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Original Article

BANANA PEEL OXALATE OXIDASE-DETECTION, PURIFICATION, CHARACTERIZATION AND PHYSIOLOGICAL ROLE

SHADMA ANJUM[**], SHANTHY SUNDARAM[*], G. K. RAI. [**]

[*]Centre for Biotechnology, University of Allahabad, Allahabad, [**]Centre of Food Technology, University of Allahabad, Allahabad.
Email: shanthy_s@rediffmail.com

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ABSTRACT

Objective: Enzymes like oxalate oxidase (EC 1.2.3.4) and superoxide dismutase (EC 1.15.1.1) from germin family are known to generate active oxygen species. In the mammalian system, excess accumulations of oxalate causes kidney stones. Oxalate oxidase, an H_2O_2-generating enzyme, used for detection of oxalate. The aim of the present work is to screen out the activity of enzymes from all three stages of banana (Musa paradisica L. Variety "Bhusawal") peel and to isolate, purified and characterized oxalate oxidase from this. With that describe the physiological role of both oxalate oxidase and superoxide dismutase in the plant.

Methods: Oxalate oxidase activity can be detected directly in SDS-PAGE gel. Purification was done by using ion-exchange chromatography and SDS-PAGE Gel.

Results: Highest activity 5.99+0.021 unit /mg of oxalate oxidase were detected in leaky ripe stage of banana peel after purification. In crude extract of unripe banana peel activity of superoxide dismutase were found high (2.41unit/mg) compared to oxalate oxidase (0.269+ 0.020 unit/mg). Their occurence in different ripening stage of banana peel shows its role in plant defense mechanism.

Conclusion: The purified enzyme of oxalate oxidase from banana peel is useful in the determination of oxalate content in common food, which is necessary for the prescription of the low oxalate diet for a patient with urinary and kidney stone where as superoxide dismutase work against ageing.

Keywords: *Musa paradisica*, Oxalate oxidase, Oxalate and overripe fruit, Germin like protein.

INTRODUCTION

Banana Peel (Musa paradisica: local name Bhusawal Keli) is extensively grown and exported in eastern Utter Pradesh like Kaushambhi [1]. According to Food and Agriculture Organization statistic's database, India is the biggest producer of banana and mango and second largest producer of lime, in the world. In fruit production, U. P ranks fifth among all states[2]. Banana is a fruit, other than mango and guava, extensively grown in Utter Pradesh. Kauhshambi is the leading state in U. P, where the production of banana is high. The banana is highly perishable fruits so the processing of fruit and its part, is the major issue of the researcher. The main by-product of banana processing industry is peeled, which represents almost 30% of fruit [3]. However, no attention is being paid to the banana peel, which is unfortunate because they have medicinal properties due to the presence of various polyphenol and bioactive molecules [3, 4]. The waste part of a banana peel is also a potential source of oxalate degradation. Active oxygen species play significant roles in many plant processes. A number of enzymes are known to generate active oxygen species (Elstner et al., 1994) and one of those are oxalate oxidase (oxalate: oxygen oxidoreductase, EC 1.2.3.4) and superoxide dismutase, both belong to the same germ in family, a H_2O_2-generating enzyme, used for clinical detection [5]. Enzyme and gene expression data suggest that both oxalate oxidase and germin like protein genes, and their encoded proteins play roles in plant defense responses [6]. Oxalate oxidase in banana peel was first suggested by Richardson [7]. Production of hydrogen peroxide during the oxidation of oxalate by an oxalate oxidase destroys fungal toxins and microbes, serving as a defense mechanism and also worked in peroxide catalyzed cross linking reactions and strengthening the cell walls [8]. Dahiya (9) reported oxalate oxidase in strawberry fruit. Hu and Guo [10] purified and characterized oxalate oxidase from wheat seedling. Kanauchi [11] suggested that enzyme activity was increased during germination in malt. Determination of oxalate in different food matrices is of great interest because a high oxalate concentration level in a foodstuff may cause the formation of insoluble kidney stones as a result of unbalanced nutrition habits. Current analytical methods for determination of oxalate in the food industry are time consuming, often require highly trained staff and need relatively expensive instrumentation [12]. One approach is to degrade oxalate by enzymatic method utilizing a low-cost source of, an oxalate oxidase enzyme. Banana peel could serve the purpose. During the ripening of the banana peel, proteins denoted germins are expressed in the later stages of ripening because germin expression is so closely linked with the onset of germination in cereal seedling [13]. Dietray hyperoxaluria manifests as a sequence to frequent intake of oxalate rich food like spinach, lettuce, amaranthus, kulfa, beet, banana, tea, cocoa, beer, almond and cashew. These increase risk of kidney stone formation. The above problem originates due to oxalate accumulation and could be reduced by degrding the oxalate level in the human body [14].

Our most- recent study evaluated the activity of germin like protein (oxalate oxidase and superoxide dismutase activity) and describes some characteristics of a cell wall bound oxalate oxidase in banana peel in different stages of ripening. (Musa paradisica L. variety "Bhusawal keli"). Due to cost effective approach, this work is the primary step for the commercialization of oxalate oxidase from the natural resources. In the present study, we have purified and characterized a soluble oxalate oxidase from banana peel and studied its role in fruit ripening. Thus, the proper utilization of peel will provide employment to the people of eastern U. P. by exploiting it for its use in commercial purpose in the food industry, medicinal and pharmaceutical field.

