

EVALUATION OF PEROXIDASE ACTIVITY IN *BETA VULGARIS* L., *CUCUMIS SATIVUS* L. AND *RAPHANUS SATIVUS* L.

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Abstract

Peroxidase contents were evaluated in three vegetables i.e., *Beta vulgaris* L., *Cucumis sativus* L. and *Raphanus sativus* L. using different extraction strategies. Different types of buffers of pH 5.2, 6.0 and 7.0 were used and compared with crude extracts. The results showed a significant difference among three vegetables as regards the presence of protein contents and peroxidase after the statistical analysis. *B. vulgaris* showed better peroxidase activity in extracts without buffer (17.69 U/ml/hr) as compared to different buffers i.e., at pH 5.2 (4.93 U/ml/hr), at pH 6.0 (3.23 U/ml/hr) and at pH 7.0 (3.03 U/ml/hr). Enzyme activity in extract of *C. sativus* without buffer was 0.92 U/ml/hr. Whereas, at pH 5.2 it was 0.57 U/ml/hr, at pH 6.0 observed as 7.63 U/ml/hr and at pH 7.0 turned out to be 50.9 U/ml/hr. Peroxidase activity in extract of *R. sativus* without buffer, was 6.20 U/ml/hr. Observations for peroxidase activity at pH 5.2, 6.0 and 7.0 turned out to be 1.32 U/ml/hr, 14.33 U/ml/hr and 17.17 U/ml/hr, respectively. Specific enzyme activity observed in extracts of *B. vulgaris* L. without buffer was 12.47, at pH 5.2 was 12.68, at pH 6.0 was 4.51 and at pH 7.0 was 1.54. Specific enzyme activities in extracts of *C. sativus* turned out to be 1.80 for extraction without buffer, 4.97 at pH 5.2, 17.41 at pH 6.0 and 37.07 at pH 7.0. Extracts of *R. sativus* L. showed specific enzyme activities as 7.50 (without buffer), 9.62 (at pH 5.2), 10.08 (at pH 6.0) and 22.37 (at pH 7.0). The protein contents of *B. vulgaris* in different extracts were 0.064 mg/ml (without buffer), 0.019 mg/ml (at pH 5.2), 0.035 mg/ml (at pH 6.0) and 0.083 mg/ml (at pH 7.0). The protein contents in extracts of *C. sativus* were observed to be 0.030 mg/ml (without buffer), 0.005 mg/ml (at pH 5.2), 0.010 mg/ml (at pH 6.0) and 0.069 mg/ml (at pH 7.0). The protein contents in extracts of *R. sativus* were observed to be 0.041 mg/ml, 0.007 mg/ml, 0.070 mg/ml and 0.038 mg/ml for without buffer, at pH 5.2, at pH 6.0 and at pH 7.0, respectively. Results for enzyme activity, specific activity and protein contents for the three selected vegetables under study revealed statistically significant observations in crude extracts for *Beta vulgaris*, whereas, in *Cucumis sativus* and *Raphanus sativus* the peroxidase activity was higher in buffer of pH 7.0. However, protein contents were higher for the *Beta vulgaris* (0.064 mg/ml) as compared to other vegetables studied in this work.

Introduction

The peroxidases (E.C.I.II.1.7) are a large family of ubiquitous isozymes that catalyze a wide range of substrates mainly H₂O₂, reactive oxygen species and many organic metabolites (Laidler, 1954; Reed, 1975). These enzymes along with catalases, superoxide dismutase and glutathione reductases etc. have antioxidant properties and act as oxidoreductase (Reed, 1975). When these enzymes fail to remove reactive oxygen species, consequent free radical assembly causes severe cellular damages (Ferguson and Dunning, 1986). Superoxide dismutase (SOD) removes the toxicity of the O₂ producing H₂O₂ as a byproduct which is also toxic and need to be removed. Reaction is characterized by the coupled-series of oxidation-reduction reaction which involves the ASC3 and glutathione which are catalyzed by the enzymes ASC peroxidase, DHA reductase and glutathione reductase. Same kinds of reaction occur in the chloroplasts (Grodén and Beck, 1979; Nakano and Asada, 1981).

