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Comparative study of the increased production & characterization of Bromelain from the peel, pulp & stem pineapple (Anannus commas)

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Abstract: Crude Bromelain was extracted from peel, pulp & stem of the pineapple by using sodium acetate buffer. Then those were purified separately by ammonium sulphate precipitation, Dialysis followed by ion-exchange chromatography. Enzyme Activity & Specific activity of peel, pulp & stem bromelain were estimated and protein fold and total enzyme yield were calculated. Enzyme kinetics was studied for bromelain by taking effect of temperature, pH, Substrate Concentration, Activator & Inhibitor. Finally peel part gave the better yield of enzyme & the specific activity was more than the pulp & stem.

Keywords: Crude Enzyme; Dialysis; Ion exchange chromatography; Lowry method; Stem bromelain; Pulp bromelain; Peel bromelain; Protein fold; Enzyme yield; Enzyme Kinetics.

Introduction:

Bromelain:

Pineapple (*Ananas comosus*) is one of the tropical plants that have been used as traditional medicines from a long time. It was originated from tropical South America and were discovered by Europeans in 1943(Bartholomew et al 2003). The Pineapple (*Ananas comosus*) is cultivated extensively in Hawaii, Philippines, Caribbean, Malaysia, Australia, Mexico, South Africa and Brazil. Brazil is the second producer worldwide with more than 58,000 hectares planted. Bromelain is an enzyme that is beneficial for health and is found naturally in pineapples. Bromelain is a general name for a family of sulfhydryl protective enzymes obtained from various species of Bromiliaceae. Almost all parts of pineapple plants such as fruit and peel and stem contain bromelain. Bromelain enzyme is also produced in the pineapple. Fruit bromelain differs extensively from stem bromelain & peel bromelain in amino acid composition, but it apparently has a similar mechanism of action. These enzymes performs important role in proteolytic modulation at cellular matrix, in numerous physiologic process, including tissue morphogenesis, tissue repair, angiogenesis and tissue modulation, decreasing bruises, swelling, pain and healing time.

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Bromelain enzyme has EC number: EC 3.4.22.4 which means; it is in the group of hydrolase, sub class- Protease, sub-sub-class thiol proteinase. Sulfhydryl proteolytic fraction is the primary component of bromelain. Bromelain is a glycoprotein, having a molar mass of about 33,000 Dalton. Cystein endopeptidase is a component of bromelain present in pineapple's stem, leaves and skin having a strong preference for Arg-Arg-|NHMec amongst other substrates. It exhibits a broad specificity for protein cleavage. It is stable in pH 3 to 7 and temperature 40^oC to 60^oC.

Extraction of crude bromelain can be done by taking Sodium Acetate buffer of pH 7.0 from parts of pineapple (peel, pulp, stem) separately. Ammonium salt precipitation, Dialysis and DEAE cellulose anion exchange chromatography is used to purify crude bromelain. Assay of bromelain can be performed by titrymetric method of gelatine hydrolysis. Protein concentration of enzyme is estimated by Folin's method by plotting standard graph. Enzyme kinetics can be done by taking effect of pH, temperature, substrate concentration, Activator, Inhibitor into consideration. Enzyme specific activity, percentage of yield can calculated after the estimation of enzyme activity.

Materials & Methods:

Extraction of Enzyme from Pineapple Peel, pulp & Stem:

The pineapple stem, peel & pulp are taken and cut into pieces and grinded with 0.1M. Sodium acetate buffer (p^H 7).The juice was filtered with the help of cheese cloth and centrifuged at 8000 rpm for 10 minutes. Then Sodium Benzoate was added at a concentration of 1gm/kg for storage. The Filtrate was used as "crude extract."

Enzyme Assay for Crude Extracts:

Crude enzymes (from peel, pulp & stem) were assayed bon the basis of gelatine degradation. 5.0% (w/v) Gelatin solution was prepared by heating at 80^oC in water bath with intermittent stirring for about 20 minutes. Then it was cooled to 45^oC and p^H was maintained 4.5. 30% formaldehyde & 5% hydrogen peroxide & 005N NaOH was prepared. For all the three crude enzyme samples one test and one blank has prepared separately by following the below table No - 1.

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Table No: 1

Sample	Vol.of Gelatin (ml)	Equilibrateto 45°C in waterbath for 10 min	Crude Enzyme (ml)	Incubate in water bath at 45°C for 20 minutes	Distilled H_20 (ml)	Vol. of H_20_2 (ml)	Adjust the pH to 6.9 with 0.05N NaOH	Vol. of Formaldehyde (ml)	Titrate to pH 7.8 with 0.05N NaOH	Vol.of NaOH run down (ml)
Blank	2.5ml		----		0.1ml	0.01ml		1ml		Ml
Test	2.5ml		0.1ml		----	0.01ml		1ml		ml

Then the total NaOH run down for the test and the blank was measured as follows:

- Actual amount of NaOH run down = Volume of Test – Volume of Blank

After measuring the NaOH volume, the enzyme activity of the crude peel, pulp and stem was calculated by the below formula.

Calculation of Bromelain Enzyme (Crude) activity:-

$$\text{Units / gm enzyme} = \frac{\text{(Volume of Test – Volume of Blank) (N) (14) (1000)}}{\text{mg enzyme /RM}}$$

Enzyme activity of the Crude Extracts:

1) $$\text{Units/mg enzyme of crude stem extract} = \frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$$

Vol. of Test = 1.8; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.135mg

$$\text{Units/mg enzyme} = \frac{(1.8-1.1)(0.05)(14)(1000)}{0.135/3.61} = \frac{0.7x0.05x14x1000}{0.135/3.61} = \frac{490}{0.037} = 13243.24$$

2) $$\text{Units/mg enzyme of crude pulp extract} = \frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$$

Vol. of Test = 1.7; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.12mg

$$\text{Units/mg enzyme} = \frac{(1.7-1.1)(0.05)(14)(1000)}{0.12/3.61} = \frac{0.6x0.05x14x1000}{0.12/3.61} = \frac{420}{0.033} = 12727.27$$

3) $$\text{Units/mg enzyme of crude peel extract} = \frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$$

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Vol. of Test = 1.6; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;
mg enzyme (obtained from standard curve) = 0.095mg

$$\text{Units/mg enzyme} = \frac{(1.6-1.1)(0.05)(14)(1000)}{0.095/3.61} = \frac{0.5\text{x}0.05\text{x}14\text{x}1000}{0.095 / 3.61} = \frac{350}{0.026} = 13461.53$$

Purification of Crude Enzyme:

The crude enzymes were purified by ammonium sulphate purification technique followed by dialysis and Ion Exchange chromatography.

Ammonium salt precipitation:

The crude peel, pulp and stem bromelain were precipitated separately by 44.4% of ammonium sulphate salt precipitation process. 20ml each from all the three crude enzymes are taken separately in 3 different beakers. 8.88gms of ammonium sulphate salt was added to the beakers pinch by pinch; which contained 20ml crude peel, pulp and stem separately. This process took place in ice cold condition by proper stirring. Then all the three ammonium sulphate precipitated enzyme samples were kept in 4^0C over night. Next day all the three enzymes are centrifuged one by one separately at 10,000rpm for 12min and the supernatants collected from each crude(peel, pulp and stem) were dissolved separately in 15ml of 10mM Tris HCl of pH 7. Then this ammonium sulphate precipitated samples were assayed like the same process as the crude sample and the activity of the enzymes were calculated.

Enzyme activity of the extracts after ammonium salt precipitation:

1) $$\text{Units/mg enzyme of stem extract} = \frac{(\text{Vol. of test} - \text{Vol. of blank})(N)(14)(1000)}{\text{mg enzyme/ RM}}$$

Vol. of Test = 2.0; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;
mg enzyme (obtained from standard curve) = 0.095mg

$$\text{Units/mg enzyme} = \frac{(2.0-1.1)(0.05)(14)(1000)}{0.095/3.61} = \frac{0.9\text{x}0.05\text{x}14\text{x}1000}{0.095/3.61} = \frac{630}{0.026} = 24230.76$$

2) $$\text{Units/mg enzyme of pulp extract} = \frac{(\text{Vol. of test} - \text{Vol. of blank})(N)(14)(1000)}{\text{mg enzyme/ RM}}$$

Vol. of Test = 1.9; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;
mg enzyme (obtained from standard curve) = 0.09mg

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Units/mg enzyme = $\frac{(1.9-1.1)(0.05)(14)(1000)}{0.09/3.61} = \frac{0.8x0.05x14x1000}{0.09/3.61} = \frac{560}{0.024} = 23333.33$

3) $\frac{\text{Units/mg enzyme of peel extract}}{\text{mg enzyme/ RM}} = \frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 1.8; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.055mg

Units/mg enzyme = $\frac{(1.8-1.1)(0.05)(14)(1000)}{0.055/3.61} = \frac{0.7x0.05x14x1000}{0.055/3.61} = \frac{490}{0.015} = 32666.66$

Dialysis:

The peel, pulp and stem ammonium sulphate samples were placed in the three separate dialysis bags (after activation by 2% Sodium bicarbonate). Then all three bags were kept in separate beakers contained 100ml of 25mM Tris HCl. Dialysis process was done in cold condition overnight on magnetic stirrer. Then on next day all the three samples were removed from their respective dialysis bags and placed in separate beakers and assayed to measure the enzyme activity.

