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Table of Content

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Full Length Research Paper

Physicochemical properties of rhodanese from the kidney of cane rat (*Thryonomys swinderianus)*

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We report the properties of partially purified rhodanese, a cyanide detoxifying enzyme isolated from the kidney of the greater cane rat commonly known as grasscutter (*Thryonomys swinderianus*). *T. swinderianus* kidney rhodanese had a specific activity of 1.43 µmole/min/mg protein, a fold of 2.31 and a 55% recovery. The apparent K_m values of 0.408 and 0.316 mM were obtained for both sodium thiosulphate ($Na_2S_2O_3$) and potassium cyanide (KCN) respectively. Substrate specificity study showed that *T. swinderianus* rhodanese can use other substrates. The enzyme showed its maximum activity at pH 8.0 and 50°C. The assay for the effect of metal ions showed that the enzyme is not affected or inhibited by metal ions such as Na^+, Fe^{3+} but inhibited by Hg^{2+}. The continued existence of *T. swinderianus* after consumption of food crops with high cyanogenic glycosides suggests that the animal has a functional cyanide detoxification mechanism.

Keywords: Rhodanese, cyanide, kinetics, detoxification, cane rat, grasscutter.

INTRODUCTION

Due to its inhibitory effect on cellular respiration, cyanide is considered as a toxic compound for aerobic organisms. Various sources of cyanide reported include photo-synthetic bacteria, algae, fungi, plants and foods (such as beans, almonds and cashew nuts, etc) and even in the animal kingdom (ATSDR, 1997; EPA, 2003). Its presence has been reported in the roots of tubers such as cassava and potato grown in tropical countries. Cyanides are very reactive poisons that can be deadly and may be released into the environment through industrial usage or vehicle exhaust (ATSDR, 2006). Some plant species contain cyanogen glycosides which upon ingestion release hydrogen cyanide, a poisonous gas (Conn, 2008). Plants, such as beans, cassava, and peas used as food in tropical countries has been reported to contain cyanogenic glycosides. Cassava and peas are

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known to contain linamarin coexisting with lotaustralin (Uyoh et al., 2007).

Rhodanese (EC 2.8.1.1) is a ubiquitous enzyme and is widely distributed in plants (Nagahara et al., 1999; Aminlari et al., 2000). It is a detoxification enzyme that involves in the conversion cyanide (a toxic compound) to thiocyanate a less toxic compound (Agboola and Okonji, 2004; Lee et al., 1995). Various studies have reported the presence of rhodanese in the cytosol and other organelles (Nagahara et al., 1999; Steiner et al., 2018; Itakorode et al., 2019). Its detoxification potential has been reported in the liver tissues of diverse animals (Lee et al., 1995; Akinsiku et al., 2009). The highest concentration of rhodanese has been reported to be found in the liver of mammals, though, significant amounts can also reside in the kidney and other tissues (Cipollone and Visca, 2007). In spite of its widely studied detoxification role, research had made known that the enzyme also involved in other physiological activities such as energy metabolism and iron sulphur centres formation (Ogata and Volini 1990; Aussignargues et al., 2012). Grasscutter (*Thryonomys swinderianus*) feeds generally on plant debris, cassava and grasses which have been demonstrated to contain a high content of cyanogenic glycosides and on consumption release cyanide, a poisonous compound (Conn, 2008). This study, therefore, attempts to characterize rhodanese from *T. swinderianus* kidney for the purpose of biochemical comparison and to further understand the cyanide detoxification mechanism of the animal.

MATERIALS AND METHODS

The animal, grasscutter (*T. swinderianus*) was obtained from hunters in Abagboro village in Ile-Ife, Osun state, Nigeria. It was transported to the laboratory within 10 min of its slaughter. It was then dissected to remove the kidney.

Extraction of intracellular rhodanese

The kidney was rinsed properly with distilled water to remove blood stains and weighed. 20 g of the kidney tissue was cut into pieces and then homogenized in Tris buffer, (100 mM, pH 7.2) with a warring blender. The homogenate was filtered and then centrifuged at 10, 000 rpm at 4°C for 30 min. The supernatant obtained was checked for rhodanese activity and protein content.

Enzyme assay

Modified method of Lee et al., (1995) was used to determine rhodanese activity. Briefly, the assay mixture consists of 50 mM (borate buffer pH 9.4), 200 mM $Na_2S_2O_3$, 200 mM KCN, and 100 µl of the enzyme in 1 ml solution. The mixture was incubated with 15% formaldehyde (0.5 ml) for 1 min, followed by the addition of an appropriate volume of Sorbo reagent (Sorbo, 1951). The absorbance was then read at 460 nm using a visible spectrophotometer. One rhodanese unit (RU) was taken as the amount of the enzyme, which under the given conditions produced an optical density reading of 1.08 at 460 nm per min. Protein content was determined using the Bradford (1976) method.

Purification procedure

The crude enzyme was subjected to 70% ammonium sulphate precipitation and left overnight at 4°C. After centrifugation for 30 min, the precipitate obtained was re-suspended in a small amount of 0.1 M Tris buffer (pH 7.2) and dialyzed. The dialysate was assayed for rhodanese activity and protein concentration. 5 ml of the dialysate was applied to pretreated CM-Sephadex c-50 column equilibrated with 0.1 M Tris-HCl buffer (pH 7.2). Unbound protein was removed with 0.1 M Tris-HCl buffer, pH 7.2, followed by step-wise elution with 0.5 M NaCl in the same buffer. At the rate of 20 ml per hour, fractions of 2 ml were collected and monitored for protein and rhodanese activity. The active fractions from the column were then pooled.

Characterization procedure

Kinetic parameters determination

The kinetic parameters (Km and Vmax) of the enzyme were determined using potassium cyanide and sodium thiosulphate as substrates. This was done by varying concentrations of each substrate between 10 and 60 mM at a constant concentration of the other substrate. The Lineweaver-Burk double reciprocal plot was used to determine the kinetic parameters (Lineweaver and Burk, 1934).

Substrate specificity

The ability of the enzyme to use different sulphur compounds was investigated using copper sulphate, ammonium sulphate, ammonium persulphate, 2-mercaptoethanol, Sodium sulphite, and sodium sulphate decahydrate. The activity was determined as described above and expressed as a percentage activity of the enzyme using sodium thiosulphate as the control.

Effect of pH and temperature and metal ions on rhodanese activity

To check the effect of pH on enzyme activity, the enzyme was assayed using buffers of different pH: Citrate buffer (50 mM, pH 3-5), phosphate buffer (50 mM, pH 6-7), 50 mM Tris-HCl buffer (pH 8) and borate buffer (50 mM, pH 9-10). To study the effect of temperature, the enzyme was assayed at temperatures between 30 and 100°C. Influence of metals such as $HgCl_2$, NaCl, $FeCl_3$ and $MnCl_2$ was investigated at 0.1, 0.2 and 0.3 mM concentrations. The reaction mixture without the salt served as the control with 100% activity.

RESULTS

Purification of rhodanese

T. swinderianus kidney rhodanese had a specific activity of 1.43 µmol/mg protein, a fold of 2.31 and a yield of 55%. Table 1 summarized the result of the purification of *T. swinderianus* kidney rhodanese. The elution profiles after CM Sephadex c-50 ion-exchange chromatography is shown in Figure 1.

Table 1. Summary of purification of *T. swinderianus* kidney rhodanese.