Generally in the redox reactions catalyzed by peroxidases, the hydrogen peroxide acts as the electron acceptor to liberate oxygen from many kinds of substrates (Brill, 1996). The electron donor is often reduced cytochrome-c in plants, which at first reacts with H₂O₂. Whereas, in horseradish the peroxidases reduce the peroxide by utilizing the electrons derived from cytochrome-c which are transported from various intramolecular sites. However, one of the two reducing electrons originates from compactly bound haem group, derived by each of the peroxidase. In horseradish

peroxidase the electron is derived from the porphyrin ring of the haem group (Dolphin *et al.*, 1971).

Peroxidases are the enzymes which are the most heat stable than other enzymes present in plants and do not show thermal inactivation (El-Gendy and Marth, 1981). The isoenzymes varied in their heat stability: the anionic isoenzymes were found to be more heat stable than the cationic isoenzyme (Moulding *et al.*, 1988). Peroxidase activities are also related to lignification in plants (Siegel, 1956; Harkin and Obst, 1973).

Fresh vegetables are a rich source of valuable contents such as minerals, vitamins (such as C, E, A), phytochemicals (such as folates, glucosinolates, carotenoids, flavonoids, phenolic acids, lycopene), selenium and dietary fibers (Jiménez-Monreal, *et al.*, 2009). Several organizations such as Europe Food Safety Authority (EFSA), Food and Agriculture Organization (FAO), United States Department of Agriculture (USDA), World Health Organization (WHO) etc., recommend increased fruit and vegetable utilization in diet (Allende *et al.*, 2006). The reason is that these offer defense against cardiovascular diseases, cancer, cataract and muscular degeneration (Del Caro *et al.*, 2004) due to their important constituents among which antioxidant enzymes play a vital role.

Peroxidase applications are very wide in the field of health sciences as a diagnostic tool. A large number of peroxidases are used in the techniques such as ELISA (Enzyme Linked Immuno-Sorbant Assay), where POD forms antispecies-antibody-enzyme conjugate due to high sensitivity, high turnover rate and rapid availability (Zia *et al.*, 2001). Peroxidases are also used as tracer macromolecules in the

biochemical, biomedical and cytological analyses where the pathway can be detected by histochemical analysis in the organisms. It has wide applications in the food industry (Reed, 1975).

So far in Pakistan, many attempts have been made by many workers to isolate and characterize food source peroxidases from tomato (Malana, 2000), turnip, radish, horse radish roots, horse radish legume (Rehman *et al.*, 1999), soy bean (Habib *et al.*, 2003) etc. However local production of the enzyme is negligible and our commercial requirements are fulfilled by importing the enzyme for canned food industry or research institutes for experimental work. Thus, there is a strong need to produce and purify enzymes to meet our local demands (Habib *et al.*, 2003). Vegetables are the inexpensive source of POD. So this source must be explored for the commercial production of POD (Rehman *et al.*, 1999). However, the recovery of the enzyme depends on the strategy followed and the pH of the extraction buffers.

Hence, the main objective of present study was to calculate the enzyme activity in common salad vegetables in order to find out the best and cheapest source for POD to meet the local demands and to work out the best extraction procedure.

Materials and Methods

Present study was planned and conducted to estimate the activity of peroxidase (POD) enzyme in three locally available vegetables i.e., cucumber (*Cucumis sativus* L.), radish (*Raphanus sativus* L.) and beet root (*Beta vulgaris* L.) that were purchased from the local market. Each time new and fresh vegetables were purchased instead of growing any particular variety, firstly to make the sample random in order to draw general conclusions and also to ensure best activity of POD and its purification.

Preparation of reagents

Bradford reagent: Dissolved 100mg of Coomassie blue (G-250) in 50mL of 90% ethanol. Then 100mL of 85% H_3PO_4 was added and raised the volume up to 1L with distilled water. Reagent was filtered through Whatman filter paper No.1 and stored in an amber color reagent bottle and was used within one month.

Phosphate Buffer (pH 7.0): For the preparation of 1L phosphate buffer following solutions were prepared:

Solution A: Dissolved 136.09g of KH_2PO_4 in distilled water to make 1L of solution.