Enzyme activity of the extracts after dialysis:

1) Units/mg enzyme of stem extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 2.3; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.075mg

Units/mg enzyme = $\frac{(2.3-1.1)(0.05)(14)(1000)}{0.075/3.61} = \frac{1.2x0.05x14x1000}{0.075/3.61} = \frac{840}{0.020} = 42000$

2) Units/mg enzyme of pulp extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 2.1; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.07mg

Units/mg enzyme = $\frac{(2.1-1.1)(0.05)(14)(1000)}{0.07/3.61} = \frac{1.0x0.05x14x1000}{0.07/3.61} = \frac{560}{0.024} = 36842.10$

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3) Units/mg enzyme of peel extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 2.0; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.03mg

Units/mg enzyme = $\frac{(2.0-1.1)(0.05)(14)(1000)}{0.03/3.61} = \frac{0.9x0.05x14x1000}{0.03/3.61} = \frac{630}{0.008} = 78750$

Ion- Exchange Chromatography:

The dialysed enzymes samples were further purified by Ion Exchange chromatography. DEAE Cellulose ion exchange were prepared by washing 1g DEAE in 25ml of 50mM Tris HCl, 2 to 3 times and keeping that in fridge overnight at 4^0C. Then the DEAE were packed into the column in 1 to 1.5 cm thickness. After the packing, column was washed 2 to 3 times with 25mM Tris HCl of pH 8. Then 6 ion-exchange elutes were prepared for each dialysed sample (peel, pulp, stem) separately with 25mM Tris HCl and NaCl. Then the one dialysed enzyme was added into the column and left for 10mins for settling. Then the sample run down & was collected into test tube. After that Elute -1 was added into the column, left for same 10 min for settling & was collected into the same test tube. Like this, the elution process for Elutes 2, 3, 4, 5 & 6 were also done. Finally all the six elutes for each sample (peel, pulp, stem) were collected. Then assayed to get best elute (showing highest enzyme units) for each sample by following table no: 2.The sixth number elutes for the peel, pulp and stem separately was showing highest activity than the other elutes after the assay.

Table No: 2

Sample	Vol.of Gelatin (ml)	Equilibrate to 45°C in water bath	Crude Enzyme (ml)	Incubate in water bath at 45°C for 20 min.	Distilled H_20 (ml)	Vol. of H_20_2 (ml)	Adjust the pH to 6.9 with 0.05N NaOH	Vol. of Formaldehyde (ml)	Titrate to pH 7.8 with 0.05N NaOH	Vol. of NaOH run down (ml)
Blank	2.5ml		----		0.1ml	0.01ml		1 ml		ml
Test 1	2.5ml		0.1ml		----	0.01ml		1 ml		ml
Test 2	2.5ml		0.1ml		----	0.01ml		1 ml		ml
Test 3	2.5ml		0.1ml		----	0.01ml		1 ml		ml
Test 4	2.5ml		0.1ml		----	0.01ml		1 ml		ml
Test 5	2.5ml		0.1ml		----	0.01ml		1 ml		ml
Test 6	2.5ml		0.1ml		----	0.01ml		1 ml		ml

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Enzyme activity of the extracts after ion exchange chromatography:

1) Units/mg enzyme of stem extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 3.6 Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.035mg

Units/mg enzyme = $\frac{(3.6-1.1)(0.05)(14)(1000)}{0.035/3.61} = \frac{2.5x0.05x14x1000}{0.035/3.61} = \frac{1750}{0.0096} =$ 182291.67

2) Units/mg enzyme of pulp extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 3.9; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.045mg

Units/mg enzyme = $\frac{(3.9-1.1)(0.05)(14)(1000)}{0.045/3.61} = \frac{2.8x0.05x14x1000}{0.045/3.61} = \frac{1960}{0.0124} =$158064.516

3) Units/mg enzyme of peel extract = $\frac{\text{(Vol. of test – Vol. of blank)(N)(14)(1000)}}{\text{mg enzyme/ RM}}$

Vol. of Test = 4.0; Vol. of blank = 1.1; N= Normality of NaOH = 0.05; RM = 3.61;

mg enzyme (obtained from standard curve) = 0.025mg

Units/mg enzyme = $\frac{(4.0-1.1)(0.05)(14)(1000)}{0.025/3.61} = \frac{3.9x0.05x14x1000}{0.025/3.61} = \frac{2030}{0.0069} =$ 294202.89

Estimation of Protein Concentration by Folin Lowry Method:

Standard graph of Protein was plotted by Folin's Lowry method by taking BSA in different concentrations and OD at 660nm as the table no: 3.

S.No	Protein (BSA) Standards Concentration (µg)	OD at 660nm
1	Blank	000
2	0.01	0.07
3	0.02	0.09
4	0.03	0.11
5	0.04	0.13

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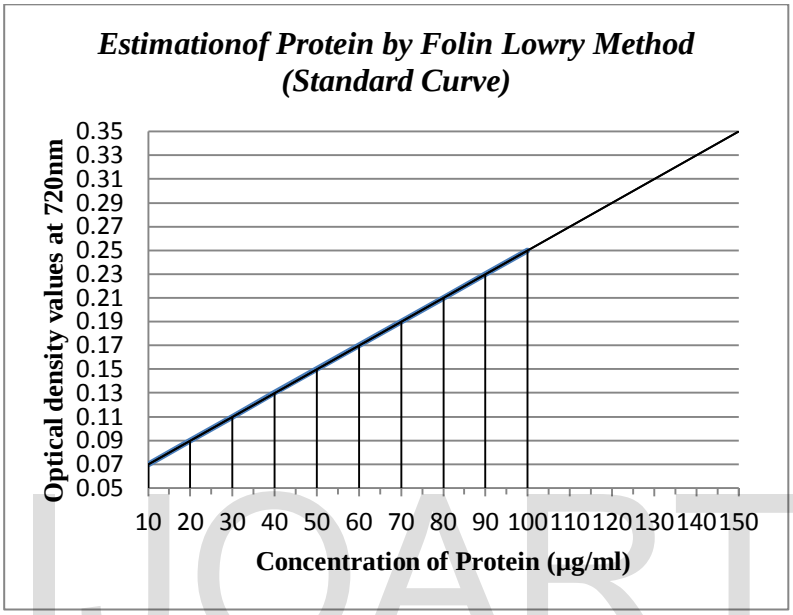
6	0.05	0.15
7	0.06	0.17
8	0.07	0.19
9	0.08	0.21
10	0.09	0.23
11	0.10	0.25



On the basis of the standard graph the concentration of protein in crude bromelain, ammonium sulphate precipitated bromelain, dialysed bromelain and ion-exchanged sample bromelain were estimated which are given below.

- Estimation of protein concentration for crude bromelain:

Sl.No	Sample	Final O.D.
	Blank	----
2.	Crude Extract of Stem	0.32
3.	Crude Extract of Peel	0.29
4.	Crude Extract of Pulp	0.24

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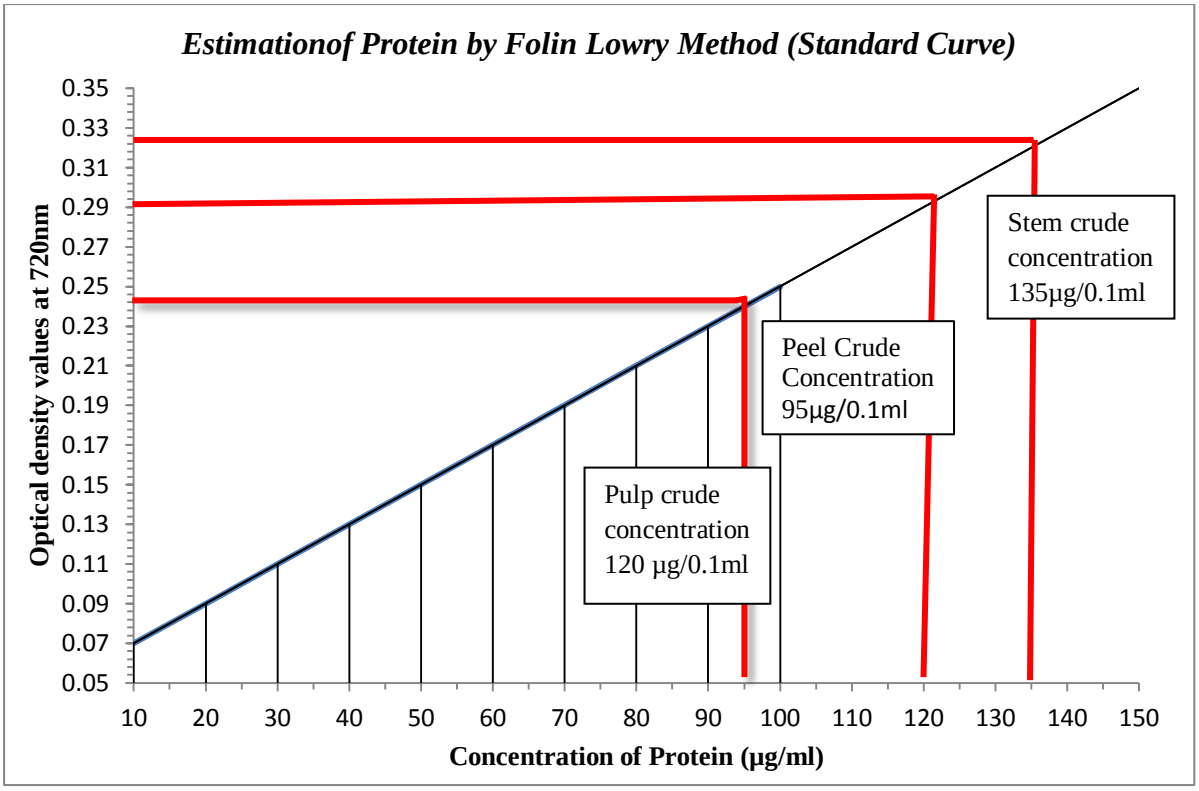

