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Biochemical Properties of Rhodanese from Almond (*Prunus amygdalus*) Nuts

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Abstract

Study of the characteristic pattern of enzymes are useful in the understanding of certain physiological and biochemical processes. Thiosulfate: cyanide sulfurtransferase (rhodanese) is a ubiquitous multifunctional enzyme, that is believed to function in cyanide detoxification. The present study was conducted to determine the activity of rhodanese in almonds (*Prunus amygdalus*) that belong to the rose family, rosaceae. Rhodanese from the almond nuts was purified by ammonium sulphate precipitation, ion exchange and affinity chromatography. The molecular weight of the enzyme was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The purified rhodanese from the almond nuts had a specific activity of 5.09 RU/mg with yield of 0.06%. A K_m value of 11.14 mM with V_{max} 0.46 RU/ml/min were obtained from KCN while a K_m value of 13.95 mM with V_{max} of 0.48 RU/ml/min was obtained from $Na_2S_2O_3$. The substrate specificity studied indicated that Mercapto-ethanol (MCPE), Ammonium per sulfate $((NH_4)_2S_2O_8)$, Ammonium sulfate $((NH_4)_2SO_4)$, Sodium sulfate (Na_2SO_4) and Sodium metabisulfate $(Na_2S_2O_5)$ cannot be substituted for sodium thiosulphate $(Na_2S_2O_3)$ as sulphur donor for rhodanese catalytic reaction. The optimum activity of the enzyme was observed at 50°C and an optimum pH of 8. The effect of metals on rhodanese from Almond nut showed that at 1 mM concentration of the metals used did not pronouncedly affect the activity of the enzyme metals except that of $HgCl_2$ and $MnCl_2$. However, the divalent metals including $MnCl_2$, $HgCl_2$, $CaCl_2$, and $BaCl_2$ inhibited the enzyme at 10 mM concentration. The molecular weight obtained from sodium dodecyl sulphate polyacrylamide gel electrophoresis was estimated to be 35 kDa. The study validates the expression of rhodanese activity in almond nut. The characteristic property of rhodanese in the plant may be exploited for bioremediation of cyanide polluted soil.

Keywords: Rhodanese, Bioremediation, cyanide, *Prunus amygdalus* and sulphurtransferase

1.0 INTRODUCTION

Cyanide is a potent cytotoxic agent that destroys the cell by inhibiting cytochrome c oxidase of the mitochondrial electron transport chain. It could be released to the environment through transportation accidents, pipe failures and tailings dam-related releases [1]. Non-point sources of cyanide released to water include runoff from cyanide-containing anti-caking salts such as sodium ferrocyanide used on roads, migration from landfills, agricultural and atmospheric fallout and washout [2]. In bitter almond, HCN levels ($1062 \pm 148.70\text{mg/kg}$) are approximately 40 times higher than levels ($25.20 \pm 8.24\text{mg/kg}$) found in sweet almond [3]. This implies that the amount of amygdalin contained in the bitter almond largely exceeds the amount contained in the sweet one. After enzymatic hydrolysis, the amygdalin which is the most important cyanogenic glycoside in *Prunus* species releases a high level of hydrocyanic acid and a benzaldehyde which is responsible for the bitterness [4]. Knowing that the acute lethal dose of cyanide for mammals is as low as 0.5 mg CN/kg of the body weight [5], the acute oral lethal dose of HCN for humans is reported to be 0.5–3.5 mg/kg of body weight and the consumption of 50 bitter almonds is deadly for adults [3]. However, for young children, 5–10 almonds are fatal [6].

Almonds (*Prunus amygdalus*) are prunes that belong to the rose family, the Rosaceae. They were traditionally placed in a sub-family, the Prunoideae (or Amygdaloideae), but sometimes, they are placed in their own family, the Prunaceae (or Amygdalaceae). More recently, it has become apparent that *Prunus* evolved from the sub-family, Spiraeoideae [7]. The fruit consists of an outer hull and a hard shell with the seed (“hut”) inside. Two major types of almonds are grown commercially, which can be categorized as sweet almonds (*Prunus amygdalus dulcis*) and bitter almonds (*Prunus amygdalus amara*). The sweet almond is more popular for obvious reasons. Like the olive, the almond provides food and oil and both are produced with little effort from the former. Amygdaline differentiates the bitter almond from the sweet almond. In the presence of water (hydrolysis), amygdaline yields glucose, benzaldehyde and hydrocyanic acid (HCN). The skin of almonds should always be removed before use, as it contains irritating properties. While almonds may cause allergy or intolerance, its cross reactivity with peach allergens (lipid transfer proteins) and tree nut allergens is common. The symptoms range from oral allergy syndrome and contact urticaria to systemic symptoms including anaphylaxis such as urticaria, angio-oedema and gastrointestinal and respiratory symptoms [8]. Although the edible portion of the *Prunus amygdalus* is its nuts, which are commonly known as almonds and it is a popular, nutritious food [9], the nuts possess various pharmacological properties including lipid lowering [2], immunostimulant [10], anti-oxidant [11], anti-stress [12], Pre-Biotic Potential [13], and cholesterol Lowering Action [14].

