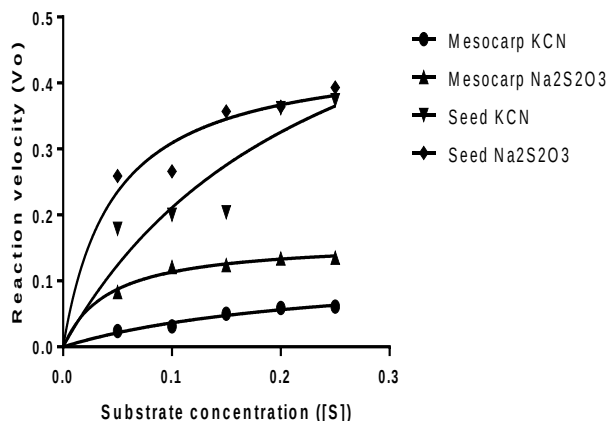


Figure 1: The nonlinear regression (Michaelis–Menten model) displaying the link between varying concentrations of Potassium Cyanide (KCN), sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and the reaction velocity by rhodanese in *Anacardium occidentale* mesocarp and seed

Table 3: K_m and V_{max} of Cashew Mesocarp Rhodanese

Substrate	K_m (mM)	V_{max} ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	R^2
KCN	0.2394 ± 0.1091	0.1235 ± 0.0325	0.9513
$\text{Na}_2\text{S}_2\text{O}_3$	0.0422 ± 0.0091	0.1611 ± 0.0086	0.9499

Table 4: K_m and V_{max} of Cashew Seed Rhodanese

Substrate	K_m (mM)	V_{max} ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	R^2
KCN	0.2373 ± 0.2491	0.7116 ± 0.4292	0.7353
$\text{Na}_2\text{S}_2\text{O}_3$	0.0460 ± 0.0194	0.4511 ± 0.0492	0.8185

Effect of pH on rhodanese activity in *Anacardium occidentale* mesocarp and seed

The activity of rhodanese from cashew mesocarp and seed was found to be optimum at pH 5 and pH 7, respectively. Graphs showing the relationship between pH and rhodanese activity are presented in Figures 2 and 3.

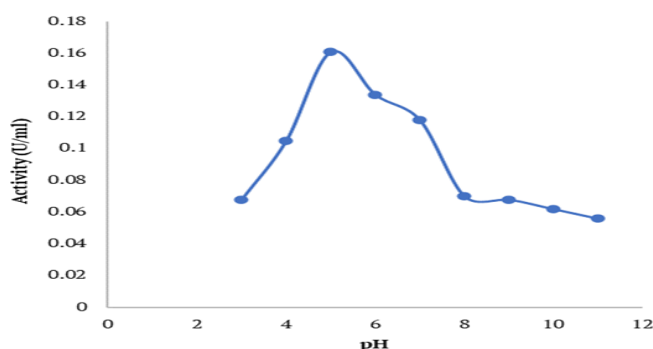


Figure 2: Effect of pH on Cashew mesocarp rhodanese

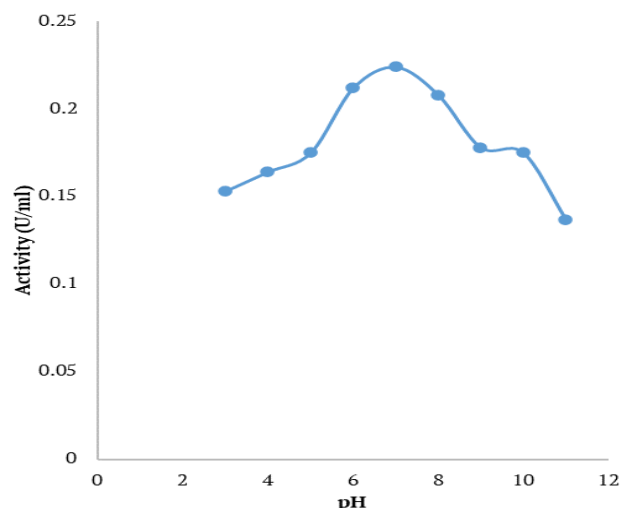


Figure 3: Effect of pH on Cashew seed rhodanese

Effect of temperature on rhodanese activity in *Anacardium occidentale* mesocarp and seed

By conducting assays at various temperatures from 30°C to 80°C , the impact of heat on rhodanese was measured. Rhodanese gave an optimum activity at 50°C for the cashew mesocarp and 30°C for the cashew seed. The result is shown in Figures 4 and 5.