Estimation of protein concentration after Ammonium Salt Precipitation:

S.No	Sample	O.D at 660nm
	Blank	----
2.	Extract of Stem	0.24
3.	Extract of Pulp	0.23
4.	Extract of Peel	0.16

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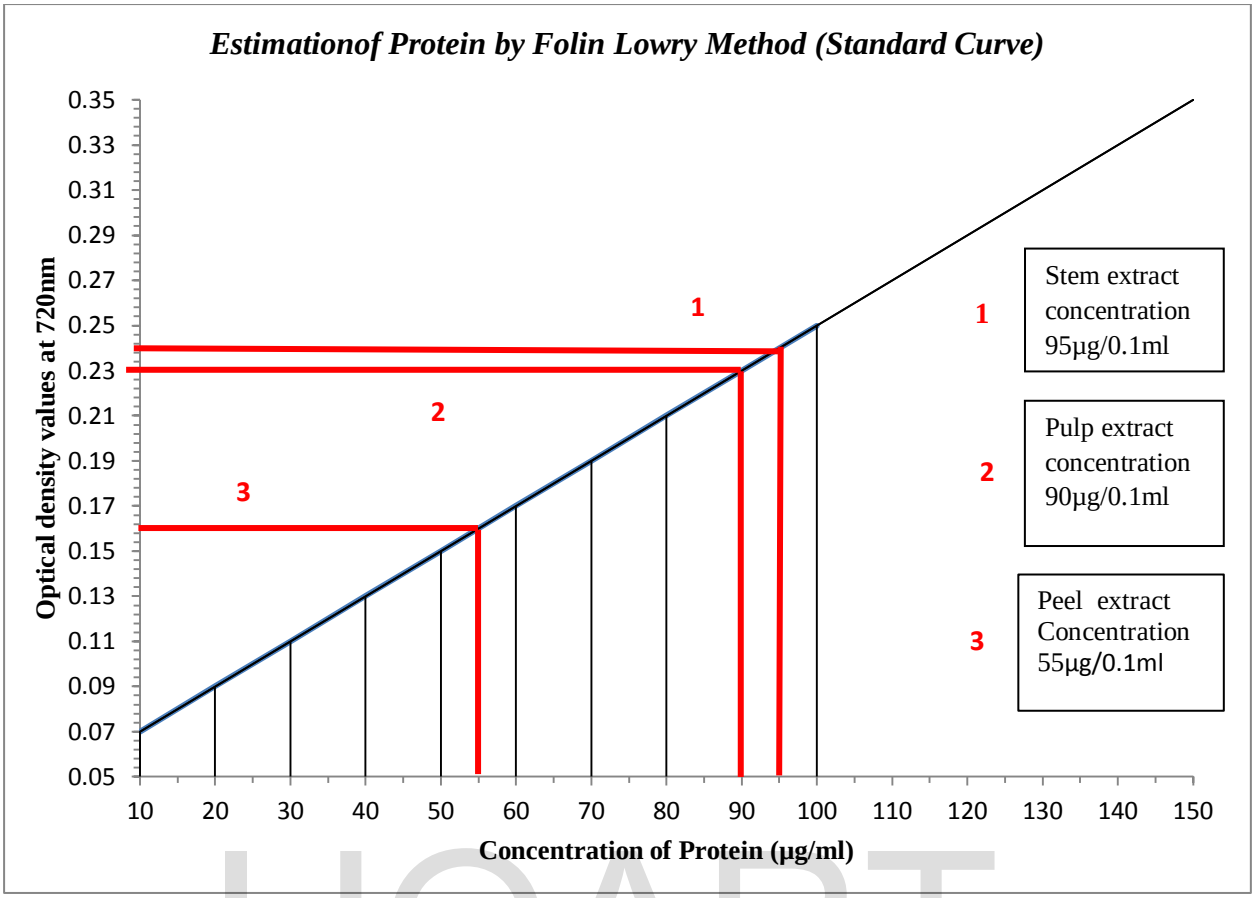

Estimation of protein concentration after Dialysis:

S.No	Sample	O.D at 660nm
1.	Blank	----
2.	Extract of Stem	0.20
3.	Extract of Pulp	0.19
4.	Extract of Peel	0.11

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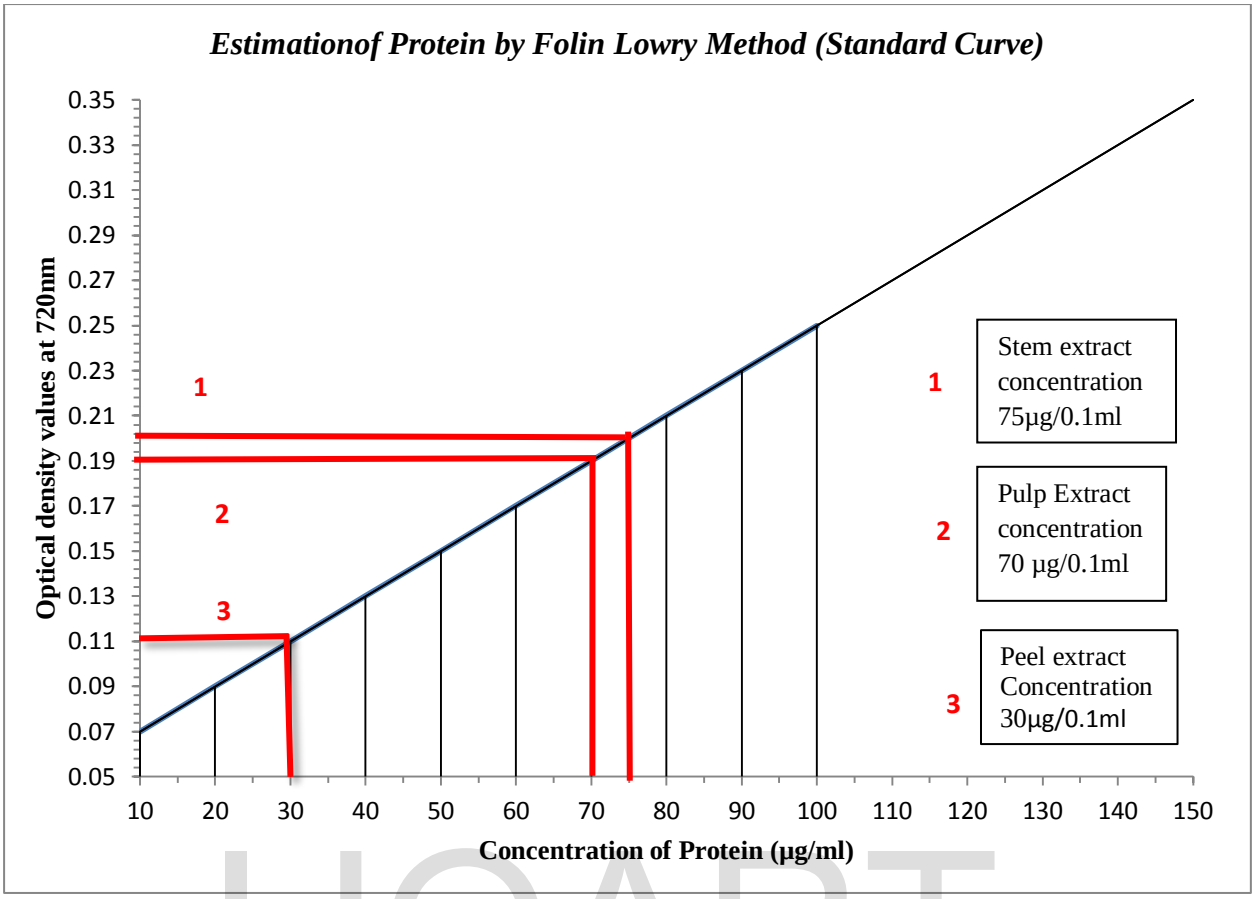