MATERIAL AND METHODS

Chemicals and Reagents

Acetone, Folin Denis reagent, Tricholroacetic acid and thiobarbituric acid, N-N dimethylaniline,3-methyl-2-benzothiozolonone hydrazone, horseradish peroxidase, oxalic acid, $CuSO_4$ (Sigma Chemical Co, Delhi, U. P, India), acrylamide, bis acrylamide, ammonium persulfate, molecular weight marker, silver stain were used were purchased from Science Corporation (Allahabad, U. P, India) and Milli-Q quality distilled water were used.

Sample collection and extraction

Banana (*Musa paradisica*: var. Bhusawal Keli) fruits were purchased from the local market of Kaushambi, Uttar Pradesh, India, at different stages of ripening without any ethylene and stored at 20°C for 24hr before being extracted.

Proteins were extracted from the banana peel at three different stages of ripening. Stage I, Pre-ripening (Green, hard fruit), Stage II, ripening (yellow, some softening), Stage III, late-ripening (yellow-brown, very soft), which were previously ground.

Preparation of membrane bound Enzyme

Membrane-bound enzyme was prepared according to Lathika et al. [15] using an extraction buffer, containing 0.02 M HEPES pH7.9 at 1:11 ratio (w: v) in a chilled mortar and pestle. The resulting enzyme was treated as membrane-bound germ in like protein. The sample was tested for enzyme activity and protein.

Solubilization of membrane bound oxalate oxidase

Membrane-bound enzyme was solubilized according to Lathika et al. [15]. Lysis buffer containing Triton X- 100 and 0.01 M Tris HCl (pH 7.4) was added to the suspension and kept on ice for half an hour. Immediately, 1 mM PMSF was added to the supernatant to degrade the activity of polyphenol and others biomolecule. The homogenate was centrifuged at 15,000×g for 20 min at 40C. Supernatant was collected and tested for activity and protein. Both supernatant and pellet were collected, and the pellet was re-suspended in a minimum quantity of extraction medium and stored at 4°C until use. Proteins were quantified using Lowry's method [16]. After centrifugation at 10,000×*g* for 20 min, the supernatants corresponding to 25 µl was loaded with 50% glycerol and dye on a 12% native PAGE gel.

Assay of oxalate oxidase

Oxalate oxidase activity of from the supernatant and the pellet was detected using 3-methyl-2-benzothiazolinone hydrazone hydrochloride hydrate (MBTH), N, N-dimethylaniline (DMA) and horseradish peroxidase and oxalate (substrate). The resulting indamines dye was determined at 595 nm. The reaction was carried out in a test tube wrapped in black paper. Hydrogen peroxide generated was extrapolated from a standard curve. It is prepared in different concentration [17, 18].

Unit of enzyme

One unit of enzyme is defined as the amount of enzymes required to generate 1 nmol H_2O_2/min under standard assay conditions.

Assay for Superoxide dismutase: NBT reduction system

SOD activity was assayed using the nitroblue tetrazolium (NBT) method [19]. Superoxide dismutase activity of banana peel tissue extracts in 70% acetone, water, ethyl acetate, chloroform and hexane were determined by photochemical reduction of NBT, according to Giannopolitis and Ries [20] with slight modifications, using the assay system consisting of methionine, riboflavin and NBT. NBT was reduced to the blue formazan by O_2^-, which has a strong absorbance at 560 nm. However, the presence of SOD inhibits this reaction. Two sets of each sample were used. The reaction mixture consists of 1.3µM riboflavin, 13 mm methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2) and the appropriate volume of extract. Distilled H_2O was added to bring in the final volume of 3 ml. The test tubes were inverted twice. One set of the reaction tube was covered with a black cloth as a control. The other set was placed approximately 30 cm below a blank of two 15-W fluorescent lamps. The reaction was initiated by turning the light on for 10 min. Following light exposure, the tubes were covered with a black cloth to prevent further reaction. Illuminated mixtures lacking enzyme developed maximum color, while the non-illuminated mixture did not develop color and was used as control. The absorbances of the above mixtures were taken, using a spectrophotometer at 560 nm. From the graph; the volume of enzyme extracts corresponding to 50% inhibition of the reaction was calculated and considered as one enzyme unit. Protein concentration was determined according to Bradford using bovine serum albumin as a standard [16]. SOD activity was expressed as enzyme Unit /gm fresh wt. of banana peel.

Purification of oxalate oxidase

All steps for the purification of solubilized enzyme oxalate oxidase were carried out at 4°C which are as follows:

Step I ammonium sulfate (0- 35%) precipitation

Solid ammonium sulphate was added to solubilized enzyme to get 0–35% saturation according to the method [21]. The solution was stirred and centrifuged at 10,000×*g* for 20 min. The pellet was separated from the supernatant and dissolved in 15 ml 0.02 M HEPES buffer, pH 7.0, and dialyzed against the same buffer (pH 7.0, 0.01 M) at 4°C for 24 h under constant stirring through a dialysis tube [22]. The resulting solution was considered as sample P_1.

Step II ammonium sulfate (35-80%) precipitation

The supernatant was used further for complete precipitation of solubilized protein. Ammonium sulphate was added to the supernatant to get final 35–80% saturation. Supernatant was discarded, and the pellet was dissolved in the same buffer after dialysis the resulting solution was considered as Sample P2.