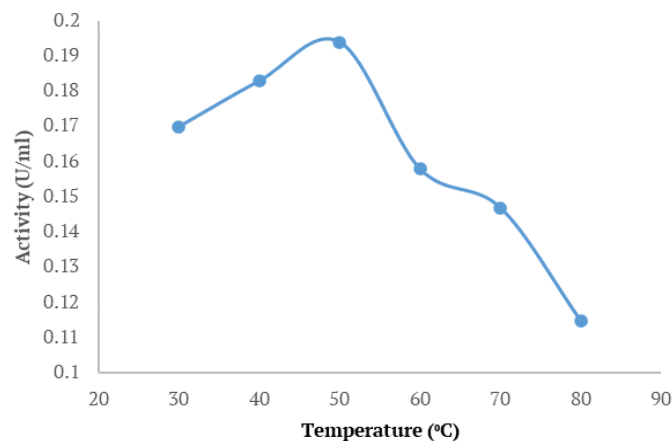


Figure 4: Effect of temperature on cashew mesocarp rhodanese

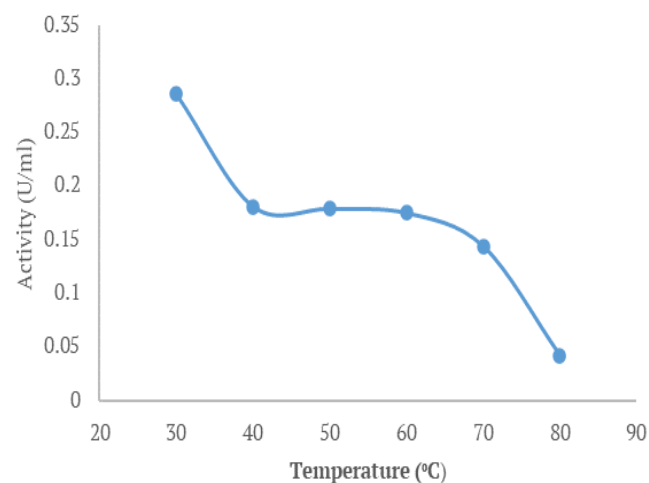


Figure 5: Effect of temperature on cashew seed rhodanese

Substrate specificity of rhodanese from *Anacardium occidentale* mesocarp and seed

By examining the affinity of rhodanese from cashew mesocarp and seed to various sulphate compounds, substrate specificity was examined. The result is shown in Figure 6 and figure 7.