Estimation of protein concentration after Ion exchange chromatography:

S.No	Sample	O.D. at 660nm
1.	Blank	----
2.	Extract of Stem	0.10
3.	Extract of Peel	0.12
4.	Extract of Pulp	0.14

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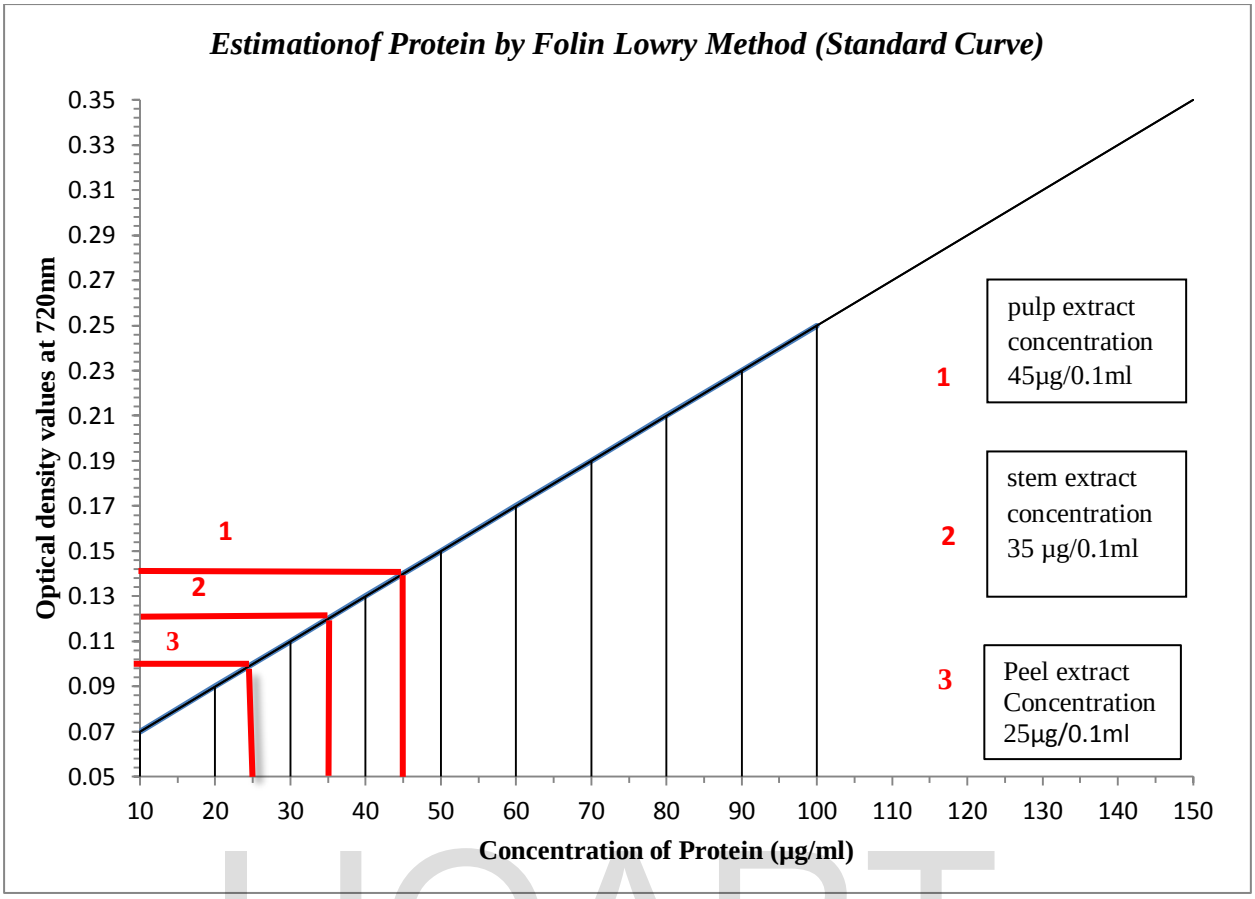

Specific activities of crude peel, pulp and stem extracts were then calculated.

Enzyme activity of crude extracts of stem, peel and pulp of pineapple:

Sample	Protein concentration	Enzyme activity	Specific activity
Crude stem	135µg or 0.135mg	13243.24	98098.0741
Crude pulp	120µg or 0.120mg	12727.27	106060.583
Crude peel	95 µg or 0.095mg	13461.53	141700.316

Then the specific activities, protein fold, % of enzyme yield were calculated for ammonium sulphate precipitated samples, dialysed and ion exchange chromatography samples for peel, pulp and stems.

Specific activity, protein fold and % of yield of stem, peel and pulp extracts after ammonium salt precipitation:

Sample	Protein concentration	Enzyme activity	Specific activity	Protein fold	% yield
Stem	95 µg or 0.095mg	24230.76	255060.632	2.600	182.96
Pulp	90 µg or 0.09mg	23333.33	259259.222	2.444	183.33
Peel	55 µg or 0.055mg	32666.66	593939.395	4.191	242.66

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Specific activity, protein fold and % of yield of stem, peel and pulp extracts after dialysis:

Sample	Protein concentration	Enzyme activity	Specific activity	Protein fold	% yield
Stem	75 µg or 0.075mg	42000	560000	2.25	312.89
Pulp	70 µg or 0.07mg	36842.10	526315.714	2.03	289.47
Peel	30 µg or 0.03mg	78750	2625000	4.410	585

Specific activity, protein fold and % of yield of stem, peel and pulp extracts after ion-exchange chromatography:

Sample	Protein concentration	Enzyme activity	Specific activity	Protein fold	% yield
Stem	45µg or 0.045mg	158064.516	3512544.8	6.272	1193.5
Pulp	35µg or 0.035mg	182291.667	5208333.34	9.895	1423.29
Peel	25µg or 0.025mg	294202.89	11768116	4.483	2185.50

Study of Enzyme Kinetics:

The ion exchange peel

Effect of substrate concentration on enzyme activity (stem extract):

Sl No	Vol of Gelatin (ml)	Distilled water (ml)	Equilibrate at 45°C in water bath for 10min	Enzyme (ml) Stem extract	Incubate in water bath at 45°C for 20 mins	Vol. of H_2O_2 (ml)	Enzyme (ml) Stem extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run Down (ml)	
Blank	---	5		---		0.01ml	0.1ml		1ml		0.5	---
2	2.5	2.5		0.1ml		0.01ml	----		1ml		1.3	0.8
3	3.0	2.0		0.1ml		0.01ml	----		1ml		1.6	1.1
4	3.5	1.5		0.1ml		0.01ml	----		1ml		1.8	1.3
5	4.0	1.0		0.1ml		0.01ml	----		1ml		2.0	1.5
6	4.5	0.5		0.1ml		0.01ml	----		1ml		2.7	2.2

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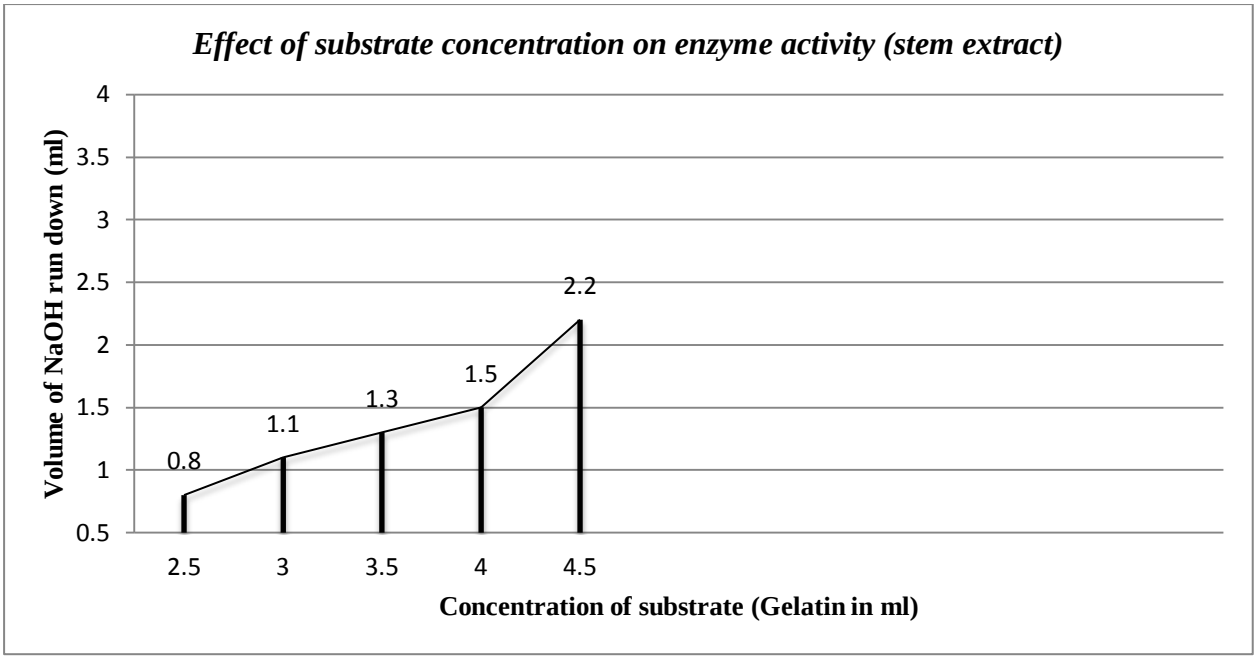