Step III DEAE Sephadex ion exchange chromatography

The dialyzed enzymes were loaded on to CM Sephadex C-50 (weak cation exchange) column (1.5×100 cm) previously equilibrated with 0.05 M HEPES buffer pH 7.0. The column was eluted in the same buffer at the flow rate of 0.5 ml/min [21, 23]. Fraction was collected and monitored for protein and oxalate oxidase activity. The bound proteins were eluted by a linear gradient of NaCl (0-0.9 mM) in 0.05 M HEPES buffer pH 7.0 and 3 ml were collected from the flow rate of 0.5 ml/min and again tested for protein and oxalate oxidase activity. The active fractions were pooled, and their specific activity was calculated.

Step IV SDS PAGE gel filtrations

Purification by SDS-PAGE was done according to the method [23, 24] with slight modification. SDS-PAGE used not only for evaluating purity, but also as an active step in the purification process. The first step in purifying proteins from polyacrylamide gels is to locate the electro phoresed protein of interest in the gel. For that stain a side strip of the gel and then aligns that strip of gel with the unstained gel portion and cut out the band of gel that aligns with the stained protein of interest in the reference strip. Bands of gel just above and below the region presumed to contain the protein of interest may also be excised and processed [24].

Elution of protein from gel matrix

Placed excised gel pieces in clean screw-cap culture or micro centrifuge tubes and add 0.5-1 ml of elution buffer (0.1 M Tris-HCl, 0.2 M NaCl, and 0.1 mM EDTA; pH 7.5) so that the gel pieces are completely immersed. Crushed the gel pieces using a clean pestle and incubate in a rotary shaker at 30°C overnight. Centrifugation was done at 5,000-10,000 × g for 10 minutes and carefully pipettes supernatant into a new micro centrifuge tube [24]. An aliquot of the supernatant may be tested for the presence of oxalate oxidase activity by subjecting it to SDS-PAGE and native PAGE.

Characterization of Enzyme

SDS-polyacrylamide gel electrophoresis

SDS-PAGE electrophoresis was used for protein profiling of banana peel at three different SDS-PAGE electrophoresis was used for protein profiling of banana peel at three different stages of ripening. The samples were subjected to SDS-PAGE using a 12.0% uniform gel. Electrophoresis was carried out as described by Laemmli (24) with the following modifications [21]. The samples were added to 6× sample buffers containing SDS. Glycerol, 2-mercaptoethanol and Bromophenol Blue in 0.5 M Tris-HCl buffer (pH 6.8) and then heated at 100°C for 3 min before adding 25 µL of each mixture per well. The gels were run at 100 volt at a gel thickness of 1.5 mm. Molecular weight standards were from Bio- Rad Laboratories, Inc (Gurgaon, Haryana, India). The gel was stained with silver stain.

After isolation of oxalate oxidase, crude purification of the enzyme was carried out at 4°C using solid ammonium sulphate which was

added to the crude enzyme to give a final 0-80% saturation. The resulting solution was further centrifuged at 10,000 × g for 30 min. The pellet was dissolved in distilled water, dialyzed against 0. 01M potassium phosphate buffer.

Native PAGE of oxalate oxidase

Oxalate oxidase activity in semi-native SDS-PAGE gel was detected by immersing the gel in a cocktail of oxalic acid, 0.5 mg ml^{-1} 3-3 diaminobenzidine and 5 Unit/ml horseradish peroxidase in 50 mM citrate, pH 4.0, containing 40% ethanol [8]. Enzyme activity was visualized after 45 min at room temperature. A negative control, to rule out oxalate oxidase activity, omitted oxalate from the reaction cocktail [24].

RESULTS AND DISCUSSION

Banana peels in different stages of ripening were analyzed for the enzymatic activities which have a beneficial effect upon human health. Fruit ripening has been described as oxidative phenomena [4], which require a turnover of active species such as H2O2 and superoxide anion. Superoxide dismutase and peroxides may play a protecting role against the oxidative damage [27].

Absolute protein values obtained from unripe, ripe and leaky ripe peel samples expressed in g/25g fresh weights are 0.201, 0.324 and 0.422 respectively. The soluble component of banana peel contains enzymatic activity after being analyzed by both types of assay mixtures. Assays were performed using two types of assay mixtures, the first containing the conventional substrate oxalate (+), and the second prepared for the absence of oxalate (–). The oxalate oxidase activity measured using (–) oxalate assay mixture was not as expected. It was due to the presence of interfering species in the crude extract of banana.

Peel which may interact with the dye precursor and/or horseradish peroxidase causing a change in intensity of the dye within the assay mixture. Due to the absence of oxalate in (-) oxalate assay mixture. The following activities were oxidase activity rather than oxalate oxidase activity. In crude extracts of leaky ripe sample, the activities of oxalate oxidase were highest (0.724+0.027 unit/mg), whereas in a partially purified sample of it, the specific activity was 5.99+ 0.021 unit/mg. Fig. 1 (b) shows the superoxide dismutase activity in the extracts of the banana peel at three different stages of ripening, and the highest activity was found in the unripe sample. It is 0.875+0.038unit/mg, shown in fig. 1(b). The oxalate oxidase activity increased approximately three times in the purified sample comparative to the crude extract measured after performing ammonium sulfate fractionation, whereas reduction in the oxidase activity was also observed after undertaking ammonium sulfate fractionation using the – oxalate assay mixture. This decrease was likely to be due to the removal of possible interfering species present in the extract prior to ammonium sulfate fractionation. The precipitate obtained from 80% saturation with ammonium sulfate was dissolved in water and dialyzed.