Purification steps	Total protein (mg)	Total activity (µmole/min)	Specific activity (µmole/min/mg)	Purification fold	% yield
Crude sample	523.7	316.2	0.62	1	100
70% Ammonium Sulphate precipitation	221.3	196.7	0.89	1.44	62.2
Ion exchange chromatography	122.3	174.3	1.43	2.31	55

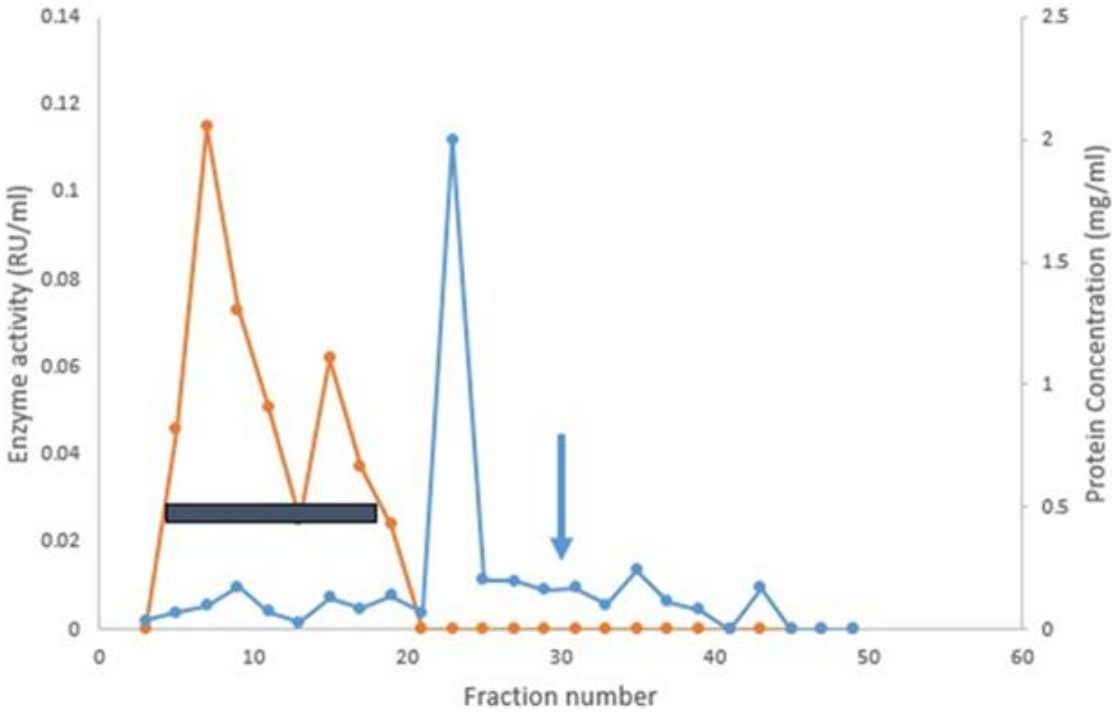

Figure 1. Elution profile of *T. swinderianus* kidney rhodanese on CM-Sephadex c-50 ion exchange chromatography.

Enzyme at 460 nm

Protein at 595 nm

Pooled fractions.

Kinetic parameters

The Line weaver-Burk plots for a fixed concentration of thiosulphate and KCN are shown in Figures 2 and 3 respectively. KCN and $Na_2S_2O_3$ had K_m values of 0.316 and 0.408 mM respectively while their V_{max} was 10.92 and 12.03 RU respectively.

Effect of pH and temperature and metal ions on rhodanese activity

The optimum pH for the activity of *T. swinderianus* kidney rhodanese was found to be 8.0 (Figure 4), while the optimum temperature for the activity of the enzyme was found to be 50°C (Figure 5). Metal ions such as Na^+, Fe^{3+} had little or no inhibitory effect on *T. swinderianus* rhodanese while Hg^{2+} had an inhibitory effect (Table 2).

DISCUSSION

Rhodanese has been reported in many organisms from bacteria to animals (Agboola and okonji, 2004; Aladesanmi et al., 2009; Itakorode et al., 2019). In this study, we report the presence of rhodanese, a cyanide

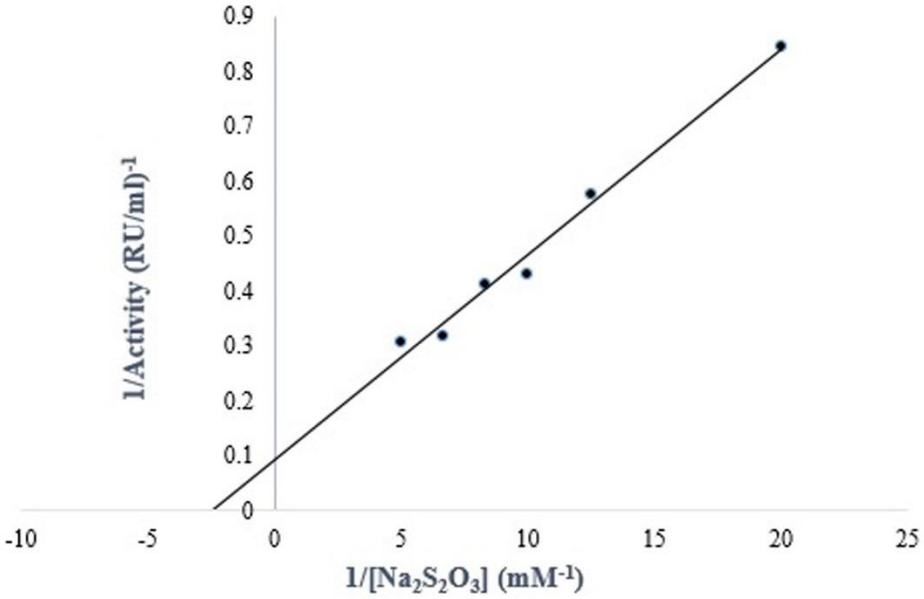

Figure 2. Lineweaver-Burk Plot for varying concentration of sodium thiosulphate between 10 mM and 60 mM and a fixed concentration KCN.

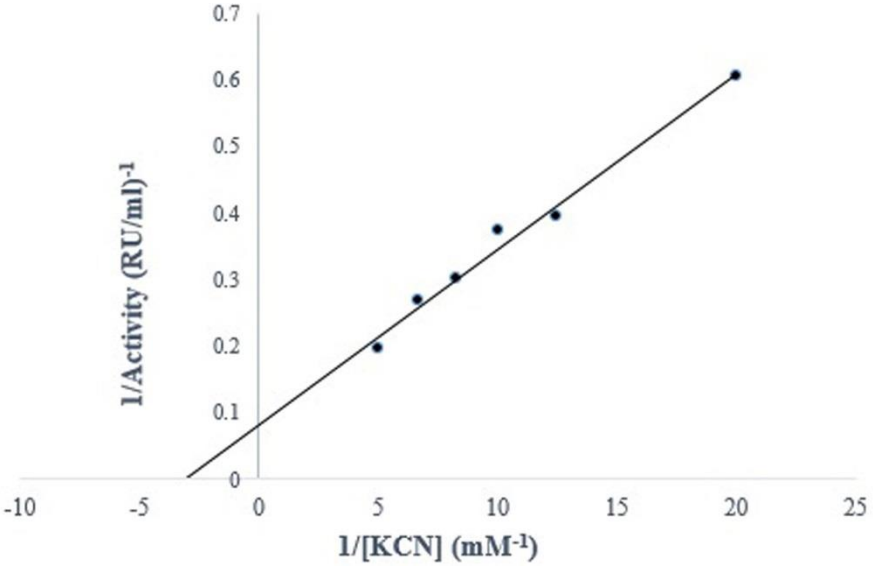

Figure 3. Lineweaver-Burk plot for varying concentration of potassium cyanide between 10 mM and 60 mM and a fixed concentration of $Na_2S_2O_3$.

detoxifying enzyme in the kidney of grasscutter (*T. swinderianus*). *T. swinderianus* feeds on cyanogenic plants such as green maize, sorghum, grasses with fairly high fibre content, cassava, etc. *T. swinderianus* kidney rhodanese was isolated and partially purified using 70% ammonium sulphate precipitation, and ion exchange chromatography on CM-Sephadex c-50. The enzyme had a specific activity of 1.43 µmole/min/mg of protein and 55% recovery. Different specific activity values have been reported for rhodanese from various sources (Aladesanmi et al., 2009; Okonji et al., 2010b). A value of 1.076 µmole/min/mg was reported for mouse liver rhodanese (Lee et al. 1995). Okonji et al., (2010b) also obtained a value of 8.4 µmole/min/mg for rhodanese from