Effect of substrate concentration on enzyme activity (peel extract):

Sl No	Vol of Gelatin (ml)	Distilled water (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Peel extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Peel extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run Down (ml)	
Blank	---	5		---		0.01ml	0.1ml		1ml		0.6	---
2	2.5	2.5		0.1ml		0.01ml	----		1ml		1.3	0.7
3	3.0	2.0		0.1ml		0.01ml	----		1ml		1.7	1.1
4	3.5	1.5		0.1ml		0.01ml	----		1ml		2.1	1.5
5	4.0	1.0		0.1ml		0.01ml	----		1ml		2.5	1.9
6	4.5	0.5		0.1ml		0.01ml	----		1ml		3.0	2.4

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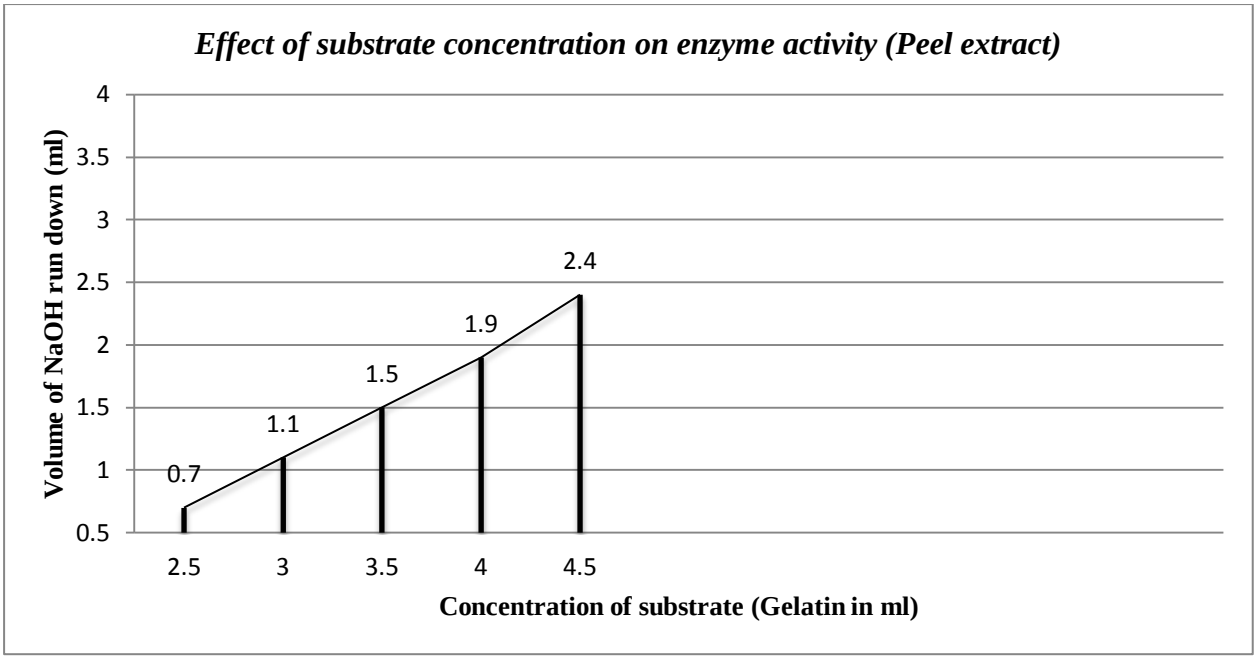

Effect of substrate concentration on enzyme activity (pulp):

Sl No	Vol of Gelatin (ml)	Distilled water (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Pulp extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Pulp extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run Down (ml)	
Blank	---	5		---		0.01ml	0.1ml		1ml		0.4	---
2	2.5	2.5		0.1ml		0.01ml	----		1ml		1.4	1.0
3	3.0	2.0		0.1ml		0.01ml	----		1ml		1.7	1.3
4	3.5	1.5		0.1ml		0.01ml	----		1ml		1.9	1.5
5	4.0	1.0		0.1ml		0.01ml	----		1ml		2.2	1.8
6	4.5	0.5		0.1ml		0.01ml	----		1ml		2.5	2.1

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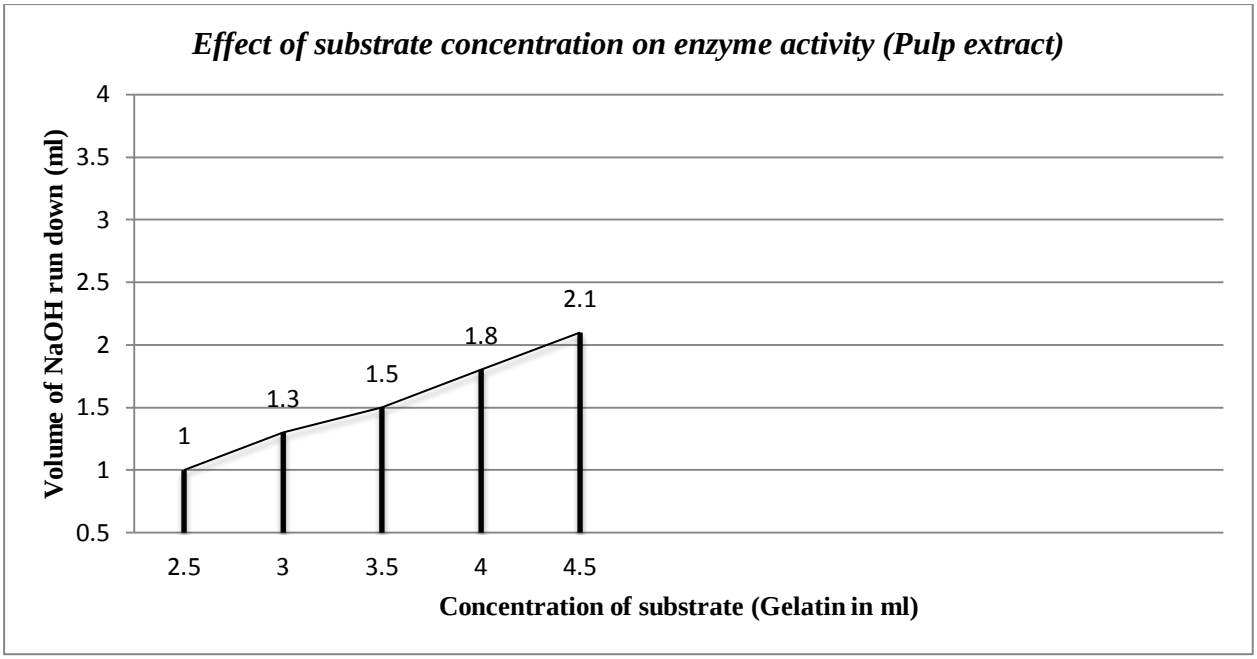

Effect of activator on enzyme activity (stem)

Sl No	Vol of $CaCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Stem elute	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml)	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down(ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		2.0	---
2	0.2	0.8	2.5		0.1		0.01	----		1ml		2.5	0.5
3	0.4	0.6	2.5		0.1		0.01	----		1ml		2.8	0.8
4	0.6	0.4	2.5		0.1		0.01	----		1ml		3.1	1.1
5	0.8	0.2	2.5		0.1		0.01	----		1ml		3.3	1.3
6	1.0	---	2.5		0.1		0.01	----		1ml		3.5	1.5

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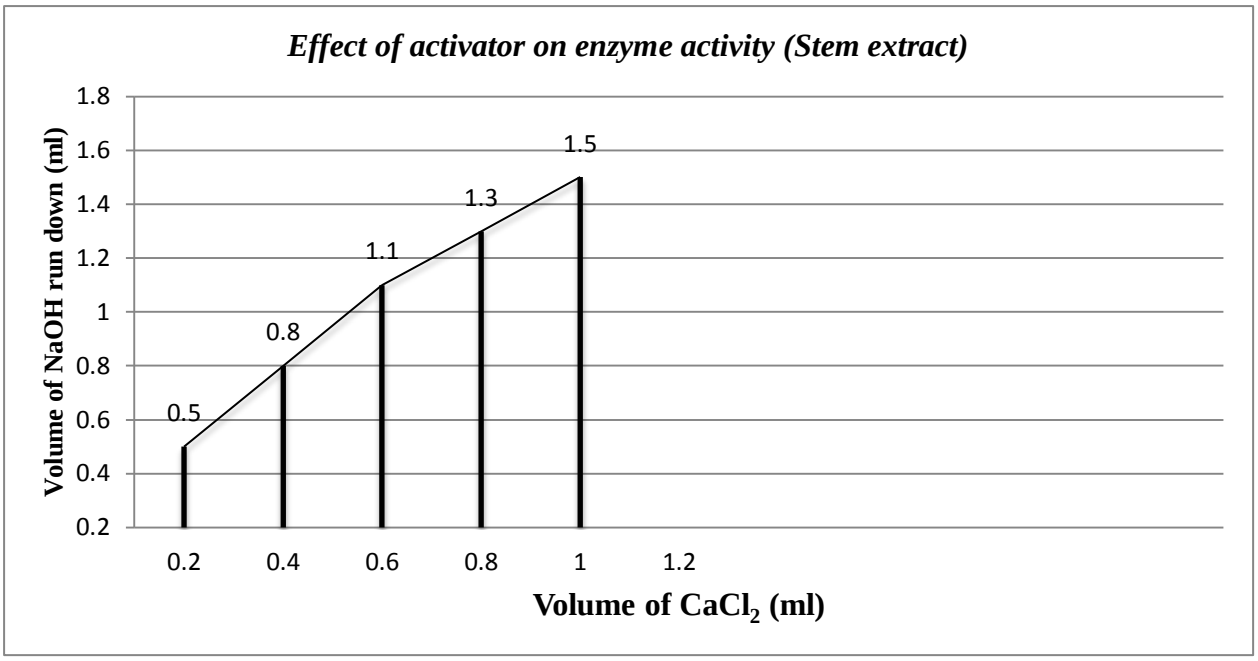