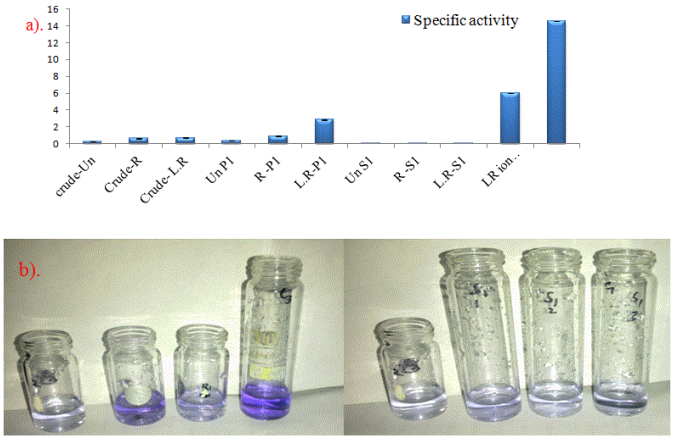

Fig. 1: Specific activity (unit/mg) of enzyme oxalate oxidase in crude and partially purified sample of unripe, ripe and leaky ripe banana peel samples (b) Biochemical evaluation of oxalate oxidase in crude extracts of banana peel by MBTH assay at different stage of ripening

UV, visible scanning by spectroscopic methods of banana peel extract

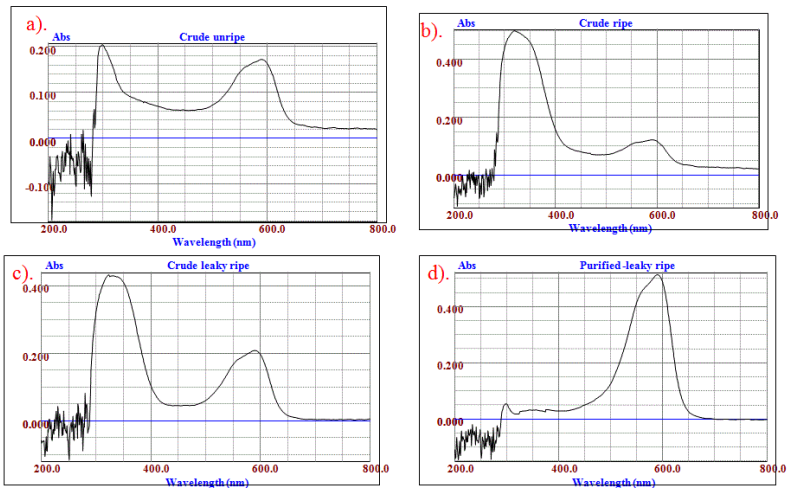

Fig. 2: UV visible scanning of oxalate oxidase activity (a) Crude unripe, (b) Ripe, (c) Crude leaky ripe and (d) Purified leaky ripe samples of banana peel homogenate obtained after 80% ammonium sulfate fractionation and compared with 10-100 mM H_2O_2 at 590 nm*

Oxalate oxidase activities by MBTH assay were examined by a spectrophotometer at 590 nm. In fig. 2 a, 2 b and 2 c different peaks were detected in the crude extracts of banana peel, where as a single peak was identified in a purified fraction obtained from the extract of leaky ripe banana peel in the region of 590 nm* as shown in fig. 2 (d). Peak at 590 nm determined the liberation of H_2O_2 when compared with standard 10-100 mM H2O2. Oxalate oxidase, an H_2O_2-generating enzyme showed the highest activity in the purified sample of leaky ripe (P-2 leaky ripe) banana peel.

Analysis of Crude and Purified Enzyme Preparation by anion Exchange and SDS-Polyacrylamide Gel Electrophoresis

The purification of the dialyzed sample of oxalate oxidase after the ammonium sulfate fractionation resulted in the elution of nearly 8.27 fold purified sample of oxalate oxidase applied at the column, and yield was approximately 19.19% as shown in fig. 3 (a). Further the oxalate oxidase active fractions eluted from the cation exchange column were pooled and purified using SDS gel purification technique. SDS-PAGE as an analytical tool with excellent ability to resolve individual components of complex mixtures, SDS-PAGE may be used not only for evaluating the purity but also as an active step in the purification process. The desired component (oxalate oxidase) obtained after SDS purification was 20.14 fold purified than the components in crude extracts after performing MBTH assay, whereas the yield was approximately 3.3%, which was quite less. We got two protein peaks; only last peak had enzyme activity. Prior to purification of banana peel oxalate oxidase by anion exchange chromatography (DEAE, cellulose) the stability of the banana peel extracts in sodium chloride was investigated as showed in fig. 3 (b).