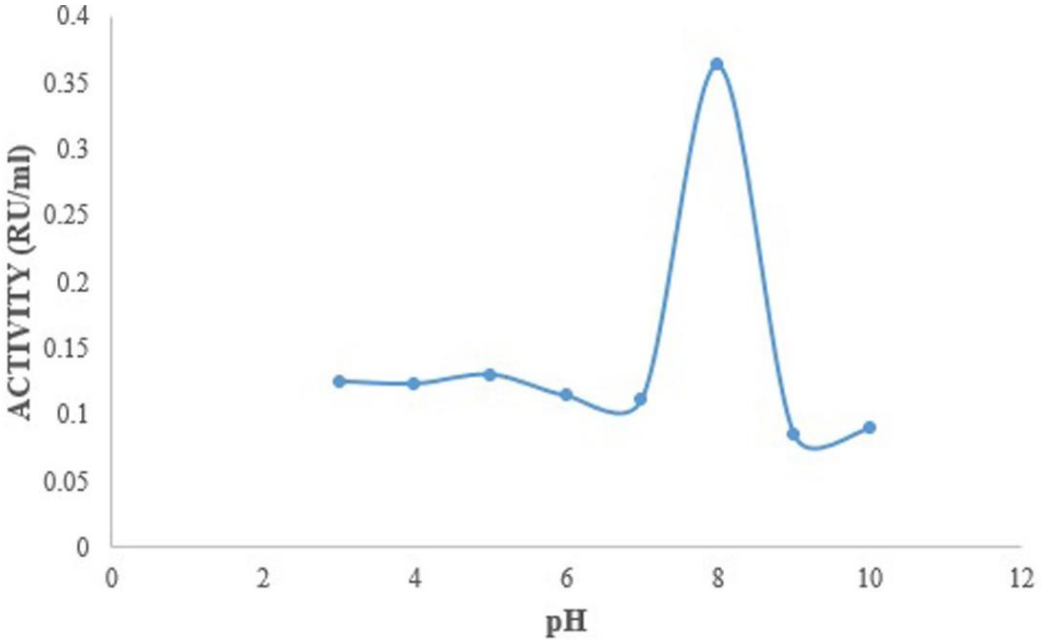

Figure 4. Effect of pH on *T. swinderianus* kidney rhodanese activity.

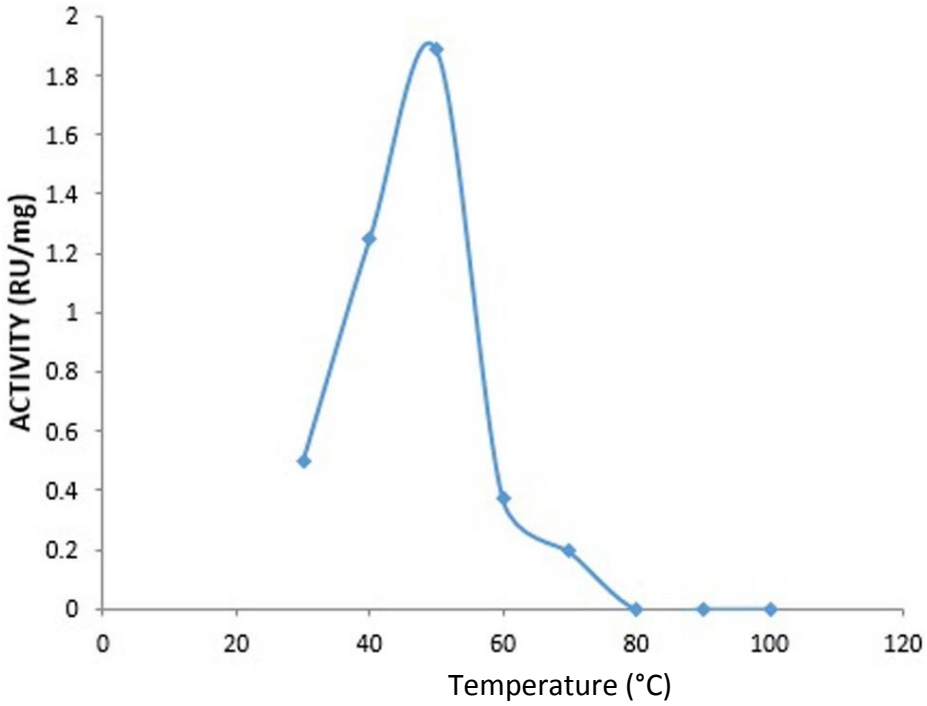

Figure 5. Effect of temperature on *T. swinderianus* kidney rhodanese activity.

the liver of mudskipper. *T. swinderianus* kidney rhodanese has low specific activity. This may be due to the source of the enzyme. In most animals studied, the liver is the richest source of rhodanese (Westley, 1973; Dudeck et al., 1980; Drawbaugh and Marrs, 1987), and this could indicate a heavy cyanide metabolizing function performed by this organ.

The kinetic parameters (K_m and V_{max}) of *T. swinderianus* rhodanese were obtained from the double reciprocal plot of Lineweaver-Burk. The K_m values of

Table 2. Effect of metal ions on *T. swinderianus* kidney rhodanese.

Metal	% relative activity		
	0.1 mM	0.2 mM	0.3 mM
$HgCl_2$	80.00 ± 0.00	40.00 ± 0.00	10.00 ± 0.00
NaCl	98.01 ± 1.91	100.00 ± 0.00	100.00 ± 0.00
$FeCl_3$	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
$MnCl_2$	52.97 ± 4.98	65.78 ± 0.95	76.00 ± 0.00

0.316 and 0.408 mM were obtained for both potassium cyanide (KCN) and sodium thiosulphate ($Na_2S_2O_3$) respectively. These results are similar but however, lower than those reported from other sources: bovine liver (Sorbo, 1953b: 19.0 and 6.7 mM for KCN and $Na_2S_2O_3$ respectively), human liver (Jarabak and Westley, 1974: 9.5 and 4.5 mM for KCN and $Na_2S_2O_3$ respectively), mouse liver (Lee et al., 1995: 12.5 and 8.3 mM for KCN and $Na_2S_2O_3$ respectively. The result from the K_m values indicates that *T. swinderianus* kidney rhodanese has a preference for thiosulphate. This may suggest a possible efficient cyanide detoxification catalytic mechanism which is important for the survival of the animal. The affinity of the enzyme for thiosulphate would enhance the biotransformation of the toxic cyanide into less toxic thiocyanate.

The results for substrate specificity study showed that *T. swinderianus* can use other sulphur containing compounds for its detoxification activity apart from thiosulphate, which corresponds with the studies reported by other researchers. Sulphur has been reported to play a prominent role in the cyanide detoxification mechanism rhodanese (Westley, 1981; Okonji et al., 2011). Wodu (2015) investigated the effect of some sulphydryl compounds such as dithiooxamide, cysteine, 2-mercaptoethanol and glutathione on sheep liver rhodanese. These monoatomic sulphur compounds never served as sulphur donors in sheep liver rhodanese as compared to the sulphur donating property of thiosulphate. In this work, the enzyme showed maximum activity with other sulphur containing compounds. This could suggest an efficient detoxification mechanism of the enzyme even in the presence of other sulphur containing compounds other than thiosulphate.