Rhodanese (thiosulphate:cyanide sulphurtransferase, EC 2.8.1.1) is a sulphur transferase that catalyses the formation of thiocyanate from free cyanide and a sulphur donor. It functions in the elimination of toxic cyanide and in the biosynthesis of cellular metabolites such as vitamins, enzymes and cofactors that include a step of sulfur transfer [15]. The enzyme is involved in the maintenance of redox homeostasis [16] and bioremediation [17]. While the presence of rhodanese activity has been well studied in some plants, animals and microorganisms, its presence has not been studied in almond seeds. This study was therefore designed to investigate the presence of rhodanese and its activity in almond nut and to compare its physicochemical properties with rhodanese from other sources.

2.0 METHODOLOGY

Fresh almond fruits were collected from Ladok Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were identified at the Department of Biology, Ladok Akintola University of Technology, Ogbomoso, Nigeria. The fresh almond fruits were washed and nuts were removed.

2.1 Enzyme Extraction and Isolation

One hundred grams (100g) of the crushed almond nuts were homogenized in three volumes of 100 mM phosphate buffer, pH 6.5 containing 10 mM sodium thiosulphate with a Waring Blender. The homogenate was filtered through a double layer of cheese cloth and then centrifuged at 4000 rpm at 10°C for 15 min using Centurion cold centrifuge (R-1880). The pellets were discarded and an aliquot of the supernatant was then assayed for rhodanese activity and protein concentration.

2.2 Ammonium Sulphate Precipitation

One hundred millilitres (100ml) of the supernatant of the crude enzyme was brought to 80% ammonium sulphate saturation by slow addition and stirring of 51.6 g solid ammonium sulphate. This was done for 1 h with occasional stirring until all the salt had dissolved completely in the supernatant. The mixture was left for 12 h at 4°C. The resulting precipitate was collected by centrifugation at 4,000 rpm for 30 min and immediately dialyzed against several changes of a 0.1 M solution of phosphate buffer (pH 7.2) at 4°C for 8 hours using a dialysis bag. The dialysate was centrifuged (to remove insoluble materials) at 4,000 rpm for 15 min and the supernatant was assayed for rhodanese activity and protein concentration.

2.3 Enzyme Assay

Rhodanese activity was measured according to the previously described method [18]. The reaction mixture consists of 0.25 ml of 50 mM borate buffer (pH 9.4), 0.1 ml of 250 mM KCN, 0.1 ml of 250 mM $\text{Na}_2\text{S}_2\text{O}_3$ and 0.1 ml of the enzyme solution in a total volume of 0.55 ml. The mixture was incubated at 37°C for 1 min and the reaction was stopped by adding 0.25 ml of

15% formaldehyde, followed by the addition of 0.75 ml of Sorbo reagent. The absorbance was taken at 460 nm.

2.4 Protein Determination

Protein concentration was determined by the method of Bradford [19] using Bovine Serum Albumin (BSA) as the standard where the protein absorbance was interpolated from a standard protein curve. The reaction mixture consists of 10 µl of the enzyme solution and 1.0 ml of Bradford reagent. The absorbance was taken at 595 nm.