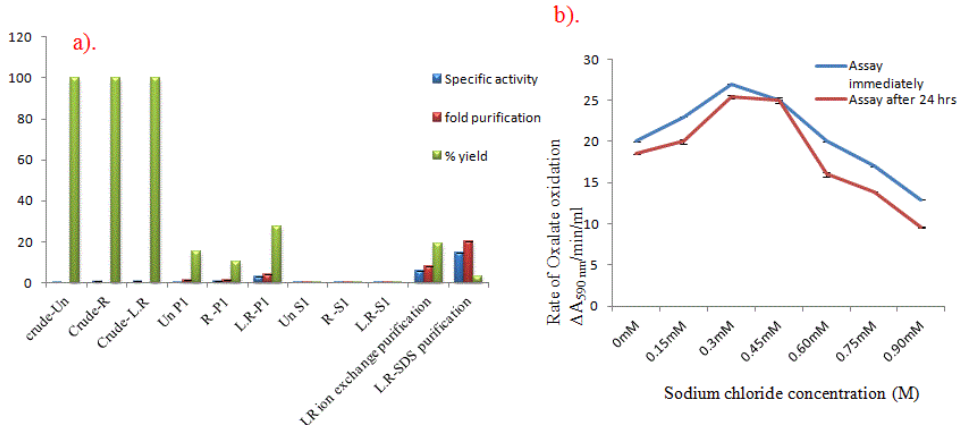

Fig. 3: Determination of (a) Specific activity, Purification factor and % yield of enzyme oxalate oxidase in crude and purified samples of banana peel (b) effect of sodium cholride on to the activity of oxalate oixdase in assays mixture using MBTH assays at A590 nm* in triplicate. Error bars are a repesentation of standard deviation, N=3

The stability studies were performed according to which the activity of banana peel oxalate oxidase remained unchanged after the immediate addition of up to 0.40 mM sodium chloride at 4°C. The components eluting prior to the sodium chloride gradient did not possess oxalate oxidase activity. Fig. 4 illustrates the chromatograms results obtained after the purification of above enzyme from banana peel using anion exchange chromatography. Two distinctive peaks eluted during the sodium chloride gradient; at about 0.1 M and 0.3 M., The fractions assayed identified oxalate oxidase activity within the first peak eluting at approximately 0.1 M sodium chloride, similar to the findings obtained by Requena and Bornemann [29].

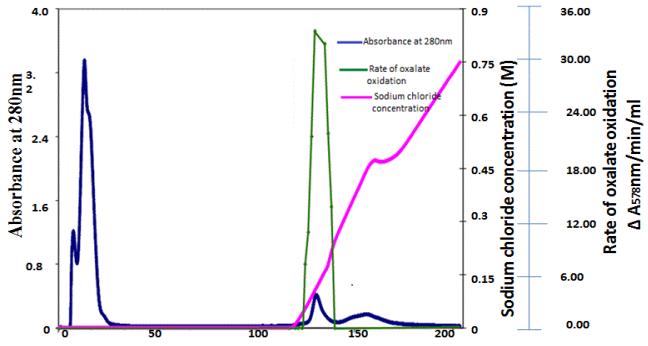

Fig. 4: Anion exchange chromatography of oxalate oxidase. ASF concentarted oxalate oxidase was purified using CM cellulose, cation exchange media and eluted with a 0 to 0.9M sodium chloride gradient. Protein content at A280 nm* was monitered spectrophometric method. All fractions eluted (5 ml) were assayed using the MBTH assay at 590 nm* in triplicate

Characterization of Molecular Mass of oxalate oxidase by SDS and Native Page gel

To examine the effect of ripening on the enzymatic activity and the accumulation of banana proteins, fractions from each purification step were subjected to SDS-PAGE analysis. Several protein bands with different R_f values indicate the heterogenicity of enzyme preparation, when crude and purified sample of banana peel were subjected to electrophoresis. SDS PAGE resolved proteins between 14 kDa and 98 kDa and led to the visualization of crude proteins of unripe, ripe and leaky ripe in lane 2, 3, 4 with molecular standards only as showed in fig. 5 whereas in lane 8, 9 and 10 separation of partially purified proteins were seen.

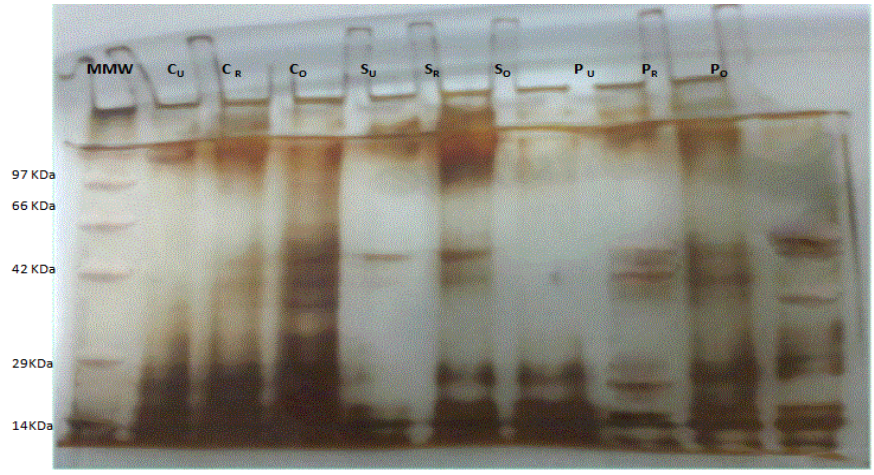

Fig. 5: Protein profiling of crude and 80% ammonium sulfate saturated fractions of unripe, ripe and leaky ripe samples by Sodium dodecyl sulfate polyacrylamide gel electrophoresis using a 12% uniform gel. MMW: Molecular weight marker; C_u: crude unripe protein; C_R: Crude ripe protein; C_O: Crude leakyripe protein; S_u: Unripe supernatant; S_R: Ripe supernatant; S_O: Leakyripe supernatant; P_u: Precipitated unripe protein; P_R: Precipitated ripe protein; P_O: Prcipitated leakyripe protein

The enzymatic staining of the gel in the oxalate oxidase gel developer solution revealed the localization of oxalate oxidase appeared as a brown band in gel as shown in fig. 6 (a). Oxalate oxidase activities were not detected in unripe banana peel, whereas in the leaky ripe sample, positive band appears with molecular weight 90-96 KDa, whereas in fig. 6 (b) the clear band of purified oxalate oxidase with a molecular mass of between 30-35 kDa were detected in lane 3 and 4. The purified enzyme exhibited a single band both on PAGE and SDS-PAGE with a molecular weight range from 90- 96 KDa and 32 - 35 kDa, respectively indicating its three identical subunits. The enzyme showed optimum activity within 15 min* when incubated at pH 4.5 in 0.01 M sodium succinate buffers at 35°C.