In this study, maximum enzyme activity was observed at pH 8.0. This value compares to the range reported for other sources (Aladesanmi et al., 2009; Hossein and Reza, 2011). Aladesanmi et al. (2009) obtained an optimum pH of 8.5 for rhodanese from the liver of land tortoise. Optimum pH as low as 6.0 and 6.5 was reported for giant freshwater prawn (*M. rosenbergii*) hepatopancreas and catfish liver rhodanese respectively (Akinsiku et al., 2009). The optimum high pH obtained may be due to the habitat of the animal as rhodanese obtained from an aquatic organism tend to show a relatively lower optimum pH. The optimum temperature of the enzyme was found to be 50°C. The optimum temperature of 50 and 55°C were reported for bovine liver and Trichoderma strain rhodanese respectively (Sorbo, 1953b; Ezzi et al., 2003).

Okonji et al., (2010b) obtained an optimum temperature of 55°C for soldier termite rhodanese. The optimum temperature of 50°C for *T. swinderianus* rhodanese indicates that the enzyme could endure a harsh condition. This optimum temperature could be an adaptive feature which sustains its survival in the kidney of *T. swinderianus* found in the tropical regions. Also, the effect of metals showed that metal ions such as Na^+, Fe^{3+} had no inhibitory effect on *T. swinderianus* rhodanese activity while Hg^{2+} had an inhibitory effect on the enzyme activity. Okonji et al., (2010b) reported the inhibition of soldier termite rhodanese by Hg^{2+} and Mn^{2+}. The absence of inhibitory effect of metal ions such as Na^+, Fe^{3+} observed in this study may be due to constant exposure of the animal to these metal ions from inorganic fertilizers, pesticides and other compounds used in the cultivation of food crops on which the animal feed. Also, the inhibition of Hg^{2+} on the enzyme activity may be due to interactions of the metal ion with the sulphydryl groups at the active site of the enzyme, thereby causing a conformational change in the structure of the enzyme.

Conclusion

In conclusion, the study was able to establish the presence of rhodanese in *T. swinderianus* kidney and the enzyme showed similar physicochemical properties to rhodaneses obtained from other sources. The continuing existence of the animal after consumption of cyanogenic plants suggest that the animal has a means of detoxifying cyanide and our report has shown that rhodanese is one of them.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Characterisation of volatile compounds and flavour attributes of *Lablab purpureus* bean accessions

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The Lablab bean (*Lablab purpureus*) has potential of being an outstanding resource for human food and animal feed in tropical agricultural systems. The bean is however grossly underutilized due to anti-nutritional factors, which may affect its nutritive value and organoleptic properties. In this study, twenty-four (24) lablab bean accessions were assayed for sensory flavor characteristics and volatile compounds to identify acceptable selections for adoption and incorporation into a rationalized breeding program. Sensory tests were carried out by a panel of 11 trained evaluators. Volatile compounds were extracted using hexane and separated using gas chromatography. Sensory tests showed significant differences for the bitter taste ($p \leq 0.05$), with accession 10706 showing the highest odour and bitter taste levels, while accession 13096 had the lowest. Two hundred and sixty two (262) volatile compounds were identified and grouped into 12 classes. The major compounds were esters (46), terpenes and terpenoids (59), hydrocarbons (57), and alcohols (28). The retention times of the volatile compounds revealed an overall 89% similarity of the lablab bean accessions. Accessions showing lower levels of bitter taste are recommended for inclusion in the participatory evaluation stage of the breeding process.

Key words: *Lablab purpureus*, odour, flavour, taste, volatile compounds, underutilized crops.

INTRODUCTION

Lablab, *Lablab purpureus* (L. Sweet) is a legume species that grows in the tropic and the subtropical regions of Asia and Africa. It grows in a diverse range of environments and is drought tolerant (Maass et al., 2010; Ravinaik et al., 2015). The species however has remained a minor crop in most regions where it is grown (Engle and Altoveris, 2000; Maass et al., 2010). Lablab is cultivated as a cover crop since its dense green cover

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reduces soil erosion by wind or rain (Mureithi et al., 2003). It has multiple uses as human food and fodder crop for livestock (Maass et al., 2010). As human food, it is eaten as green pods, mature seeds and its leaves are used as vegetables. In spite of these qualities, lablab has not been utilised extensively.

Lablab has the potential of being an outstanding resource for tropical agricultural systems and in improving human food and animal feed as a vegetable, pulse and/or forage crop (Pengelly and Lisson, 2003). The crop has high protein quality (Mortuza and Tzen, 2009). Like other legumes, it is however reported to contain anti-nutritional factors, which may affect its nutritive value and organoleptic properties. Among the important characteristics considered in selecting dry bean varieties for production and consumption is good flavour quality (Scott and Maiden, 1998), where flavour comprises of odour and taste. In India, specific lablab cultivars are preferred and valued for their nutritional and sensory attributes (Venkatachalam and Sathe, 2007). Some dark seeded types of lablab beans have been reported to have a bitter taste that may persist even after prolonged cooking (Wanjekeche et al., 2000). Factors that have been reported to contribute to bitter taste in beans include cyanogenic glycosides (Seigler et al., 1989), polyphenols such as tannins (Bressani and Elias, 1980), minerals for example iron (Yang and Lawless, 2005); saponins (Heng et al., 2004; Shi et al., 2004) and the malliard reaction (Martins et al., 2001).

The volatile components of cooked lablab beans have particularly been associated with odour which may affect the beans overall acceptance by consumers (Kim and Chung, 2008). Seed colour and quality of lablab bean are also major selection criteria in breeding programs, in conjunction with other minor criteria, particularly where human consumption is being considered (Pengelly and Maass, 2001, Spence et al., 2010). However, there has been limited study on the quality aspects of the lablab bean. This study therefore aimed to determine the sensory characteristics and the volatile compounds of lablab bean accessions from Kenya.

MATERIALS AND METHODS

Twenty-two (22) accessions of *L. purpureus* from the Kenyan National Gene Bank and Repository Centre at the Kenya Agricultural and Livestock Research Organization (KALRO-Muguga, Kenya) and two varieties from farmers' fields were used in the analysis of volatile compounds and sensory tests (Table 1). The accessions were bulked at the KALRO field, Njoro, Kenya (0° 20'S; 35° 56'E; 2166 m above sea level (asl)). The site receives an annual rainfall of about 960 mm with average maximum and minimum temperatures of 24 and 8°C with a mean of 14.9°C. The soils at this site are well drained, deep to very deep, dark reddish brown, friable and smeary, silt clay, with humic topsoil classified as mollic andosols (Jaetzold and Schmidt, 1983). The dry seeds were harvested, cleaned and dusted with insecticide (actellic super powder) and stored at room temperature. Prior to use, the seeds were washed and air-dried to remove the insecticide before the tests were carried out.

Sensory evaluation

A panel of 11-trained evaluators tasted the dry cooked seeds from the lablab bean accessions. The samples were boiled in distilled water until cooked and five seeds from each sample were served to each panellist while warm (about 40°C) in identical containers. A complete random design (CRD) was used, with three tasting replicates, and the coded samples randomly presented. The sensory characteristics were evaluated through qualitative descriptive analyses where the bitter taste intensity of the accessions was ordered using five descriptive terms; trace, slightly intense, moderately intense, very intense and extremely intense; while the odour intensity was assayed by using a vertical mark on a 15 cm line scale as described by Quirien and Keith (2005).

For analysis of category scale for the bitter taste data, the categories were converted to numerical scores by assigning successive numbers to each category; 0 was assigned to the trace and 5 to extremely intense. For analysis of line scale odour intensity data, panelists' marks were converted to numerical scores by measuring the distance in centimetres from the left or lowest intensity point on the scale to the panelists' mark. The scores were converted using 0.5 cm = 1 unit score as suggested by Quirien and Keith (2005).