Effect of activator on enzyme activity:

Sl No	Vol of $CaCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Peel elute	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Peel elute	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down(ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		2.1	---
2	0.2	0.8	2.5		0.1		0.01	----		1ml		2.6	0.5
3	0.4	0.6	2.5		0.1		0.01	----		1ml		2.9	0.8
4	0.6	0.4	2.5		0.1		0.01	----		1ml		3.1	1.0
5	0.8	0.2	2.5		0.1		0.01	----		1ml		3.4	1.3
6	1.0	---	2.5		0.1		0.01	----		1ml		3.6	1.5

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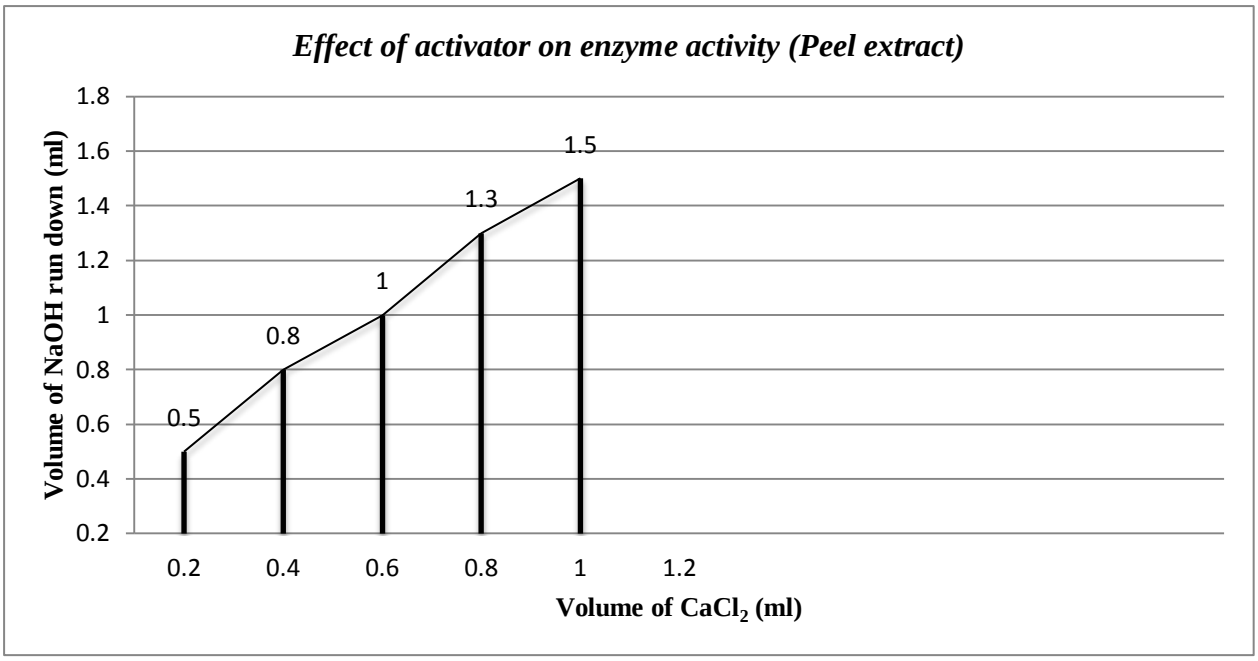

Effect of activator on enzyme activity (pulp)

Sl No	Vol of $CaCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Pulp Elute	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Pulp Elute	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down(ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		1.8	---
2	0.2	0.8	2.5		0.1		0.01	----		1ml		2.1	0.3
3	0.4	0.6	2.5		0.1		0.01	----		1ml		2.3	0.5
4	0.6	0.4	2.5		0.1		0.01	----		1ml		2.6	0.8
5	0.8	0.2	2.5		0.1		0.01	----		1ml		2.8	1.0
6	1.0	---	2.5		0.1		0.01	----		1ml		3.1	1.3

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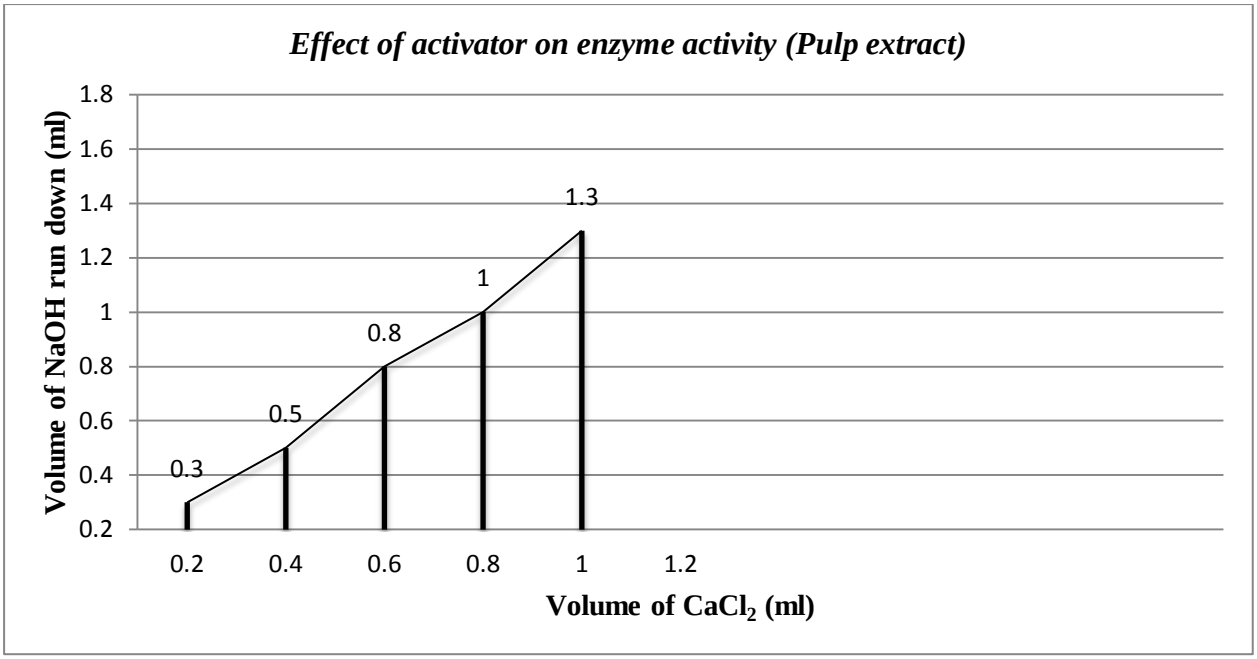

Effect of inhibitor on enzyme activity (stem extract):

Sl No	Vol of $HgCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Stem extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Stem Extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		0.8	---
2	0.2	0.8	2.5		0.1		0.01	----		1ml		2.6	1.8
3	0.4	0.6	2.5		0.1		0.01	----		1ml		2.3	1.5
4	0.6	0.4	2.5		0.1		0.01	----		1ml		1.9	1.1
5	0.8	0.2	2.5		0.1		0.01	----		1ml		1.6	0.8
6	1.0	---	2.5		0.1		0.01	----		1ml		1.3	0.5