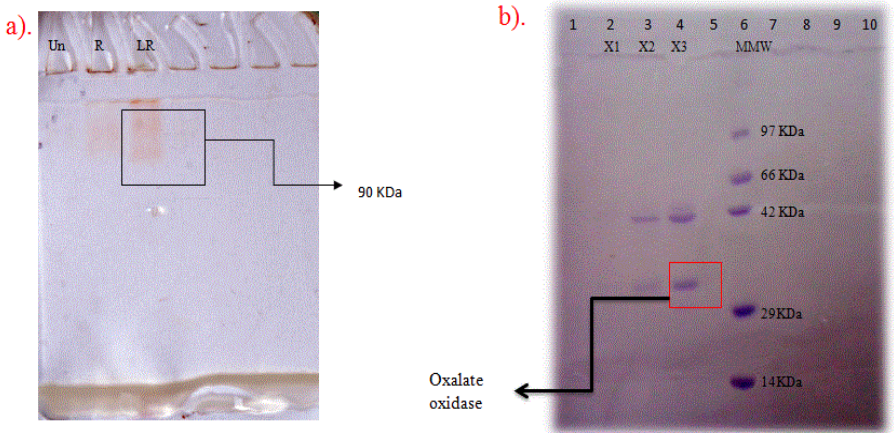

Fig. 6: Native PAGE of oxalate oxidase by active staining on gel using oxalate oxidase gel developer. Zhang. Z., Yang. L., et al (1996). Lane 1: unripe sample; Lane 2: Ripe sample; Lane 3: leakyripe sample (b) SDS PAGE analysis of oxalate oxidase from leakyripe banana peels. Lane 2: Crude leakyripe banana peel sample; Lane 3: Ammonium sulate fractionates of leaky ripe banana peel sample; Lane4: Purified sample of oxalate oxidase from leaky ripe samples

As the ripening proceeds, many changes were observed. Low molecular weight polypeptides (28, 30, and 42 kDa) increased from the immature stage to the leaky ripe stage, whereas in lane 8, 9 and 10, the 30 to 42 kDa polypeptide appeared to reach its highest concentration in a leaky stage of a banana peel as shown in fig. 5. These results were in good agreement with previously reported data [29, 30] due to de novo protein synthesis enzymatic activity in banana peel increases as the ripening proceeds, whereas according to some other reports some polypeptides were prominent in unripe fruit, decreased with ripening of banana peel. Most of the protein remains same in the stages. However, there are significant changes in the relative concentration of some polypeptides was observed. Thus, during ripening the concentration of soluble protein was increased, but another decreased. The results indicate that there is a differential protein accumulation during banana ripening, and some specific proteins in leaky ripe fruit have been detected due to the oxidative stress.

Properties of oxalate oxidase

Optimal pH: For the determination of desirable pH of enzyme, 0.1 M Succinate, citrate and phosphate buffer was used with pH ranges 3.5- 8.5 as shown in fig. 7 (a), (b) and (c). In leaky ripe banana peel oxalate oxidase exhibited optimal (89 % Relative activity) activity in a succinate buffer at pH 4.5 whereas, unripe sample exhibit two different peaks.

Optimum temperature and time

The enzyme exhibits maximum activity at 25-35^{0}C as shown in fig. 8 (a) and (b). Purified sample of oxalate oxidase from banana peel exhibited the highest activity when incubated at 35^{0}C for 20 min. The heat stability study shows that the enzyme is stable to heat after purification, whereas an increase in temperature is inversely proportional to oxalate oxidase activities in a crude sample of the banana peel.