Analysis of volatile compounds

Extraction of volatile compounds

Dry lablab seeds of the 24 accessions were ground into fine powder using a mortar and pestle. The mortar and pestle were washed and sterilised between samples. The volatile compounds were then cold extracted from the powder using gas chromatography (GC) grade hexane (BDH, England) (Mestres et al., 2000). Fifty grams (50 g) of ground seed powder was put into a separating funnel and to it added 100 ml of hexane. The solution was mixed thoroughly before filtering into a 250 ml conical flask through a filter paper (Whatman 1, diameter 125 mm). The extracts were then concentrated by evaporating the hexane using a rotavapor (BÜCHI Rotavapor R-205 Labortechnik GmbH, Essen, Germany). The concentrated sample was poured into a 5 ml sample bottle and allowed to dry through evaporation at room temperature (in the dark). The samples were stored at -20°C before gas chromatography (GC) analysis.

Gas chromatography

One hundred microliters (100 µl) of hexane were added to the sample bottle the night before GC analysis to dissolve the extracts. Five microliters of the sample was injected into the gas chromatography system (Shimadzu GC Model GC2010, Tokyo, Japan) which was fitted with a 30-m fused silica open-tubular column (ZB-5, 0.25 mm i.d., 0.25 mm film thickness, Phenomenex) with phase composition of 5% phenyl and 95% dimethylpolysiloxane. The following were the operating conditions: Initial and final temperatures with the holding times of 32°C for 5 min and 195°C for 5 min, respectively; the ramp rate was 2°C/min. Flame ionisation detector (FID) was used at 250°C and injector temperature was 220°C. The volatile compounds were identified from the chromatogram (Figure 1) obtained from each sample by

Table 1. Sensory evaluation of odour and bitter taste and predominant volatiles in 24 *L. purpureus* accessions.

Accession	Colour	Mean odour[y] score	Mean bitter taste score	Predominant volatile(s)
10706	Brown	3.60 ± 1.30	2.42 [a] ± 1.31	Isopentyl alcohol; Methyl pentanoate; Isopentyl formate
Bahati[z]	Black	3.18 ± 1.21	2.09[ab] ± 1.01	3,7,11,15-Tetramethylhentriacontane; 5,9,13-Trimethylnonacosane; 9-Methylhentriacontane
11741	Speckled	2.78 ± 1.44	2.09[ab] ±1.18	Methyl butyrate; 6-Methyldotriacontane; n-Butylmethylether
10702	Brown	3.09 ± 1.23	2.03[abc] ± 1.16	Isopentyl alcohol; Thiazole
10695	Brown	3.31 ± 1.32	2.00[abc] ± 1.11	3,7,11,15-Tetramethylhentriacontane 3,7,11-Trimethylhentriacontane;Pentanal
13083	Black	3.56 ± 1.36	2.00[abc] ± 1.08	3,7,11-Trimethylhentriacontane; 6-Methyldotriacontane; Isopentyl alcohol
11719	Brown	3.24 ± 1.57	1.94[bc] ± 0.97	Pentanal; 11,15,19-Trimethylnonacosane; 3,11,19-Trimethylhentriacontane
8. 26932	Black	3.15 ± 1.28	1.91[bcd] ± 1.18	Thiazole; Pentanal; Methyl-Pyrazine
13086	Black	3.31 ± 1.41	1.88[bcde] ± 0.99	5,9,13-Trimethylnonacosane; 4-Methyl Thiazole; 7,11,17,21-Tetramethylhentriacontane
12158	Black	3.19 ± 1.42	1.84[bcdef] ± 0.72	Pentanal; 7,11,21-Trimethylhentriacontane; 11,15,19-Trimethylnonacosane
27007	Black	3.17 ± 1.38	1.84[bcdef] ± 1.02	3,7,11-Trimethylhentriacontane; 6-Methyldotriacontane; 13-Methylhentriacontane
11723	Black	3.25 ± 1.23	1.82[bcdef] ± 0.88	Pentanal; 4-methyl Thiazole,(E)-2-Octene; Isopentyl formate,1-Octene
28663	Speckled	3.03 ± 1.21	1.79[bcdef] ± 0.89	Tetratriacontane ; Cholest-5-en-24-ethyl-3beta-ol ; 1,2,3-Benzenetriol
11736	Brown	2.80 ± 1.32	1.78[bcdef] ± 0.79	3,7,11,15-Tetramethylhentriacontane; 5,9,13-Trimethylnonacosane, 13,17-Dimethylnonacosane
12000	Brown	2.79 ± 1.40	1.73[bcdef] ± 0.91	4-methylthiazole; 15-Methyltriacontane; 3-methyl-3-buten-1-ol acetate, allyl butyrate
Njoro[z]	Black	3.01 ± 1.39	1.73[bcdef] ± 0.88	Methyl butyrate; 6-Methyldotriacontane; 3,7,11-Trimethylhentriacontane
10703	Brown	2.90 ± 1.23	1.72[bcdef] ± 0.81	Pentanal; 3,11,19-Trimethylhentriacontane; 7-Methylhentriacontane
10822	Black	3.08 ± 1.16	1.70[bcdef] ± 0.85	Isopentyl alcohol; 6-Methyldotriacontane; 3,7,11-Trimethylhentriacontane
12230	Brown	3.18 ± 1.21	1.69[bcdef] ± 0.74	7,11,17,21-Tetramethylhentriacontane; 5,9,15-Trimethylnonacosane; 6-Methyldotriacontane
13129	Brown	3.11 ± 1.25	1.67[bcdef] ± 0.89	3,11,19-Trimethylhentriacontane; Isopentyl alcohol; 7,11,17,21-Tetramethylhentriacontane
11705	Brown	2.80 ± 1.28	1.61[cdef] ± 0.79	3,11,19-Trimethylhentriacontane; 7-Methylhentriacontane; 3,7,11-Trimethylhentriacontane
12187	Brown	2.94 ± 1.17	1.50[def] ± 0.72	3,7,11-Trimethylhentriacontane; Ortho-cresol; 6-Methyldotriacontane
11722	Light Brown	3.39 ± 1.37	1.47[ef] ± 0.72	7,11,17,21-Tetramethylhentriacontane; (E)-2-Octene; 5,9,13-Trimethylnonacosane
13096	Brown	2.68 ± 1.14	1.42[f] ± 0.67	3,7,11-Trimethylhentriacontane; 7,11,17,21-Tetramethylhentriacontane; 13-Methylhentriacontane
Mean		3.11 ± 0.22	1.82 ± 0.16	
LSD		n/s	0.05	

Means within a column followed by the same letter are not significantly ($p>0.05$) different according to the LSD test. n/s= Not significant. [y]-data; transformed by square root method {SQRT = (Y+1)}. [z]farmers selection.

comparing the peak retention (kovats) indices with those found in available literature (Adams, 1995) and online database, Pherobase (El-Sayed, 2005) as discussed by Babushok et al. (2007).

Data analysis

Analysis of variance (ANOVA) was done using general linear model (GLM) procedure using the SAS software Version 8.1 (SAS Institute, Inc., 2000) to determine the differences among the sensory characteristics and among the retention times of the volatile constituents. Means were separated using Fisher's least significant difference (LSD)

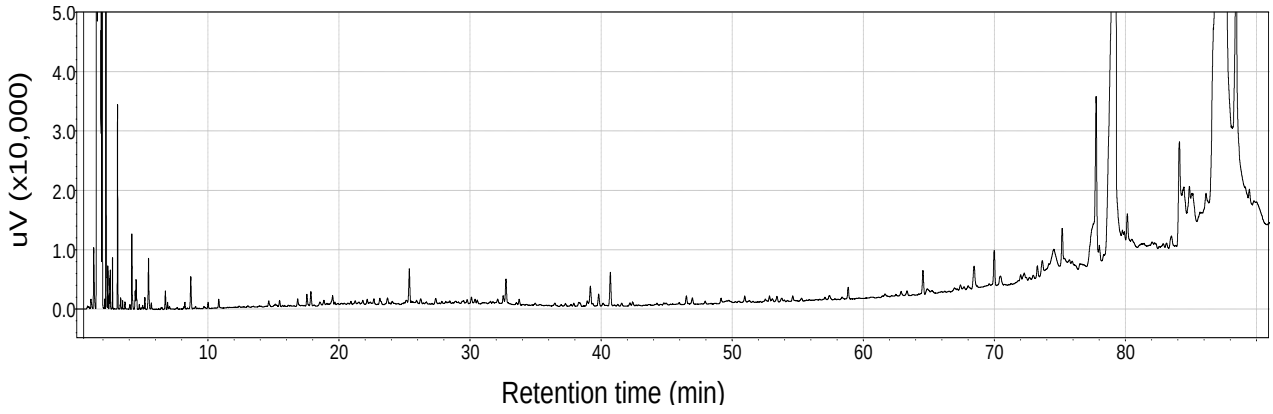

Figure 1. A gas chromatogram for *L. purpureus* seeds grown in Njoro, Kenya.

test. Correlation coefficients were determined to establish the relation between the quality variables (Bower, 2000). Pairwise similarity levels of the lablab accessions were calculated based on the retention times of the major peak areas of the volatile compounds and used to derive a dendrogram using MINITAB 11.12 statistical analysis software (MINITAB Inc, State College, Pennsylvania, USA, 1996).