Solution B: Dissolved 174.08g of K_2HPO_4 in distilled water to make 1L of solution.

Mixed 615.0ml of Solution A and 385.0ml of Solution B to get 1L phosphate buffer of pH 7.0. Adjusted pH using pH meter.

1% Dianisidine dye (stock solution): 1% solution of o-dianisidine was prepared in methanol.

Dianisidine Buffer Mixture: Added 1ml of the dianisidine stock solution to 99ml of the phosphate buffer to obtain 100ml mixture.

1% H_2O_2 solution: Mixed 1ml H_2O_2 with 99ml distilled water. This was 1% H_2O_2 solution.

SDS reagents

Acrylamide gel: 29.2g Acrylamide and 0.8g Bis-acrylamide were mixed in approximately 60-70ml double distilled water, while gently stirring. Then the final volume of the mixture was made up to 100ml to make 30% acrylamide stock solution.

Loading sample: Enzyme sample (20 μ L) and sample buffer (10 μ L) were mixed together and used as loading sample.

Reservoir buffer: Approximately 250-300ml double distilled water was taken in a beaker and 15.142g Tris salt was added to it. Then 3.8ml glycine was mixed in the solution to find a 0.192M glycine concentration, followed by 5.025ml of 10% SDS stock solution. The pH of buffer was adjusted at 8.3 with concentrated HCl to make reservoir buffer stock solution.

Sample buffer: In 5.0ml stacking buffer, 0.2g SDS was added to find a 0.2% concentration. Then 0.05ml β -mercaptoethanol (0.5%) and glycerol (0.6ml, approximately 1/10th of the total volume) were mixed in it and finally 0.005g (0.05%) bromophenol blue dye was added. This was used as sample buffer.

Separating buffer: In approximately 70-80ml distilled water, 4.43g Tris salt was added and stirred gently, followed by 4.0ml of 10% SDS solution to find 1.5M separating buffer. The pH was adjusted at 8.8 with the help of concentrated HCl.

Stacking buffer: Tris salt (30.285g) was dissolved in approximately 80-90ml double distilled water. 10ml of 10% SDS solution was mixed in order to obtain 0.4% SDS concentration. The pH of the solution was adjusted at 6.8 by adding concentrated HCl drop wise while continuous stirring the solution. After that final volume was raised up to 250ml with double distilled water to obtain 0.5M stacking buffer with pH 6.8.

Staining solution: Coomassie brilliant blue G-250 (1.25g) was dissolved in 500ml de-staining solution to obtain staining solution.

De-staining solution: Acetic acid (70ml) and methanol (300ml) were mixed and final volume of this mixture was raised to 1000ml with distilled water.

Extraction of the enzyme: Vegetables were peeled off and completely crushed using mortar pestle and grinder etc. This mashing was done in 10ml phosphate buffer using pH: 5.2, 6.0 and 7.0. When the vegetables were completely crushed, they were subjected to press filtration. For this purpose a linen cloth was used. The extract/juice of each vegetable was obtained in labeled test tubes in order to allow no mixing of the samples.

The samples were then centrifuged at 6000rpm for 10 minutes. The supernatant was obtained for every vegetable and the pellet was discarded. Samples were then stored at -20°C.

Analytical methods

Protein estimation: Protein in each vegetable extract was estimated according to the method of Bradford (1976). For this purpose 2.5ml of Bradford reagent was added to a test tube with 0.1ml of the vegetable extract sample. The absorbance of appeared blue colour was taken at 595nm on a spectrophotometer. A blank was also run parallel by replacing enzyme extract with 0.1ml of distilled water. Protein concentration in the sample was estimated from the standard curve of bovine serum albumin (BSA).