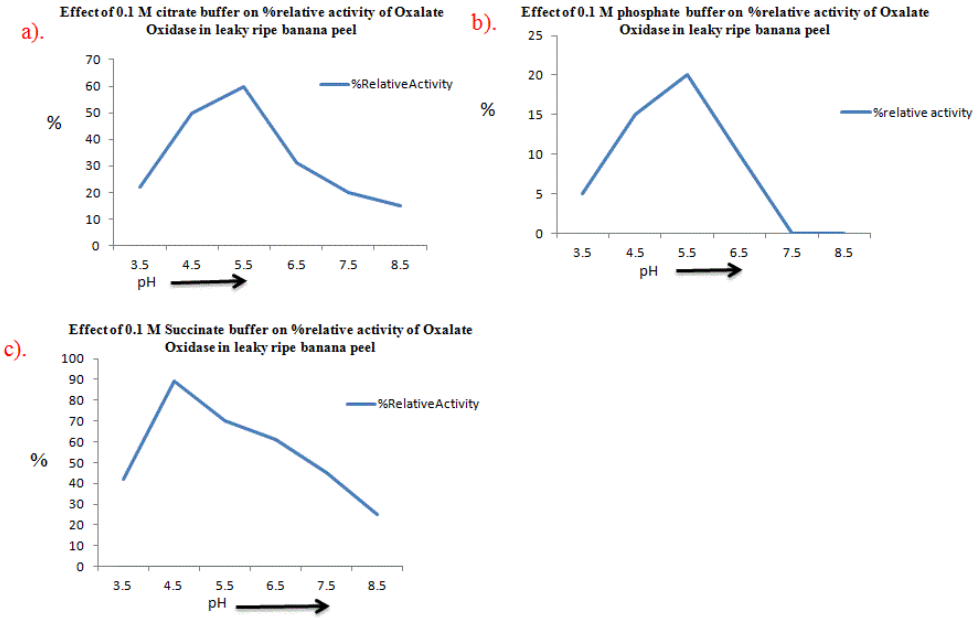

Fig. 7: Effect of (a) 0.1 M Citrate buffer (b) 0.1 M phosphate buffer 0.1 M Succcinate buffer from pH range 3.5 to 8.5 on the % reactive activity of oxalate oxidase in leaky ripe banana peel samples

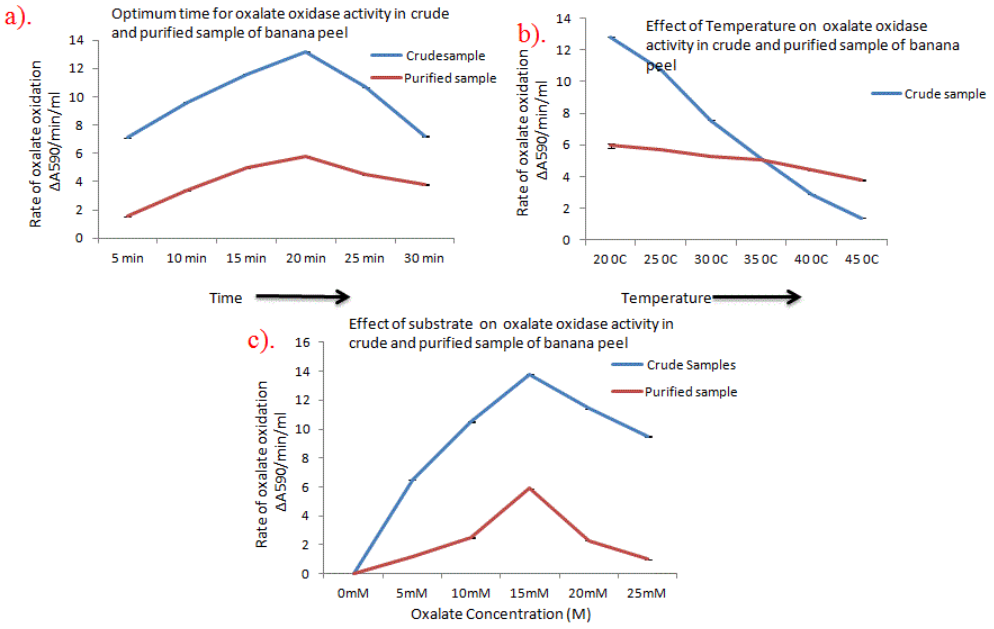

Fig. 8: (a) Determination of oxalate oxidase activity in crude and purified samples of banana peel at different time intervals. (b) Temperature variations and (c) substrate concentrations on the activity of oxalate oxidase in crude and purified samples of banana peels. The data are displayed with mean+standard deviation (bars) of three

Optimum substrate concentration

Increase in absorbance was proportional to the concentration of oxalate up to 15 mM. At 15 mM concentrations of substrate, the oxalate oxidase activity in the crude sample was 13.78+ 0.015 unit/ml whereas in a purified sample the activity was 5.89+ 0.01 unit/ml as showed in fig. 8 (c)

Physiological role of oxalate oxidase and its correlation with oxalate and superoxide dismutase

Oxalate oxidase exhibit characteristics of germ in like protein (GLP). GLP proteins are found primarily in cell wall fractions [6]. Thus the occurrence of oxalate oxidase activity and superoxide dismutase activity in banana peel tissue strongly supports that GLP transcripts are detected mainly in epidermal tissues. Fig. 9 (a) and (b) vigorously support the activity of superoxide dismutase in banana peel in early stage of ripening.

Fig. 9 (a) shows the negative staining of superoxide dismutase. Intense band in 1st and 2nd lane strongly support the activity of superoxide dismutase in an unripe and ripe banana peel sample whereas in fig. 9(b) appearance of brown band in lane 2nd and 3rd vigorously supports the activity of superoxide dismutase positive stain.