RESULTS

Sensory evaluation

The odour score ranged from 2.68 to 3.60, with a mean of 3.12 while the score for bitterness ranged from 1.42 to 2.42, with a mean of 1.82 (Table 1). There was nosignificant difference ($p>0.05$) in the odour of the 24 samples, though a significant difference was observed ($p\leq0.05$) for the bitter taste parameter. Accession 10706 exhibited the highest means for both bitter and odour taste, while accession 13096 had the least means for the same attributes. Significant positive correlation was observed between odour intensity and bitter taste of the 24 *L. purpureus* accessions ($r = 0.510$, $p\leq0.05$) and an insignificant positive correlation between the colour of the seed and the odour intensity ($r=0.046$, $p>0.05$) and bitter taste ($r=0.027$, $p>0.05$). The black, brown and speckled (dark coloured seeds with black spots) coloured accessions had varying intensities (high, medium and low) of both odour and taste (Table 1), showing no relationship of the level of bitterness and odour intensity with colour of seed. Both accessions that had the highest and lowest intensity for odour and bitter taste, 10706 and 13096, were brown in colour.

Identification of volatile compounds in *L. purpureus* accessions

The volatile compounds in the assayed *L. purpureus* accessions were easily identified from the gas chromatogram. A typical chromatogram from one of the assayed accessions is presented in Figure 1. The most common volatile constituents identified were terpenes and terpenoids, and their derivatives, which accounted for 46% of all the detected odour compounds (Table 1). A total of 262 major compounds were identified from the peaks with area measurement of above 100,000 in all the accessions (Table 2). The detected compounds were separated into 12 classes, namely alcohols (28), aldehydes (10), ketones (19), esters (46), acids (7), oxygen heterocycles (1), pyrazines (5), thiazoles (4), hydrocarbons (57), terpenes and terpenoids (59), phenols (5) and miscellaneous compounds. The most common compounds with characteristic odour description included; isopentyl alcohol (associated with fusel, alcoholic, pungent, etherial, fruity odour), 3,7,11-trimethylhentriacontane, (E)-2-Octene, 7,11,17,21-tetramethylhentriacontane/7,11,17,25-tetramethylhentriacontane, 6-methyldotriacontane, norbornene, pentanol, 4-methyl thiazole (ripe, nutty, vegetable, tomato with a green tropical nuance odour), 5,9,13-trimethylnonacosane/5,9,15-trimethylnonacosane/5,9,19-trimethylnonacosane, methyl butyrate (pungent, etherial, fruity, perfumey and fusel with a fermented, cultured, creamy undernote odour), isopentyl formate (plum, dry earthy fruit green odour), 13,17-dimethylnonacosane, 13-methylhentriacontane, 9-methylhentriacontane, 7-methylhentriacontane, santene, heptanal/n-nonane, 5-methylnonacosane, 5-methylhentriacontane, 3,11,19-trimethylhentriacontane, 3,7-dimethylhentriacontane (Table 2) Some volatile compounds were unique to only one accession (Table 2). For example, 37 volatile compounds were unique to accession 28663. The following accessions also had volatile compounds that were unique to them: Bahati, 11741, 11719, 26932, 13086, 12158, 11736, 12000, Njoro, 12230, 13129, 11705, 12187, 11722 and 13096.

Table 2. Volatile compounds in *lablab* accessions (the numbers indicated in this table correlate with accession numbers in Table 1).

Compound	In accession
Methyl butyrate	2, 3, 8,9,12,15,16,22,23,24
Ethyl propanoate	1,4
Isopentyl alcohol	1-7,9,10,12,15,17-20, 23,24
Thiazole	1,4,7,8,19
Norbornene	1,2,3,4,7,8,9,12,15,19
Pentanol	1,2,4,5,8-10,,12,15,19,23,24
2,3-Butanediol	1,4,8,9,12,19
Cyclopentanol	2,3,10,15,19,20
(E)-2-Octene	2,3,7,12,22,23
Pentanal	4,7,10,12,17
n-Butylmethylether	3
4-methyl thiazole	2,5,7,9,10,12,15,19,20,22,23,24
3-methyl-3-buten-1-ol acetate	2,15,20
Canellal	2
Labd-13E-8,15-diol	2,13
trans-totarol acetate	2,13
2-methyl 3- buten-2-ol acetate	1,8
Isopentyl formate	1,4,5,6,9,10,12,18,23,24
1-Octene	1,5,7,9,10,12
Methyl pentanoate	1,4
3,4-Hexanedione	8
Methyl-Pyrazine	8
(E)-2-Octene	5,8,9,10,12,15,19,20,23,24
Isopropyl butyrate	1,4,12,15
(E)-3-Hexenol	1,8,
(Z)-3-Hexenol	1,4,8,12
2-methyl butyl acetate	1,4,12
Santene	1,4,7,9,12,19,22,23
Ethyl pentanoate	1,4,12
5-Hydroxypentanal	8
Heptanal	1,4,8,12,15,19,22,23
n-Nonane	1,4,8,12,15,19,22,23
2-Ethyl pyrazine	8,9,22
Exo-5-norbonen-2-ol	1,4,8,9
Isovaleric acid	4,9,12,15,19,23
5-Methyl-3-heptanone	4,12,15
α-Pinene oxide	14
Ethyl heptanoate	14
(z)-Ocimenone	14
Iso-acorone	14,23
Ethyl isovalerate	15
Propyl butyrate	15
Allyl butyrate	15,20
EPI- β-santalene	15
β-Acorenol	15,20
Isocomene	19,21
3-Dodecanone	19,21
(Z)-Trimenal	19,21

Table 2. Contd.

1,7-Di-EPI-β-Cedrene	19
Ethyl Vanillin	19,20,23
Neo-Mentyl lactate	19,21
γ-Gurjunene	19,22,24
β-Himachalene	19,21,22
α-Muurolene	19,21,22
Chavicol	24
Carvacrol ethyl ether	24
Sesamol	24
Cyclosativiene	24
ethyl anthranilate	24
β-Acoradiene	24
α-Chamigrene	24
(E)-β-Ocimene	23
Endo-5-norbornen-2-ol acetate	23
Dihydro citronellol	23
Citronellol	23
Dihydro-linalool acetate	23,24
Citronellyl formate	23,24
Valeranone	23
Epi-longipinanol	19,21
(Z)-Anethole	20
Butyrophenone	20
Isopulegol acetate	20
(Z)-Jasmone	20
Isobornyl n-butyrate	20
Neo-menthyl acetate	21,23,24
n-Dodecane	21,22,23
Octanol acetate	21,22,23,24
Carvenone	21,23
Piperitone	21,23
Undec-10-en-1-al	21,23
Geranyl formate	21
n-Nonanol acetate	21
Sesamol	21
cis-2,3-Pinanediol	21
Methyl Geranate	21
Peperonal	21
Benzyl butyrate	21
(Z)-α-damascone	21
γ- Nonalactone	21
Trans-2,3-pinanediol	21
Furfuryl hexanoate	21
(Z)-ethyl cinnamate	21
β-Maaliene	21
1-phenyl-4-methyl-pentan-3-one	21
α-Cedrene	21
(E)-β-damascone	21
α-Gurjunene	21
Para-menth-1-en-9-ol acetate	21
(E)-Isoeugenol	21,24
Geranyl acetone	21,22,24

Table 2. Contd.