Peroxidase assay: Peroxidase test was performed to check the activity of peroxidase in different vegetables. The rate of decomposition of hydrogen peroxide catalyzed by peroxidase, with o-Dianisidine dye as hydrogen donor, is determined by the increase in absorbance at 432nm. For this test 1% H₂O₂ and o-dianisidine was prepared. The reaction mixture was prepared by taking two test tubes for each of the extracted sample. Added 1.5ml of 1% H₂O₂ and 1.4ml of prepared solution of o-dianisidine in buffer. Maintained temperature of the mixture at 35°C using an incubator. To one of this mixture added 0.1ml of the boiled sample. This is the reference mixture. To the other test tube added 0.1ml of un-boiled sample. Similar procedure done for every vegetable sample and incubated the test tubes at 35°C for 10min.

After 10min checked absorbance at 432nm using spectrophotometer. Adjusted the wavelength at 432nm, number of cycles as 5, and time of each cycle as 1min on the spectrophotometer. Added the boiled sample to the cuvette and placed in the spectrophotometer and pressed zero. This was the reference of the sample. Then removed the reference from the cuvette and added the unboiled sample. Took 5 readings for absorbance, after every 1 minute. The increase in absorbance was measured as E_{432nm/min}. The amount of enzyme that decomposes 1 micromole of hydrogen peroxide per minute at pH 7 and 25°C. Formula applied to calculate the activity of enzyme:

$$\text{Activity U/ml} = \frac{(E_{432\text{nm/min}}) (\text{Total Vol.}) (\text{Enz. Diln.})}{(6.58) (\text{Enz Vol.}) (\text{mg Prot/ ml})}$$

SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used to separate proteins according to their electrophoretic mobility. The SDS gel electrophoresis of samples having identical charge per unit mass due to binding of SDS results in fractionation by size and is probably the world's most widely used biochemical method for purification (Laemmli, 1970).

For this, gel plates were cleaned, dried and assembled. For 12.5% resolving gel (1.5ml separating buffer, 2.5ml 30% acrylamide solution, 1.93ml distilled water, 60µl 0.1% ammonium per sulphate and 6.0µl TEMED). After polymerization (45min), the top gel was

washed with distilled water. Stacking gel (1.0ml stacking buffer, 0.52ml 30% acrylamide, 0.44ml distilled water, 2.0ml 40% sucrose, 40µl 0.1% ammonium-per-sulphate and 4.0µl TEMED) was poured and immediately a comb was inserted for the preparation of wells and allowed to polymerize the gel. Comb was removed from the mould and wells were given three washings with distilled water. Gel was mounted in the electrophoresis apparatus and the running buffer was added at the top and bottom reservoirs of the apparatus. Loading samples (vegetables + loading dye) along with marker proteins were loaded in the wells of the stacking gel and voltage was applied 60V in the start. Then as the samples flow out of the stacking gel the voltage was increased to 110V. As the samples flow out in the running buffer, the power supply was turned off, sandwich plates from the apparatus were removed and gel is carefully collected and immersed in the staining solution for 30 minutes. After staining added destaining solution and removed it 2-3 times. Then allowed the gel to retain in the destaining solution over-night. Bands appear in the gel. This gel was placed on the filter paper and allowed it to dry at 80°C temperature under vacuum pressure. Image of the dried gel was obtained with the help of gel documentation system. Compared results with that of the ladder.

Parameters of the enzyme activity: Estimation of the enzyme using different parameters was done.

Estimation of enzyme activity in crude extract: Crude extract of the vegetables were evaluated for the enzyme activity in order to estimate the innate enzyme status.

Estimation of specific activity in crude extract: Enzyme activities of crude extracts of the vegetables were used to calculate their respective specific activities.

Effect of pH on enzyme activity: Buffers of different pH (5.2, 6.0, 7.0) were used to estimate their effect on enzyme activity. For the purpose, o-dianisidine dye was mixed with buffers of different pH (5.2, 6.0, 7.0) and the enzyme activity was estimated by the Peroxidase assay.

Effect of pH on specific activity: Enzyme activities of buffers extracts of different pH (5.2, 6.0, 7.0) were used to estimate their respective specific activities.

Evaluation of the vegetables for enzyme activity: Vegetables under study will be evaluated for maximum enzyme activity at different pH levels.

Evaluation of the vegetables for specific activity: Specific activities of vegetables under study at different pH levels will be calculated from their respective enzyme activities.