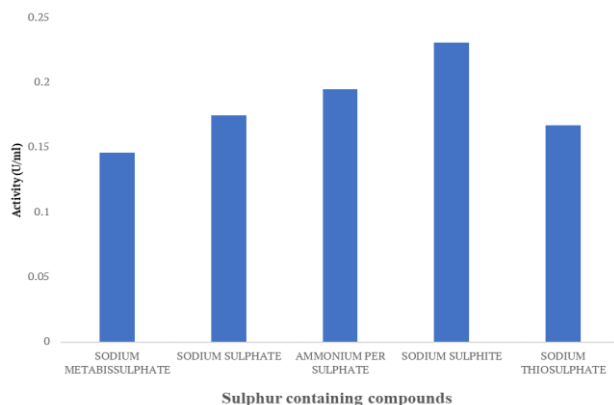


Figure 6: Effect of sulphur containing compounds on rhodanese in cashew mesocarp

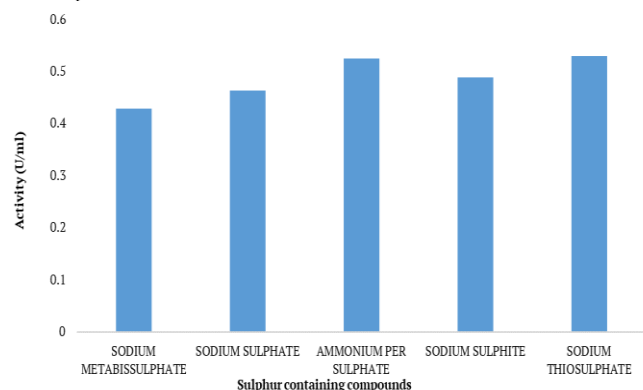


Figure 7: Effect of sulphur containing compounds on rhodanese in cashew seed

Effect of metal ions on rhodanese activity in *Anacardium occidentale* mesocarp and seed

The effect of metals (HgCl, NaCl, ZnCl₂, SnCl₂ and MnCl₂) on rhodanese in both cashew mesocarp and seed revealed that the metals impacted the activity of rhodanese at varying concentration (either by activating or inhibiting its action). The results presented in figure 8 and figure 9.

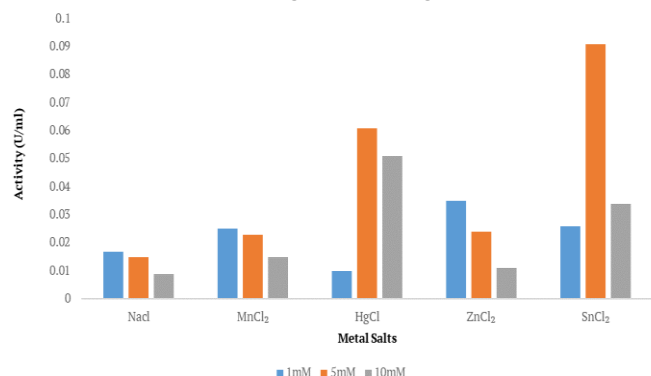


Figure 8: Effect of metal ions on rhodanese in cashew mesocarp

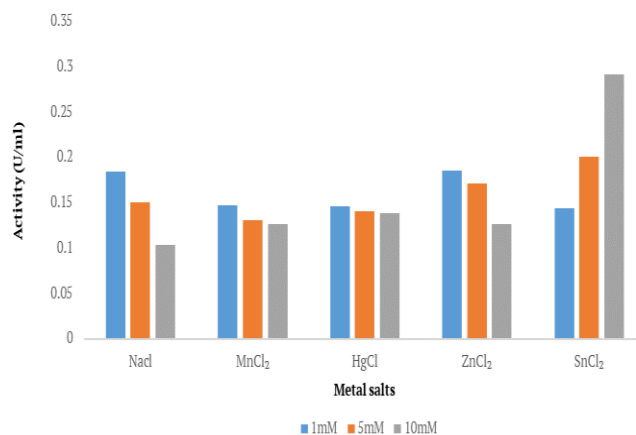


Figure 9: Effect of metal ions on rhodanese in cashew seed

Effect of inhibitors (Urea, EDTA, and Mercaptoethanol) on rhodanese activity in *Anacardium occidentale* mesocarp and seed

The effect of urea, EDTA and mercaptoethanol on rhodanese in both cashew mesocarp and seed at varying concentration (5.0 mM, 2.5 mM, 1.0 mM, and 0.5 mM) is shown in figures 10 and 11. Rhodanese activity in cashew mesocarp was increased more by EDTA and inhibited more by urea. However, rhodanese activity in cashew seed was increased more by mercaptoethanol and also inhibited more by urea.