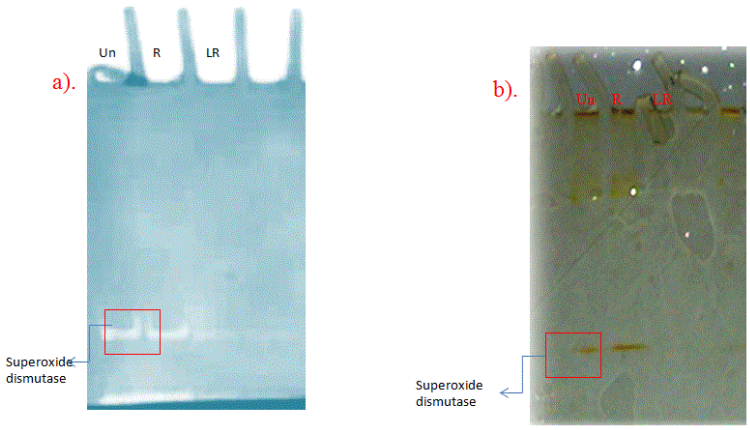

Fig. 9: Native staining of SOD on gel using gel developer according to method Gucciardo, S., *et al.*(2007). Lane 1: Unripe banana peel sample; Lane 2: ripe banana peel sample; Lane 3: Leakyripe banana peel sample. (b) Positive staining of SOD on gel using gel developer according to method Gucciardo, S., *et al.*(2007). Lane 2:unripe banana peel sample; Lane3: Ripe banana peel sample; Lane4: Leakyripe banana peel sample

In crude extract of banana peel significant activities of oxalate oxidase were detected within the range of 0.269+ 0.020 to 0.724+0.027unit/mg (unripe to leaky ripe), whereas the activities of superoxide dismutase were recorded 2.41+0.185 to 1.88+0.091 unit/mg. Superoxide dismutase converts superoxide anions into H2O2. One germ in like protein from barley does not possess oxalate oxidase or superoxide dismutas activity. However, in banana peel, both germin like protein were detected. Oxalate oxidase was detected in later stage of ripening whereas the superoxide dismutase shows activity in early stage of ripening. Enzyme and gene expression data suggest that both oxalate oxidase and germ in like protein genes play roles in plant defense responses fig. 10 (a) shows that effect of ripening and their correlation with oxalate and oxalate oxidase activity.

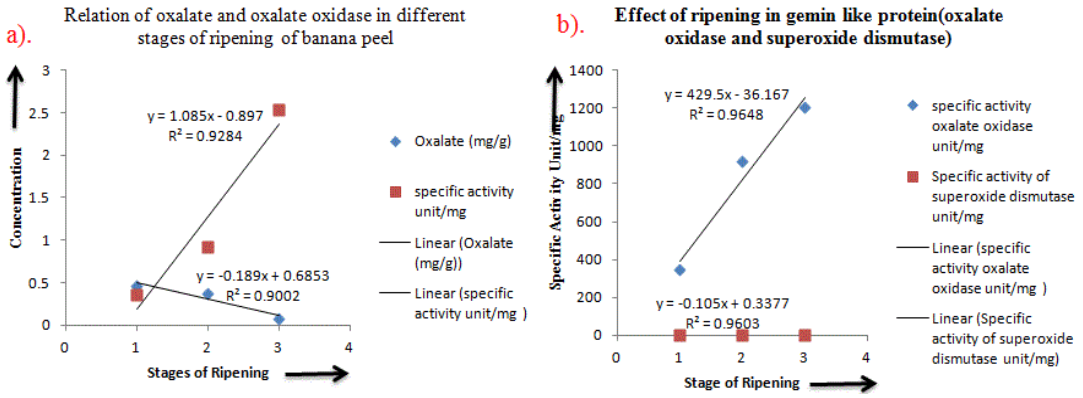

Fig. 10: Correlation between (a) Oxalate content and oxalate oxidase activity (b) Oxalate oxidase activity and superoxide dismutase activity in banana peel at different stage of ripening

Both the oxalate and oxalate oxidase are strongly correlated as the ripening proceeds the activity of an oxalate oxidase increase in positive direction whereas oxalate content decrease. The decrease in anti nutritional factors like oxalate content (-0.872 correlation with oxalate oxidase) and tannin content (-0.999 correlation with oxalate oxidase) with advancement of ripening indicated the physiological role of oxalate oxidase in fruit ripening as shown in table 1, whereas highest correlations were existed between oxalate oxidase and protein content (R=0.999). The correlations between oxalate oxidase activity and superoxide dismutase activity in banana peel were negatively correlated, and it was nearly around R= -0.999 as showed in fig. 10 (b). Thus the occurrence of oxalate oxidase activity and superoxide dismutase activity in peel tissue of banana strongly supports that germ in like protein transcripts are detected mainly in epidermal tissues and native and recombinant germin like proteins are found primarily in cell wall fractions [5].

CONCLUSION

From the above investigation, we are concluded that the waste part of the banana peel is a potential source for oxalate oxidase. The nutritional property and enzymatic activity (superoxide dismutase and oxalate oxidase "germin protein) of banana peel can be exploited as a health supplement, and it uses in medicinal and clinical industry. Besides that oxalate oxidase and GLP (germin like protein) genes play important roles in general defense against a wide variety of pathogens; they are desirable for utilization in a crop improvement program.

Production of hydrogen peroxide during the oxidation of oxalate by oxalate oxidase and free radical (O^{2}-) production by superoxide dismutase destroys fungal toxins and Microbes, serving as a defense mechanism and in strengthening the cell walls.

Thus, by managing these wastes, we will overcome problems related to environment and health hazards. Banana peel is a cheapest source material for the isolation of oxalate oxidase. It thereby properly immobilized the enzyme, we can use it in pharma and food industry.

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ABBREVATION

U. P: Utter Pradesh, W: V: Weight/volume, H_2O_2: hydrogen peroxide, SOD: Superoxide dismutase, SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis, EDTA: Ethylene diaminetetraacetic acid

CONFLICT OF INTERESTS

Declared None

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