2.5 Ion-Exchange Chromatography on CM-Sephadex C-50

CM-Sephadex C-50 cation exchanger was pretreated by boiling 5g of the resin in distilled water for 1 h. This was followed by the addition of 100 ml 0.1 M HCl for 30 min after which the acid was decanted and the resin was washed with distilled water several times to ensure the total removal of the acid. Thereafter, 100 ml of 0.1 M NaOH was added to the resin, which was decanted after 30 min, followed by the thorough rinsing of the resin with distilled water to remove all traces of the base. The resin was then equilibrated with 0.1 M phosphate buffer (pH 7.6) before it was packed into a 2.5 x 40 cm column. Six millilitres (6 ml) of the enzyme solution from the preceding step was then applied on the column. The column was washed with 0.1 M phosphate buffer (pH 7.6) to remove unbound protein, followed by a step-wise elution with 0.5 M NaCl and 1.0 M NaCl in the same buffer. Fractions of 4 ml were collected from the column at a rate of 48 ml per h. The active fractions from the column were pooled and dialyzed against 50% glycerol in 0.1 M phosphate buffer, pH 7.6. The dialyzed fraction was assayed for rhodanese activity and protein determination using Bradford reagent, the active fractions pooled were stored in a refrigerator.

2.6 Affinity Chromatography on Reactive Blue-2 Agarose

Fifteen millilitres of Reactive Blue-2 Agarose resin was packed into a 1.5 x 10 cm glass column and equilibrated with 0.05 M citrate buffer (pH 5.0). Three millilitres of enzyme solution from the preceding step was then layered on the column and washed with the buffer to remove unbound protein, followed by a step-wise elution with 1.0 M NaCl solution in the same buffer. Fractions of 2 ml were collected from the column at a rate of 12 ml per h. The active fractions from the column were pooled and dialyzed against 100 ml 50% glycerol in 0.1 M phosphate buffer, pH 7.5. The dialyzed fraction was assayed for rhodanese activity and protein.

2.7 Determination of Native Molecular Weight

The native molecular weight was determined on a Sephadex G-100 (2.5 x 90 cm). The standard proteins were bovine serum albumin (Mr 66000; 5 mg/ml), ovalbumin (Mr 45000; 5 mg/ml) and chymotrypsinogen-a (Mr 25,000; 5mg/ml). Total sample volume of each of the protein markers applied to the col-

umn was 5 ml. The proteins were eluted with phosphate buffered saline pH 7.2. Fractions of 5 ml were collected and monitored by measuring absorbance at 280 nm for the protein. The void volume (V₀) of the column was determined by the elution volume (V_e) of Blue dextran (2 mg/ml). A 5 ml aliquot of the enzyme solution was then applied to the same column and the elution volume of the rhodanese was determined.

2.8 Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Weber and Osborn [20] to determine the subunit molecular weight of the enzyme on a 10% slab gel apparatus with a notched glass plate. Gels of 1.5 mm thickness were prepared by using perplex spacers of same size. A mixture of the enzyme sample and sample buffer containing SDS (10%), bromophenol blue, β-mecaptoethanol and glycerol was prepared in a concentration of 1:1, and was then heated in boiling water bath for 3 min and then allowed to cool. Fifteen microlitre of the mixture along with the same volume of protein standards were applied to different wells on the slab and electrophoresed at a constant voltage of 70 volts to allow stacking of the proteins. The standard proteins that were used for the calibration of the gel were bovine serum albumin (66,000 Da), ovalbumin (45,000 Da), trypsin inhibitor (20,100 Da) and lysozyme (14,300 Da), which had also been denatured by mixing with SDS and heating for 3 min. After stacking was achieved, the voltage was increased to 100 volts to allow separation of the proteins in the resolving gel. After electrophoresis, the gels were stained in 0.04% Coomassie brilliant blue R-250 in 3.5% perchloric acid solution for 1h, followed by destaining in a solution containing 7.5% acetic acid to increase the sensitivity of detection. After destaining, the lengths of the gels as well as the distance of migration by the different protein bands were measured.

2.9 Determination of Kinetic Parameters

The kinetic parameters (K_m and V_{max}) of the enzyme were determined. The K_m and V_{max} of sodium thiosulphate and potassium cyanide were determined by varying concentrations of sodium thiosulphate and potassium cyanide between 10 mM and 50 mM at a fixed concentration of 25 mM potassium cyanide and sodium thiosulphate respectively in 50mM buffer pH 9.4. The kinetic parameters were determined from the double reciprocal plot.

2.10 Effect of pH on the Rhodanese Activity

The effect of pH was studied by assaying the enzyme using different buffers: 0.1 M citrate buffer (pH 3.0-6.0); 0.1M phosphate buffer (pH 7.0-8.0) and borate buffer (pH 9.0-10.0). The reaction mixture of 1.0 mL contained 0.25 mL of buffer and other reagents as contained in the normal rhodanese assay protocol [20, 21].