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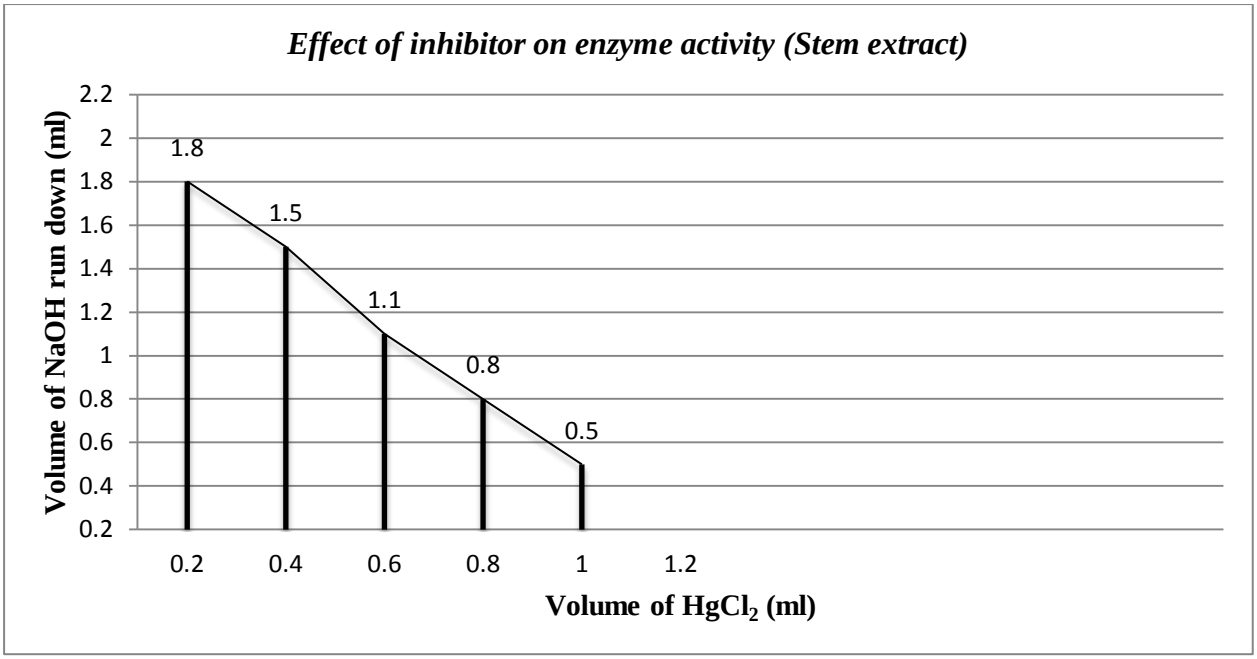

Effect of inhibitor on enzyme activity (Peel extract):

Sl No	Vol of $HgCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Peel extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Peel Extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		1.3	----
2	0.2	0.8	2.5		0.1		0.01	----		1ml		2.8	1.5
3	0.4	0.6	2.5		0.1		0.01	----		1ml		2.5	1.2
4	0.6	0.4	2.5		0.1		0.01	----		1ml		2.1	0.8
5	0.8	0.2	2.5		0.1		0.01	----		1ml		1.9	0.6
6	1.0	---	2.5		0.1		0.01	----		1ml		1.6	0.3

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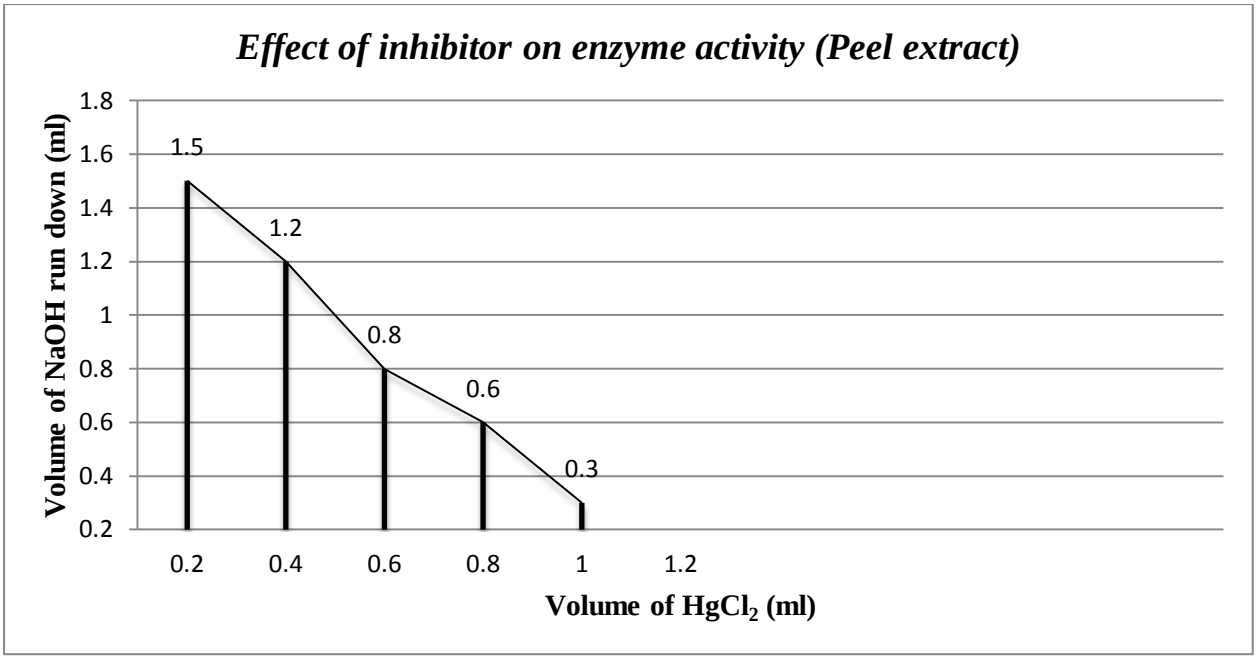

Effect of inhibitor on enzyme activity (Pulp extract):

Sl No	Vol of $HgCl_2$ (ml)	Distilled water (ml)	Vol.of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) Pulp extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) Pulp Extract	Adjust both the test and blank to p^H 6.9 with 0.05N NaOH	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Blank	---	1.0	2.5		---		0.01	0.1ml		1ml		1.2	---
2	0.2	0.8	2.5		0.1		0.01	----		1ml		3.4	2.2
3	0.4	0.6	2.5		0.1		0.01	----		1ml		3.0	1.8
4	0.6	0.4	2.5		0.1		0.01	----		1ml		2.7	1.5
5	0.8	0.2	2.5		0.1		0.01	----		1ml		2.3	1.1
6	1.0	---	2.5		0.1		0.01	----		1ml		1.8	0.6

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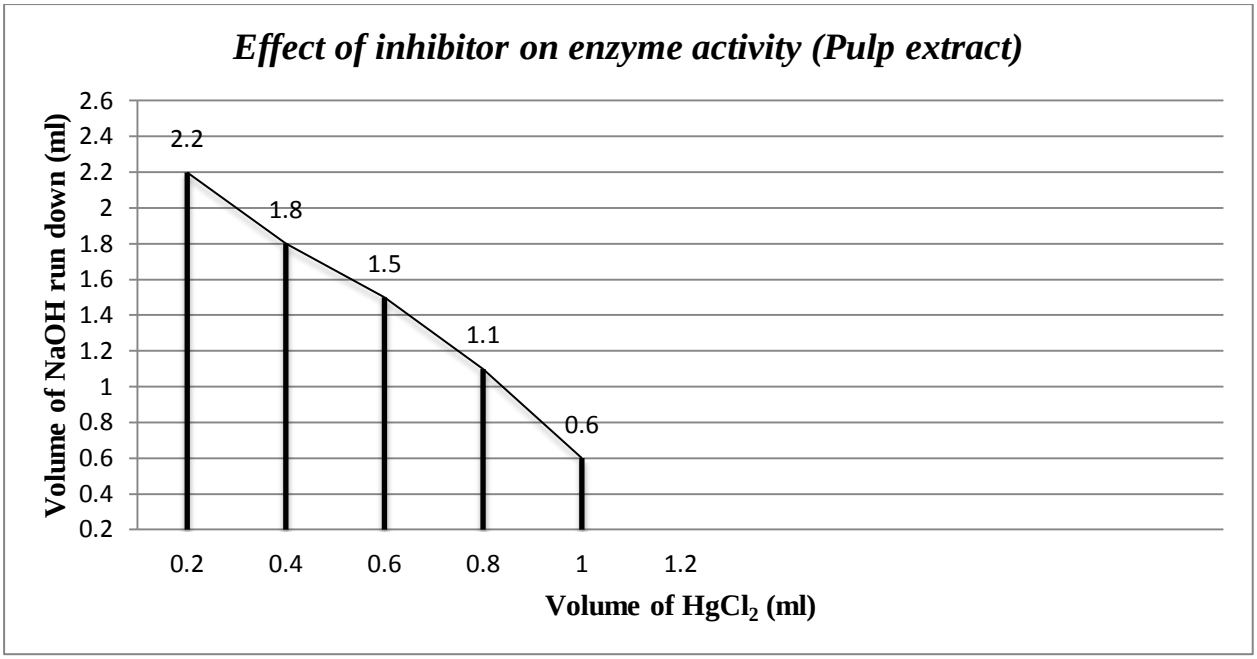

Effect of p^H on enzyme activity (stem extract):