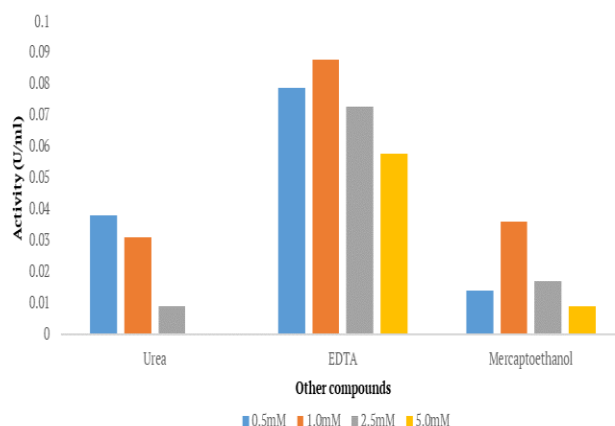


Figure 10: Effect of Urea, EDTA and mercaptoethanol on rhodanese in cashew mesocarp

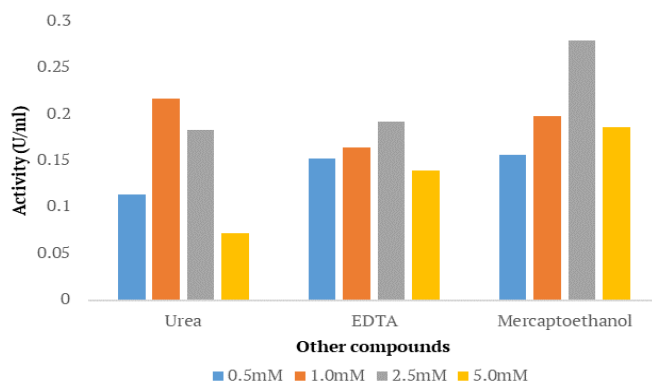


Figure 11: Effect of Urea, EDTA and mercaptoethanol on rhodanese in cashew seed

DISCUSSION

Cyanogenic glycosides are plant compounds that can release hydrogen cyanide, a potent cytotoxic agent (Ehigie *et al.*, 2019a; Ogbuagu *et al.*, 2019; Okonji *et al.*, 2015) that inhibits cytochrome oxidase activity, thereby killing the cell (Ilesanmi *et al.*, 2020). Cyanogenic glycosides from several sources (Tewe and Iyayi, 2023; Zona, 2022; Onojah and Odin, 2015) occur due to developmental (endogenous), ecological (exogenous), genetic and environmental factors (Lachowicz *et al.*, 2024; Okunola *et al.*, 2022). When cyanide is ingested, the bodily mechanisms of its detoxification are activated (Aladesanmi *et al.*, 2017), resulting in cyanide's transformation into thiocyanate with less toxicity (Ehigie *et al.*, 2019b; Okonji *et al.*, 2017). Both 3-mercaptopyruvate sulphurtransferase and rhodanese are reportedly the major enzymes linked with detoxifying cyanide (Rao *et al.*, 2023; Okunola *et al.*, 2022).

In this scientific study, rhodanese was extracted and partially purified using "80% ammonium sulphate precipitation" from cashew seeds and mesocarp. Rhodanese produced a specific activity of 0.442 $\mu\text{mol}/\text{min}/\text{mg}$ of protein and 1.82% yield for the mesocarp, while the seed showed a specific activity of 0.462 $\mu\text{mol}/\text{min}/\text{mg}$ of protein and 21.644% yield. For rhodanese, various sources have reported varying values of specific activity. According to related research, Rhodanese from fruit bat liver was found to be 131 $\mu\text{mol}/\text{min}/\text{mg}$ of protein (Agboola and Okonji, 2004) and 20.1 $\mu\text{mol}/\text{min}/\text{mg}$ of protein (Fagbohunka *et al.*, 2004). According to Akinsiku *et al.* (2010), the protein values for catfish rhodanese I and II (cRHDI and cRHDII) were 73 $\mu\text{mol}/\text{min}/\text{mg}$ and 72 $\mu\text{mol}/\text{min}/\text{mg}$, respectively. Mudskipper liver rhodanese was found to be 8.4 $\mu\text{mol}/\text{min}/\text{mg}$ of protein by Okonji *et al.* (2011).