2.11 Effect of Temperature on the Rhodanese Activity

The enzyme was assayed at temperatures between 30°C and 100°C to investigate the effect of temperature on the activity of the enzyme and to determine the optimum temperature of the enzyme activity. The assay mixture was first incubated at the indicated temperature for 10 min before initiating reaction by the addition of an aliquot of the enzyme which had been equilibrated at the same temperature. The rhodanese activity was assayed routinely as previously described [20, 21].

2.12 Effect of Salts on the Enzyme Activity

The salts tested were NaCl, KCl, BaCl₂, HgCl₂ and MnCl₂ at 0.001 mM, and 0.01 mM from stock solutions of 0.1 mM in a typical rhodanese assay mixture. The metallic chlorides were dissolved in distilled water. The reaction mixture without the salts was taken as control with 100% activity [23].

2.13 Substrate Specificity

The substrate specificity of the enzyme was investigated by testing its activity towards structurally similar compounds. The compounds include Mercapto-ethanol (MCPE), Ammonium per sulfate (NH₄)₂S₂O₈, Ammonium sulfate (NH₄)₂SO₄, Sodium sulfite (Na₂SO₃), Sodium metabisulfate (Na₂S₂O₅). The solutions of the compound (250mM) were prepared in 50mM borate buffer, pH 9.4 and assayed as described above.

3.0 RESULTS

3.1 Purification

The result of the purification protocol for Almond Rhodanese showed a specific activity 5.09 RU/mg with yield of 0.06%. The purification procedure is summarized in Table 1. Figure 1 and 2 shows elution profile of Ion-exchange chromatography on CM-Sephadex C-50 and Reactive Blue-2 Agarose Affinity Chromatography respectively.

Figure 1: Elution profile on CM-Sephadex-C-50 ion exchange chromatography for Rhodanese from almond nut.

Figure 2: Elution profile on Blue-2 Agarose Affinity Chromatography for Rhodanese from almond nut.

Table 1: The purification profile of rhodanese from almond nut

	Total Protein (mg)	Total Activity (U)	Specific Activity (u/mg)	Yield %	Purification Fold
Crude Enzyme	190.88	478.69	2.51	1.00	1.00
80% (NH ₄) ₂ SO ₄ Precipitation	29.7	85.77	2.89	0.18	1.15
CM-Sephadex C-50 Ion Exchange Chromatography	11.32	54.24	4.79	0.11	1.91
Reactive Blue-2 Agarose Affinity Chromatography	5.26	26.79	5.09	0.06	2.03

3.2 Kinetic Parameter

Rhodanese followed Michaelis-Menten kinetics with a K_m for sodium thiosulphate (Na₂S₂O₃) of 13.95 mM with V_{max} of 0.48 RU/ml/min estimated from a double reciprocal plot (Table 2 and Figure 3). The K_m for potassium cyanide (KCN) of 11.14mM with V_{max} 0.46 RU/ml/min estimated from double reciprocal plot (Table 2 and Figure 4).

Table 2: The Kinetic Values of the Potassium cyanide and Potassium cyanide substrates.

Substrate	K _m	V _{max}
Potassium cyanide	11.14	0.46
Sodium thiosulphate	13.95	0.48

Figure 3: Lineweaver-Burk Plot for Varying Concentration of Sodium Thiosulphate Lineweaver-Burk plot of 1/V against 1/[S] at varying concentrations of Na₂S₂O₃ between 10 mM and 50 mM and a constant concentration of KCN at 25 mM.

Figure 4: Lineweaver-Burk Plot for Varying Concentration of Potassium Cyanide
Lineweaver-Burk plot of $1/V$ against $1/[S]$ at varying concentrations of KCN between 10 mM and 50 mM and a constant concentration of $\text{Na}_2\text{S}_2\text{O}_3$ at 25 mM.

3.3 Effect of pH and Temperature

The effect of pH on the enzyme was determined using assay buffer ranging from 3 to 11. The pH assay was done to determine the optimum pH of enzyme activity. There was an increase in the enzyme activity between pH 8.0 and 9.0. An optimum pH of 8.0 was observed (figure 5). The effect of temperature on the enzyme was determined by assaying at different temperatures between 30°C to 100°C to investigate the effect of temperature on the enzyme activity. The optimum activity was observed at 50°C (Figure 6).