β-Thujaplicin	21
Citronellyl isobutyrate	21
Lavandulyl isovalerate	21
(Z)-Cinnamaldehyde	22
Cis-Sabinene hydrate acetate	22
Neo-iso-dihydro carveol	22
Cis-Carveol	22
Nordavanone	22
cis-Ascaridole	22
(E)-Ocimenone	22
Neral	22
Trans-sabinene hydrate acetate	22
Ambersage	22
Dec-9-en-1-ol	22
Trans-carvone oxide	22
Carvacrol, ethyl ether	22
Iso-3-thujyl acetate	22
(E,E)-2,4-decadienal	22,24
Dimethoxy-(Z)-Citral	22
Methyl geranate	22
Piperonal	22
Citronellyl acetate	22
α-Cyclogeraniol	22
Cyclosativene	22
3,4-Dihydro-coumarin	22
(E)-β-DAMASCENONE	22
Daucene	22
β-Patchoulene	22
(Z)-Cinnamyl acetate	22,24
1,7-di-Epi-α-cedrene	22,24
Iso-italicene	22
1,7-di-Epi-β-cedrene	22
α-Himachalene	22
Laciniata furanone H	22
Spathulenol	22
α-Acorenol	22
(E)-dihydro apofarnesal	21
6-Methyl-α-(E)-ionone	21
γ- Dehydo-AR-himachalene	21
β-Vetivenene	21
α-Cadinene	21
n-Tridecanol	21
Carotol	21
5-Cedranone	21
(Z)-3-Hexenyl phenyl acetate	21
Khusimol	21
(6E,10E,14E,18Z)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	21
1-EPI-Cubenol	20
Caryophyllene alcohol	19,21,24
(E)-Sesquilavandulol	19,24
6-Methyl-6-(3-methylphenyl)-heptan-2-one	19,21,22,23,24

Table 2. Contd.

3-Iso-Thujopsanone	19,21,22,23,24
β-Eudesmol acetate	19,21
β-Bisabolenol	19,21
Dodecanoic acid, butylester	19,21
Cyclopentadecanolide	19,21
3-Methylheptacosane	8
3,15-Dimethylheptacosane	8
Pentacosanoic acid	8,15
7-Methyltriacontane	17,19
5-Methylnonacosane	1,4,5,6,7,8,12,15
9-Methylnonacosane	15
13-Methylnonacosane	16
15-Methylnonacosane	16
Hentriacontane	23
3,11,19-Trimethylhentriacontane	4,7,8,9,10,17,20,21
3,7,11-Trimethylhentriacontane	1,4,5,6,9,10,11,12,16,18,20,21,22,24
5,9,13-Trimethylnonacosane	1,2,5,9,12,14,15,16,18,19,21,23
5,9,15-Trimethylnonacosane	1,2,5,9,12,14,15,16,18,19,21,23
5,9,19-Trimethylnonacosane	1,2,5,9,12,14,15,16,18,19,21,23
7,11,21-Trimethylhentriacontane	10,12,19
7,11,25-Trimethylhentriacontane	10,12,19
9,13,19-Trimethylnonacosane	7,8,10,19
9,15,19-Trimethylnonacosane	7,8,10,19
11,15,19-Trimethylnonacosane	7,8,10,19
3-Methylhentriacontane	9,16
5-Methylhentriacontane	6,7,9,11,14,15,16,18
7-Methylhentriacontane	5,6,8,9,17,19,21,22,24
9-Methylhentriacontane	2,4,7,10,11,14,15,16,18
13-Methylhentriacontane	9,11,14,16,17,18,21,22,24
15-Methylhentriacontane	22
15-Methyltriacontane	1,15,22
11-Methylhentriacontane	1,12
3,7-Dimethylhentriacontane	1,2,12,14,20,21,22
7,11-Dimethylhentriacontane	6,16,19
7,11,17,21-Tetramethylhentriacontane	1,4,5,6,9,11,12,16,18,19,20,23,24
7,11,17,25-Tetramethylhentriacontane	1,4,5,6,9,11,12,16,18,19,20,23,24
6-Methyldotriacontane	1,2,3,6,11,12,15,16,18,19,20,22,23
3,7,11,15-Tetramethylhentriacontane	24,5,7,9,10,14,15,17,20,21
3,15-Dimethylhentriacontane	9
13,17-Dimethylnonacosane	2,6,9,14,15,19,21,22,23,24
12,16-Dimethyloctacosane	3,8,
5,17-Dimethylnonacosane	3
3,7-Dimethylhentriacontane	3,7,9,10
9,21-Dimethylhentriacontane	5,6,7,9,16,19
Cholest-5-en-24-methyl-3beta-ol	6,8,18
Tritriacontane	7
14-Methyloctacosane	8
1,3,5-Benzenetriol	9
5beta-cholestan-3alpha-ol	10
Cholest-5-en-3beta-ol	16
Oroselone	13
Isoincensole acetate	13

Table 2. Contd.

(Z)-9-Octadecenoic acid	13
Intermedine	13
n-docosane	13
Phylloclandnol	13
(Z,Z,Z)-3,6,9-Tricosatriene	13
(2S,12S)-2,12-Diacetoxyheptadecane	13
Incensole oxide	13
Isopimarol	13
Trans-Totarol	13
Trans-14-Isopropylpodocarpa-8,11,13-trien-13-ol	13
Palustrol	13
Trans-Ferruginol	13
3-α-14,15-dihydro-Manool Oxide	13
4-EPI-Abietol	13
7-Methyltricosane	13
5-Methyltricosane	13
dehydroabietol	13
Cis-Ferruginol	13
3-Methyltricosane	13
Methyl nidoresedate	13
Methyl Strictate	13
Abietol	13
n-Tetracosane	13
Integerrimine	13
cis-Ferruginol acetate	13
Heneicosanoic acid	13
11-Methyltetracosane	13
Neo-Abietol	13
1,2,3-Benzenetriol	13
Jacobine	13
Docosenoic acid	13
n-Pentacosane	13
Docosanoic acid	13
Tetratriacontane	13
Cholest-5-en-24-ethyl-3beta-ol	13
Tricyclene	22
4,5-dimethyl-Thiazole	22
β-Citronellene	22
3-Methyl valeric acid	22
2,6-Dimethyl-2-heptanol	22
2,4,5-Trimethyl thiazole	22
n-Decane	22
2,3,5-Trimethyl pyrazine	22
2-Acetyl-pyrazine	22
Propyl tiglate	22
Lavender lactone	22
Ortho-cresol	22
Artemisia ketone	22
γ-Terpinene	22
Cis-vertocitral	22
2-Acetyl-2-methyl pyrazine	22
Ortho-Guaiacol	22

Table 2. Contd.

Para-mentha-2,4 (8)-diene	22
Maltol	22
Ipsdienol	22
Veratrole	22
Iso-isopulegol	22
β-Pinene oxide	22
Meta-cresol acetate	22
Menthol	22
cis-Pinocamphone	22
para-Cymen-8-ol	22
cis-Pinocarveol	22
3-Decanone	22
(Z)-3-Hexenyl butyrate	22
Myrtenol	22

Identification references: [a] = Adams (1995) and [b] = The pherobase (El-Sayed, 2005).