In addition, the K_m and V_{max} values of rhodanese from *Anacardium occidentale* seed and mesocarp were obtained. K_m values of 0.0422 ± 0.0091 mM and 0.2394 ± 0.1091 mM were obtained for $\text{Na}_2\text{S}_2\text{O}_3$ and KCN, respectively, when considering *Anacardium occidentale* mesocarp. However, K_m values of 0.0460 ± 0.0194 mM and 0.2373 ± 0.2491 mM were obtained for $\text{Na}_2\text{S}_2\text{O}_3$ and KCN, respectively, when considering *Anacardium occidentale* seed. These observed results for K_m of rhodanese are similar to one another but lower than those reported for freshwater prawn (*Macrobrachium rosenbergii*) hepatopancreas rhodanese by Okonji *et al.* (2008), which reported K_m values of 10.0 mM and 2.56 mM for KCN and $\text{Na}_2\text{S}_2\text{O}_3$ respectively. Additionally, catfish liver gave a K_m of 25.4 mM for KCN and 18.6 mM for $\text{Na}_2\text{S}_2\text{O}_3$ (Akinsiku *et al.*, 2010).

Similarly, for *Escherichia coli* rhodanese, K_m of 78 mM for KCN and 17 mM for $\text{Na}_2\text{S}_2\text{O}_3$ were reported (Alexander and Volini, 1987). A lower K_m generally indicates greater apparent substrate affinity under the assay conditions; however, K_m alone should not be interpreted as a direct measure of overall cyanide-detoxification efficiency. According to the K_m values, rhodanese from *Anacardium occidentale* has a greater affinity for thiosulphate (with K_m = a lower value than that of KCN) compared to KCN, consistent

with the principle that a lower K_m indicates a higher substrate affinity, which may increase the likelihood of effectively reducing the mechanism of cyanide toxicity. However, the high substrate concentration used may exceed physiological relevance and could influence enzyme kinetics.

From this current study, with elevation in pH from 4.0 to 5.0 for cashew mesocarp and from pH 5.0 to 7.0 for cashew seed, an increase in activity was observed. This study observed an optimum activity to be at pH 5.0 for the *Anacardium occidentale* mesocarp and optimum activity at a pH of 7.0 for the *Anacardium occidentale* seed. Our observation of optimum activity at pH of 7.0 for the *Anacardium occidentale* seed rhodanese is consistent with that of bitter water melon (*Momordica charantia*) leaves, and the *Anacardium occidentale* mesocarp rhodanese optimum pH of 5.0 is similar to that of garden snails as reported by Okonji *et al.* (2020).

The optimum temperature for cashew mesocarp was 50°C and 30°C for cashew seed. An optimum temperature of 50°C for rhodanese in cashew mesocarp shows that the enzyme can maintain its function under slightly hot weather, whereas that of cashew seed indicates more stability at a state close to room temperature (25°C). Additionally, Okonji *et al.* (2011) found the same 50°C as the ideal temperature for mudskipper liver rhodanese.

Furthermore, substrate specificity results in this current study showed that the activity of rhodanese in *Anacardium occidentale* mesocarp and seed was activated by all the compounds used: "ammonium persulphate, sodium metabisulphate, sodium sulphate, sodium sulphite and sodium thiosulphate". However, rhodanese is highly specific for thiosulphate as a sulphur donor (Okonji *et al.*, 2015); thus, the observed effects of sulphate-containing compounds are likely due to ionic or metal-mediated interactions rather than true substrate activity. These observations should not be interpreted as evidence that all the tested compounds function as physiological sulphur donors for rhodanese. Sodium thiosulphate remains the principal reference sulphur donor used in the assay, and the apparent activity observed with other compounds may reflect differences in ionic strength, enzyme conformation, substrate interaction or assay response.