SDS-PAGE analysis: The three vegetables were evaluated for differential bands on the gel after running SDS-PAGE and then preserving and viewing the gel by gel documentation system.

Statistical analysis: Treatment effects were compared by the method of Snedecor and Cochran (1980). Significance has been presented as Duncan's multiple range test in the form of probability (P) values.

Table 1a. Mean squares from analysis of variance (ANOVA) of data for protein contents in extracts of different vegetables (n = 3).

Source of variation	Df	B ₀	B ₁	B ₂	B ₃
Main effects					
Vegetable	2	8.693**	1.754*	0.002***	0.001***
Error	6	6.855	1.866	9.356	3.444

***, **, * = Significant at 0.05, 0.01 and 0.001 levels, respectively
ns = Non-significant

Table 1b. Evaluation of vegetables for protein contents (Mean ± S.E.; n=3).

Vegetable	Protein contents (mg/ml)			
	B ₀	B ₁	B ₂	B ₃
<i>Beta vulgaris</i> L.	0.064±0.01 ^a	0.019±0.002 ^a	0.035±0.005 ^b	0.083±0.000 ^a
<i>Cucumis sativus</i> L.	0.030±0.004 ^b	0.005±0.005 ^b	0.010±0.015 ^c	0.069±0.002 ^b
<i>Raphanus sativus</i> L.	0.041±0.002 ^b	0.007±0.004 ^b	0.070±0.003 ^a	0.038±0.002 ^c
LSD (α=0.05)	0.016	0.008	0.019	0.003

Table 2a. Mean squares from analysis of variance (ANOVA) of data for Peroxidase activity in extracts of three vegetables (n = 3).

Source of variation	Df	B ₀	B ₁	B ₂	B ₃
Main effects					
Vegetable	2	220.733***	16.292**	93.732 ^{ns}	1814.450***
Error	6	6.790	0.743	35.090	10.370

, * = Significant at 0.01 and 0.001 levels, respectively
ns = Non-significant

Table 2b. Comparison of peroxidase activities in extracts of vegetables (Mean±S.E.; n=3).

Vegetable	Peroxidase activity (Units/ml/hr)			
	B ₀	B ₁	B ₂	B ₃
<i>Beta vulgaris</i> L.	17.69±4.34 ^a	4.93±1.10 ^a	3.23±1.96 ^a	03.03±1.62 ^c
<i>Cucumis sativus</i> L.	0.92±0.79 ^c	0.57±0.23 ^b	7.63±2.83 ^a	50.90±3.29 ^b
<i>Raphanus sativus</i> L.	06.20±0.92 ^b	1.32±0.98 ^b	14.33±9.67 ^a	17.17±4.20 ^a
LSD (α=0.05)	5.206	1.722	11.835	6.432

Table 3a. Mean squares from analysis of variance (ANOVA) of data for specific activity in extracts of three vegetables (n = 3).

Source of variation	df	B ₀	B ₁	B ₂	B ₃
Main effects					
Vegetable	2	85.572***	45.292 ^{ns}	125.500 ^{ns}	955.879***
Error	6	3.130	628.727	28.251	15.536

*** = Significant at 0.001 level
ns = Non-significant

Table 3b. Comparison of specific activities in extracts of vegetables (Mean ± S.E.; n=3).

Vegetable	Specific activity			
	B ₀	B ₁	B ₂	B ₃
<i>Beta vulgaris</i> L.	12.47±2.63 ^a	12.68±4.21 ^a	4.51±2.61 ^b	1.54±1.40 ^c
<i>Cucumis sativus</i> L.	01.80±1.10 ^c	4.97±6.02 ^a	17.41±5.72 ^a	37.07±1.82 ^a
<i>Raphanus sativus</i> L.	07.50±1.13 ^b	9.62±7.69 ^a	10.08±6.73 ^{ab}	22.37±6.42 ^b
LSD (α=0.05)	03.534	10.780	10.619	07.874

Results

Evaluation of vegetables for protein contents: Analysis of variance showed significant results for all the vegetables under study (Table 1a). When the vegetable means were compared at crude extract B₀ and buffer extract at pH 5.2, results of *C. sativus* and *R. sativus* were at par with each other while mean values for *B. vulgaris* (0.064 mg/ml and 0.019 mg/ml) were comparably higher (Table 1b). At buffer extract of pH 6.0 maximum protein contents were revealed by *R. sativus* whereas at buffer extract of pH 7.0 maximum protein contents were showed by *B. vulgaris*. Overall protein contents were maximum in *B. vulgaris* as compared to the other two vegetables under study.