The volatile constituents identified were significantly different among accessions ($p \leq 0.05$). The level of similarity of the accessions based on the volatile profiles was derived from the retention times, and an average similarity coefficient of 89% was observed using MINITAB 11.12 statistical software (MINITAB Inc, State College, Pennsylvania, USA, 1996). Accession 11741, was the most distinct and isolated in the dendrogram derived from the similarity coefficients (Figure 2). The other accessions showed a similarity of up to 94.9%. Accessions 10695, 10706, 27007, 13096, 11705, Njoro, 10702, 26932, 10703 and 11736 formed a tight clade in the dendrogram and had the highest similarities (Figure 2).

DISCUSSION

The utilization of the lablab bean for human consumption has been hampered by anti-nutritional factors that have impact on bean flavour besides the undesirable food colouring effect of the black variety (Waldmueller, 1992; Wanjekeche et al., 2000). In this study, it was established that the assayed lablab accessions had different bitter taste levels, with some being bitterer than others, similar to results obtained by Wanjekeche et al. (2000). Studies have shown that lablab accessions are highly diverse in most agro-morphological traits with diversity being greater in South East Asia than in Africa (Maass et al., 2010; Ravinaik et al., 2015). Based on the scores for bitter taste, the following accessions are recommended for inclusion in a rationalised breeding programme; 13096, 11722, 12187 and 11705. This study also established that the colour of the seeds did not have any relationship with the bitter taste and odour of the bean, with the accessions with highest and lowest bitter taste scores having a brown colour. This was contrary to findings from other studies that had shown a close relationship between intensity of seed colour and odour (Khah and Arvanitoyannis, 2003). This study however revealed a relationship between taste and odour ($r=0.8$). The correlation between the two quality parameters in this study is not unexpected and may be explained by the fact that both contribute to food flavour (Nursten, 1977). However, it should be noted that the correlation is not very strong, and further analysis on more accessions may need to be carried out to confirm the relationship.

Though the volatile profiles of the assayed accessions were different, there was a high level of similarity between accessions indicating a narrow genetic base for the lablab germplasm in Kenya. Analysis of molecular diversity of a larger gene pool of lablab accessions including those in this study had also revealed low genetic diversity of the accessions (Kimani et al., 2012). These findings suggest that any crop improvement efforts in Kenya are likely to be compromised by the low genetic diversity of progenitors in hybridization programmes and meaningful genetic gains can only be realised through deliberate germplasm enrichment programmes. Germplasm can be introduced into Kenya from South East Asia and other parts of Africa where some of the wild and domesticated lablab landraces have been established to be genetically diverse (Maass et al., 2005; Tefera, 2006). Some wild and undomesticated lablab can still be found to occur naturally in several African countries and these can be a rich source of genes for crop improvement (Verdcourt, 1979).

Data from the present study revealed that the volatile compounds identified in assayed accessions were slightly different from those identified in other studies. The compounds identified that were common in this study and that of Kim and Chung (2008) on lablab include pentanal, geranylacetone, heptanal, pentanol and (Z)-3-hexenol.

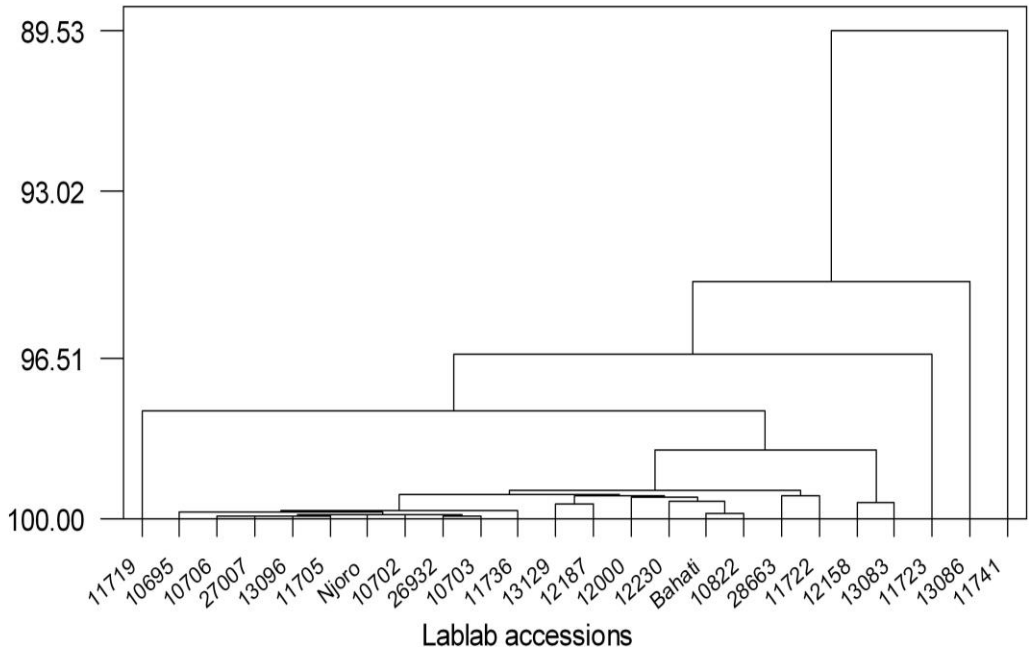

Figure 2. Similarity dendrogram of 24 *L. purpureus* accessions based on analysis of the volatile compounds on a gas chromatogram.

Differences in volatile compounds between the two studies may be due to the use of different extraction methodologies, growing conditions and the genetic differences of the accessions in the two studies. The compound 2,3,5-trimethylpyrazine that was also identified in the Kenyan lablab accessions is thought to be the characteristic compound in the fermented bean aroma (Seo et al., 1996) and has also been identified in soypaste (Zhang et al., 2010). (Z)-3-Hexenol was identified in French beans (Hinterholzer et al., 1998) using gas chromatography/olfactometry (GC/O). Among the compounds identified in this study, 2,3-butanediol, heptanal, pentanol and pentanal were found to be present in the volatile isolate of kidney beans and soybean (van Ruth et al., 2004, 2005) isolated in a model mouth system and sampling of the headspace (van Ruth and Roozen, 2000). The presence of the odd numbered long alkanes identified in this study unlike in the other studies is due to the fact that the alkanes have poor volatility in steam (Radulovic et al., 2006) used in extraction methods. Some of the n-alkanes identified in this study such as n-docosane, hentriacontane, triacontane and tetracontane have also been identified in vanilla beans (Ramaroson-Raonizafinimanana et al., 1997). Pentanal, geranylacetone, heptanal, n-nonane, n-decane and n-docecane were also extracted and identified from dry beans (*Phaseolus vulgaris*), isolated by headspace solid phase microextraction (HS-SPME) (Oomah et al., 2007). Worth noting in this study is the accessions 11741 and 11719 that stood out distinctly in the dendrogram. These two accessions were most preferred by farmers, and ranked high in taste in a study carried out on sensory and cooking quality of lablab (Shivachi et al., 2012). The predominant volatile compounds in the two accessions are however different, indicating that their preference is attributable to different volatiles.

Apart from the volatile compounds assayed in this study, there are other major biochemical components of beans that would affect flavour but which were not evaluated here. These include lipids, carbohydrates, proteins, saponins, minerals and phenolic acids. Further, it should be noted that less than 5% of all the volatiles identified in foods have actually been found to contribute to the odour (Grosch, 2000). The compounds identified in this study are therefore not exhaustive and the actual volatiles that influence the characteristic odour of *lablab* may be identified by more sensitive quantitative measurements and more systematic sensory experiments of a large sample of lablab beans grown in different environments. The analysis carried out in this study is however important in the identification of the major volatiles that need to be assayed in more detailed studies. This is important because the flavour produced by the volatiles, which contribute to odour, may either be desirable or undesirable to consumers who are not familiar with the lablab. Volatiles associated with desirable flavour should be identified and positively

selected for, while those associated with non-desirable flavour should be selected against.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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