Compounds like copper sulphate, ammonium sulphate and sodium sulphate are not established as sulphur-transfer substrates under the conditions examined for rhodanese because sulphate (SO_4^{2-}) is fully oxidised and not a sulphur donor. First reported were the unique properties of rhodanese to replace thiosulphate by Sorbo (1953). According to Okonji *et al.* (2011), rhodanese uses different sulphur compounds from a sulphane pool. The significance of the sulphane-sulphur pool in the detoxifying mechanism of rhodanese cyanide was also reported by Westley (1981). It was further noticed that sulphate-containing compounds had more impact on activating the activity of rhodanese in *Anacardium occidentale* seeds compared to rhodanese in *Anacardium occidentale* mesocarp.

The observed increase in apparent enzyme activity in the presence of these compounds is likely due to metal ion interference and non-specific physicochemical effects, rather than true catalytic enhancement (Okonji *et al.*, 2015; Akinsiku *et al.*, 2010). As reported, ionic species can alter enzyme conformation by changing the ionic strength of the medium or interacting with charged amino acid residues (Okonji *et al.*, 2015). The consequence is a temporary stabilisation of the enzyme or improvement in substrate accessibility without directly participating in catalysis (Alexander and Volini, 1987; Akinsiku *et al.*, 2010). Additionally, trace metal ions (such as Mg^{2+} and Ca^{2+}) present as contaminants in reagents may interact with critical thiol (-SH) groups at the rhodanese active site, modifying its nucleophilic properties and influencing enzyme activity (Agboola and Okonji, 2004; Okonji *et al.*, 2015). Similarly, the apparent “activation” may arise from spectrophotometric artefacts in the rhodanese assay, as sulphur-containing compounds and reducing agents can interfere with the ferric–thiocyanate detection system by altering the redox state of iron or contributing to background absorbance (Akinsiku *et al.*, 2010). In addition, increased turbidity or light scattering caused by certain salts can lead to artificially elevated absorbance readings and overestimation of enzyme activity (Ehigie *et al.*, 2019a; Ehigie *et al.*, 2019b).

Additionally, metal ions comprising Na^+ , Zn^{2+} and Mn^{2+} had no inhibitory effect on *Anacardium occidentale* mesocarp and seed rhodanese activity. On the other hand, 5 mM of Hg^{2+} and Sn^{2+} had an inhibitory influence on the activity of rhodanese in *Anacardium occidentale* mesocarp, whereas only 10 mM of Sn^{2+} had an inhibitory action on the rhodanese activity in *Anacardium occidentale* seed. These metals may block rhodanese by interaction with sulfhydryl groups at its catalytic site or by enabling modifications to its tertiary and secondary structure. The suggested structural misalignment is well reported due to incubation of proteins with metallic ions.

CONCLUSION

According to this research, the isolation and partial purification of rhodanese in *Anacardium occidentale* mesocarp and seed was carried out to provide an understanding of the properties and activity of rhodanese. This study demonstrated rhodanese-like activity in *Anacardium occidentale* mesocarp and seed and characterized selected physicochemical and kinetic properties of the partially purified enzyme preparations. The preparations showed activity in assays containing $Na_2S_2O_3$ and KCN, with distinct optimum pH and temperature conditions for the mesocarp and seed. The findings suggest that rhodanese activity in these plant tissues may contribute to cyanide detoxification; however, further studies including purification, stability analysis, and catalytic efficiency evaluation are required. Also, there is a need to further purify the rhodanese enzyme in *Anacardium occidentale* mesocarp and seed to obtain a purer enzyme whose physicochemical properties can be investigated. Therefore, we recommend

additional purification steps such as ion-exchange chromatography, gel filtration and SDS-PAGE for purity validation.

AUTHORS' CONTRIBUTIONS

BSF; Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing - review and editing. REO; Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing - review and editing. KTO; Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. AOA; Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. OAF; Investigation, Methodology, Validation, Visualization Writing - original draft. ENE; Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing - review and editing. All authors have read and agreed to the published version of the manuscript.

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Nothing to disclose

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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