Evaluation of vegetables for peroxidase activity: Variation was observed in the results of analysis of variance for POD activities and the three vegetables under study produced significant effect in all extracts except at buffer extract of pH 6.0 (Table 2a). The POD activities in extracts of *B. vulgaris* were higher, in all the buffers, than those for *R. sativus* and *C. sativus* except at buffer extract of pH 7.0 where as *R. sativus* showed maximum POD activity (Table 2b).

Evaluation of vegetables for specific activity: Specific peroxidase activity was high significant for the buffer extract of pH 5.2 (12.47) and buffer extract of pH 6.0 (12.68) of *Beta vulgaris* (Table 3a), for buffer extract of pH 6.0 and buffer extract of pH 7.0 of *Cucumis sativus*. The specific activity for the *Raphanus sativus* was also considerable after *Beta vulgaris*. Except buffer extract of pH 7.0 and crude extract of *Beta vulgaris* and *Cucumis sativus* all value were significant (Table 3b).

Estimation of protein contents in crude extracts of vegetables: Results of protein contents (Table 4) were significant for all the selected vegetables. But the results were highly significant for the *Beta vulgaris* (0.064 mg/ml). The protein contents of the *Cucumis sativus* were less than the other two vegetables.

Estimation of peroxidase activity in crude extracts of vegetables: The activity ANOVA results showed high significance for the *Beta vulgaris* (17.69 U/ml/hr). Whereas, *Cucumis sativus* showed significant results except *Raphanus sativus* (Table 4).

Estimation of specific activity in crude extracts of vegetables: The results of ANOVA showed that specific activity was significant for the *Beta vulgaris* and *Raphanus sativus* except *Cucumis sativus* showing values 12.47, 1.80 and 7.50 respectively (Table 4).

Effect of pH on protein contents: Analyses of variance revealed a variable pattern for protein contents at different pH levels for vegetables under study. The results for pH factor for *B. vulgaris* were non-significant, whereas for *C. sativus* and *R. sativus* results were significant at 0.01 and 0.001 levels, respectively.

Comparison of means suggested that there was no statistical difference in protein contents at all pH levels of buffers for *B. vulgaris*. However, the data for other two vegetables showed considerable variation. The protein values at pH 7.0 for *R. sativus* were greater (0.050 mg/ml) whereas, at pH 6.0 (B_2), *C. sativus* showed higher protein contents (0.070 mg/ml). Thus, the most effective pH was observed to be 7.0 to reveal better protein contents in all the vegetables.

Effect of pH on peroxidase activity: The comparison, between the three vegetables was made on the basis of the ANOVA. Results revealed that the effect of pH was significant for the *Beta vulgaris* and *Cucumis sativus* where as it was less significant for the *Raphanus sativus*, the significance was at 0.001 for *Beta vulgaris* and *Cucumis sativus* and 0.05 for the *Raphanus sativus*. Activity was higher for the crude extract for *Beta vulgaris*, whereas, the *Raphanus sativus* and *Cucumis sativus* the activity was higher at pH 7.0.

Effect of pH on specific activity: Similarly the effects of pH were significant for the *Beta vulgaris* and *Cucumis sativus*, whereas, results were found to be non-significant for the *Raphanus sativus*.

SDS-PAGE analysis: SDS-PAGE was carried out of the extract of the mashed vegetables. Unstained precision plus protein standards containing molecular weights 250-10kDa was run in parallel with extracts. Peroxidase (purchased commercially from Sigma) was run as a standard. It was observed that the molecular weight of the standard was about 30kDa. Almost in all the samples bands were observed in each lane parallel to the standard. The depth of the band showed the presence of peroxidase in each extract (Fig. 1).