Figure 5: Effect of pH on Rhodanese Activity from Almond

Figure 6: Effect of Temperature (°C) on Rhodanese Activity from Almond nut

3.4 Substrate Specificity

Substrate specificity of Rhodanese was investigated by testing its activity towards structurally related sulfur compounds in a typical Rhodanese assay mixture. The activity was expressed as a percentage activity of the enzyme using sodium thiosulphate as the control (Table 3).

Table 3: Percentage Substrate Specificity of each Sulphur Compound

Substrate	% Activity
Sodium thiosulphate	100
Sodium Metabisulphite	24.99
Ammonium persulphate	31.62
Mecaptoethanol	27.7

Effect of Metals

The effect of metal ions (NaCl , KCl , HgCl_2 , BaCl_2 , MnCl_2) at concentrations of 1 mM and 10 mM using their chloride salts was studied. The enzyme activity was inhibited by HgCl_2 and BaCl_2 in a concentration dependent manner while KCl , NaCl and MnCl_2 had no pronounce effect on the enzyme as shown in the Table 4.

Table 4: Effect of Metals on Rhodanese from almond

Chloride Salt	% Residual Activity (1 mM)	% Residual Activity (10 mM)
HgCl_2	75	63
KCl	98	94
CaCl_2	87	67
NaCl	97	92
BaCl_2	80	60
MnCl_2	78	65

3.5 Molecular Weights on SDS PAGE

Figures 7 and 8 show the electrophoretogram and calibration curve respectively, obtained for the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE). The molecular weight estimated from the SDS-PAGE was estimated to be 35 KDa.

Figure 7: Photograph of SDS-PAGE of almond nut rhodanese.

Lane 1=Standard Proteins; The standard molecular weight markers are: i=Lysozyme (14.3 kDa), ii=Trypsinogen (24.0 kDa), iii=Pepsinogen (34.5), iv=Ovalbumin (45.0 kDa), and v= BSA (66.0 kDa). Lane 2 = almond nut rhodanese

Figure 8: Log M_w versus R_f for the estimation of Molecular Weight of Proteins

4.0 DISCUSSION

Cyanides generally are compounds of varying degrees of chemical complexity. They contain a CN moiety, to which humans are exposed in gas, liquid and solid form from a broad range of natural and anthropogenic sources [24]. Cyanogenic glycosides are glycosides containing cyanide moiety. Cyanogenic glycosides are contained in several edible plants, whose concentrations can vary widely as a result of both developmental (endogenous) and ecological (exogenous) comprising of genetic and environmental factors, location, season and soil types [24, 25]. Plants can readily take up cyanide and metallo-cyanides when present around their root zone [27]. While Anosike and Ugochukwu [28] showed strict correlation between cyanide content and rhodanese levels in six different cassava varieties, the presence of rhodanese activity has been studied in cassava leaf [29], cabbage [30], and ginger and snake tomatoes plants [17, 31]. There is no doubt about the existence of rhodanese in plants as molecular and biochemical studies have proven their existence in plants. At the close of the 20th century Saito and Haltzfeld [32] confirmed the existence of rhodanese in plants by characterizing for the first time in plants two cDNAs encoding rhodanese isoforms in *Arabidopsis thaliana* AtRDHI and AtRDH2.

In this study, the enzyme had a specific activity of 5.09 RU per mg of protein and 0.06% recovery. A purification fold of 12.5 fold and specific activity of 8.81 mole was obtained for sheep liver rhodanese [33]. Jack et al., [34] purified rhodanese from marine fish (*Lutjanus goreensis*) liver to 11.4 fold and a specific activity of 19.7 μ Mmin-1mg-1 enzyme protein. A yield of 7.8 was obtained in tapioca leave [29] and a specific activity of 5.4 RU per mg of protein. Our previous study [17] also reported a purification fold of 1.02 and specific activity of 0.47 RU/mg for ginger (*Zingiber officinale*) rhizome.

Rhodanese catalysis is a non-sequential double displacement mechanism as previously reported [21, 22] for bovine liver