Discussion

Plants are known to have a number of enzymes. These plant sources are in fact easy to obtain and exploit for the extraction of enzymes. The present study has been done on some of the plants, bearing enzyme called

Table 4. Comparison of protein contents, peroxidase activities and specific activities of vegetables under study in crude extracts (Mean \pm S.E.; n=3).

Vegetable	Protein contents (mg/ml)	Enzyme activity (Units/ml/hr)	Specific activity
<i>Beta vulgaris</i> L.	0.064 \pm 0.01 ^a	17.69 \pm 4.34 ^a	12.47 \pm 2.63 ^a
<i>Cucumis sativus</i> L.	0.030 \pm 0.004 ^b	0.92 \pm 0.79 ^c	01.80 \pm 1.10 ^c
<i>Raphanus sativus</i> L.	0.041 \pm 0.002 ^b	06.20 \pm 0.92 ^b	07.50 \pm 1.13 ^b
LSD ($\alpha=0.05$)	0.0165	5.206	3.534

‘peroxidase’, that is normal components of most plant cells. Three vegetables were evaluated for the peroxidase using different buffers. The statistical analysis showed that *Beta vulgaris* showed maximum units at pH 5.2 and in the crude extract which was extracted without buffer (4.93 U/ml/hr), (17.69 U/ml/hr), respectively. The *Cucumis sativus* has maximum units at pH 7.0 (50.90 U/ml/hr) which was (50.90 U/ml/hr). Many researchers made attempts to evaluate vegetables for peroxidase such as, Halpin *et al.* (1989), Talat (1996) and Rehman *et al.* (1999) who proposed that the maximum activity of the *R. sativus* has maximum units at pH 6.0.

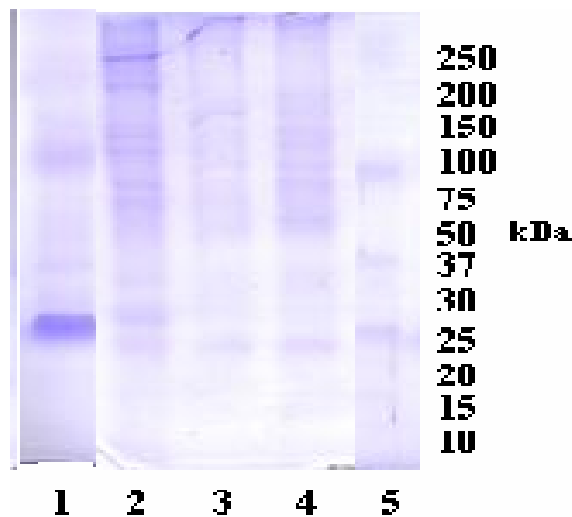


Fig. 1. SDS-PAGE for vegetable extracts; Standard of peroxidase (Lane 1), *Beta vulgaris* L. (Lane 2), *Raphanus sativus* L. (Lane 3), *Cucumis sativus* L. (Lane 4) and Molecular weight marker (Lane 5).

To seek the purity of peroxidases SDS-PAGE was employed on the extracts of vegetables. Bands for every extract were visible that confirmed the presence of peroxidase. Molecular weight estimation of the peroxidases in these extracts was found to be 30kDa. However, researchers working on the same technique found results such as, Zia *et al.* (2001), on his evaluation of tomatoes found it to be 40kDa. As reported by Jen *et al.* (1980) who found the molecular weight to be 43 \pm 20 kDa. The absorbance of enzyme extracts were recorded at 420nm after 3min interval of reaction period. It was observed there was a gradual increase in the activity of the enzyme with

increase in duration of time period. These results are in accordance with Rehman *et al.* (1999).

Protein expression is a phenotypic character that is regulated by both genotype and environmental factors. Protein profiling is a modern and probably the best tool for classification in numeric taxonomy. In the present research this approach was used to compare three vegetables on the basis of presence or absence of protein bands in SDS-PAGE. Different workers used this approach to differentiate and homogenate proteins on the basis of this character. These vegetables might also be used as peroxidase production yielding in three months and produce a lot which shows its potential to peroxidase extraction.

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