rhodanese. Researchers on rhodanese have reported various affinities between the substrates. The apparent K_m values of almond nut for KCN and $\text{Na}_2\text{S}_2\text{O}_3$ were 11.14 and 13.95 mM respectively in this study. This shows that the enzyme has a higher affinity for potassium cyanide compared to sodium thiosulphate. Lower K_m value is an indication of strong affinity of the enzyme for the substrate and high efficiency of the catalysis of the detoxification reaction. It was reported that the *P. brazzeana* root rhodanese binds to $\text{Na}_2\text{S}_2\text{O}_3$ and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ with similar affinity and to $\text{Na}_2\text{S}_2\text{O}_5$ with less affinity when all three sulphur donors are compared. Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) has been reported to be highly specific for rhodanese [22,35] although the enzyme can utilize other sulphur donors as substrate [36]. The K_m of rhodanese from *T. cucumerina* mesocarp and seed for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) were 12.5 and 10 mM, respectively [36]. Rhodanese in the cytosolic fraction of the fruit bat liver with values 19.15 mM and 13.36 mM for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) respectively [18] and Rhodanese in hepatopancreas of *Limicola flammea* with values 19.15 mM and 13.36 mM for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) respectively [37]. However, some other researchers reported a higher affinity for sodium thiosulphate as compared to potassium cyanide. The K_m values of 52.63 mM and 90 mM for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) respectively were reported for the rhodanese enzyme from ginger (*Zingiber officinale*) rhizome [17]. On the other hand, Boey et al., reported 15 mM and 66 mM for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) respectively for rhodanese from tapioca leaf [38] while Jack et al., reported 5.88mM and 11.11mM for sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) and potassium cyanide (KCN) respectively for the fish liver Rhodanese [34].

The substrate specificity study of rhodanese isolated from the almond nut has preference for sodium thiosulphate but to a far lesser extent it can also use other sulphur compounds such as ammonium persulphate, sodium metabisulphite and 3-mercaptopropanol for its catalytic activities. This is in line with the previous studies [39, 40] that reported the use of varying sulphur compounds from a sulphane pool by rhodanese. The specificity of rhodanese for sulphur from thiosulphate source. The study of the ability of different thiosulphates to substitute thiosulphate in rhodanese reaction was first reported in 1953 [22].

The effect of metals on rhodanese from Almond nut showed that at 1 mM concentration of the metals used did not pronouncedly affect the activity of the enzyme except that of HgCl_2 and MnCl_2 . However at 10 mM concentration, the divalent metals - MnCl_2 , HgCl_2 , CaCl_2 , and BaCl_2 - inhibited the enzyme. It was reported that rhodanese from bat liver was inhibited by Ba^{2+} [18] while Zn^{2+} inhibited African catfish

(*Clarias gariepinus*) liver rhodanese [41]. Mercury and zinc ions have been reported as potent inhibitors of rhodanese active site through their interactions with side chain residues [40, 18]. The rhodanese from Almond nut showed maximum activity at pH 8.0 which is consistent with the values from other plants including the snake tomato seed rhodanese [43]. The optimum pH of *Pentadiplandra brazzeana* (Baill) root rhodanese was also found to be 8.0 [34]. Different optimum pH values in the range of 7.0-11.0 have also been reported in African catfish liver [41], ginger rhodanese [17], tapioca leaf [29] marine fish [34] and sheep liver rhodanese [33]. An optimum temperature of 50°C was obtained for rhodanese isolated from Almond nut in this study. Rhodanese isolated from both Seed and mesocarp of *Trichosanthes cucumerina* was reported to have identical optimum temperature of 50°C [43]. Similar optimum temperatures were obtained in bovine liver [21], mudskipper liver [39] and ginger [17]. An optimum temperature ranging between 35 and 55°C for rhodanese enzyme in different *Trichoderma* strains [44]. The high temperature might be as a result of adaptation to harsh environmental condition. On the contrary, while the rhodanese in the cytosolic fraction of fruit Bat liver was reported to have an optimal temperature of 35°C [18], an optimum temperature of 40°C for rhodanese was reported from the liver of the catfish in Asejire Lake [41]. The subunit molecular weight obtained from Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was estimated to be 35 kDa almond nuts. The estimated value is relatively similar and in agreement with the range of 31 kDa - 37 kDa as reported by most other researchers working on rhodanese. A molecular weight of 37,000 Dalton was reported for bovine liver rhodanese [21], the same value was also reported for human liver rhodanese [45] which is a monomeric protein. A molecular weight of 34,800 Dalton and the SDS-PAGE of 34,000 Dalton was reported for mouse liver rhodanese, suggesting that the enzyme is a monomeric globular protein [23].

In conclusion, the study demonstrates the biochemical properties of the rhodanese in Almond nut which compares very well with the reports of the enzyme from other sources. The plant may perform the vital role of cyanide detoxification and possibly be exploited in remediation of polluted soil due to human activities.

Conflict of interest

The authors declare that there is no conflict of interest

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