Sl No	p^H	Vol of buffer (ml)	Vol. of Gelatin (ml)	Equilibrate at 45°C in water bath for 10 minutes	Enzyme (ml) stem extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) stem extract	Adjust both the test and blank to p^H6.9	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Test	2.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		4.0	2.1
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		1.9	
Test	3.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		6.3	2.5
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		3.8	
Test	4.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		7.9	3.2
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		4.7	

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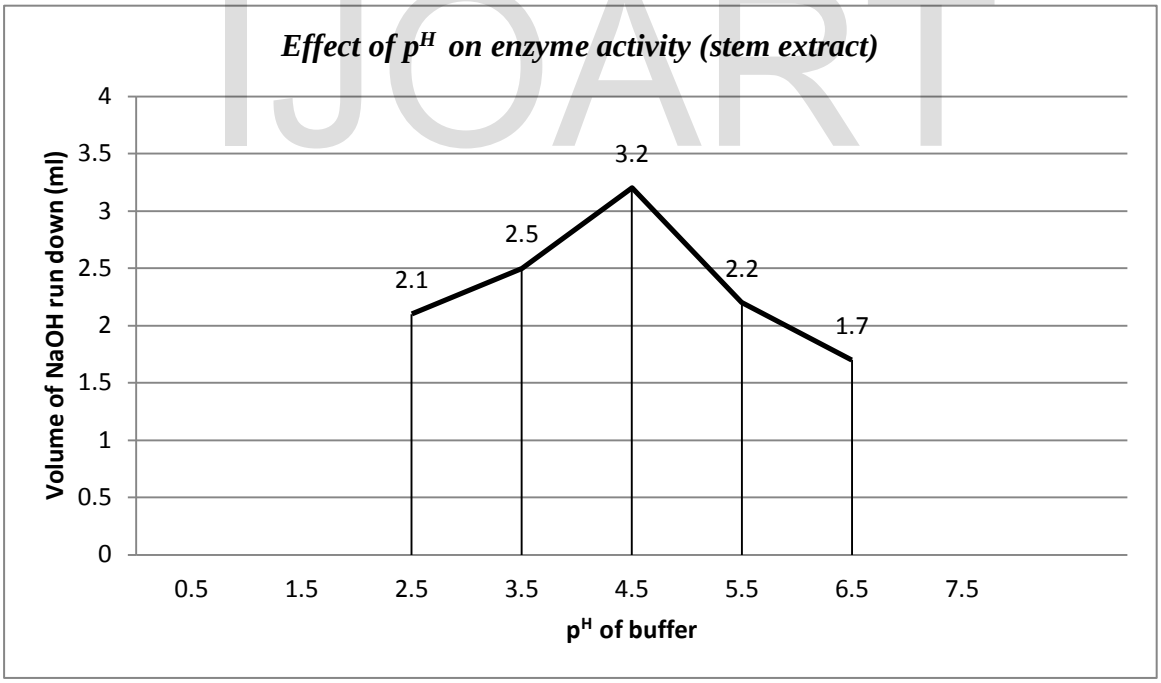
Test Blank	5.5	1ml 1ml	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		6.2 4.0	2.2
Test Blank	6.5	1ml 1ml	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		4.6 2.9	1.7



Effect of p^H on enzyme activity (peel extract):

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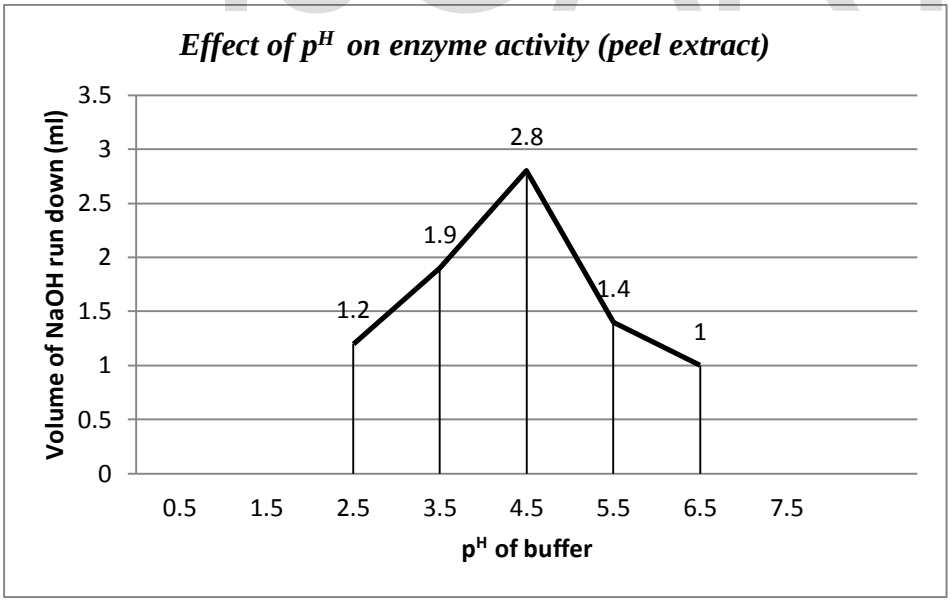
Sl No	p^H	Vol of buffer (ml)	Vol. of Gelatin (ml)	Equilibriate at 45°C in water bath for 10 minutes	Enzyme (ml) peel extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) peel extract	Adjust both the test and blank to p^H 6.9	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Test	2.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		3.8	1.2
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		2.6	
Test	3.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		5.2	1.9
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		3.3	
Test	4.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		6.9	2.8
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		4.1	
Test	5.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		4.4	1.4
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		3.0	
Test	6.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		3.3	1.0
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		2.3	



Effect of p^H on enzyme activity (pulp extract):

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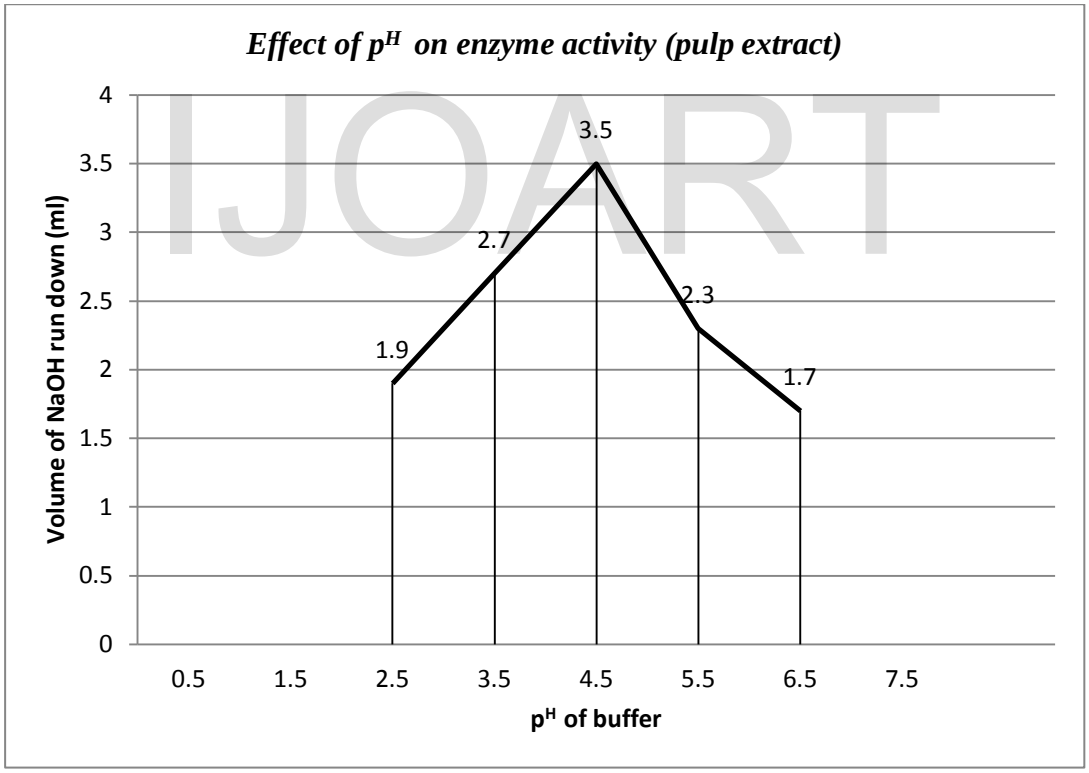
Sl No	p^H	Vol of buffer (ml)	Vol. of Gelatin (ml)	Equilibriate at 45°C in water bath for 10 minutes	Enzyme (ml) pulp extract	Incubate in water bath at 45°C for 20 minutes	Vol. of H_2O_2 (ml)	Enzyme (ml) pulp extract	Adjust both the test and blank to p^H 6.9	Vol. of formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt.of NaOH run down (ml)	
Test	2.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		4.9	1.9
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		3.0	
Test	3.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		6.8	2.7
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		4.1	
Test	4.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		8.6	3.5
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		5.1	
Test	5.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		5.8	2.3
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		3.5	
Test	6.5	1ml	2.5ml		0.1ml		0.01ml	----		1ml		4.0	1.4
Blank		1ml	2.5ml		---		0.01ml	0.1ml		1ml		2.6	



Effect of Temperature on Enzyme Activity (stem extract) :

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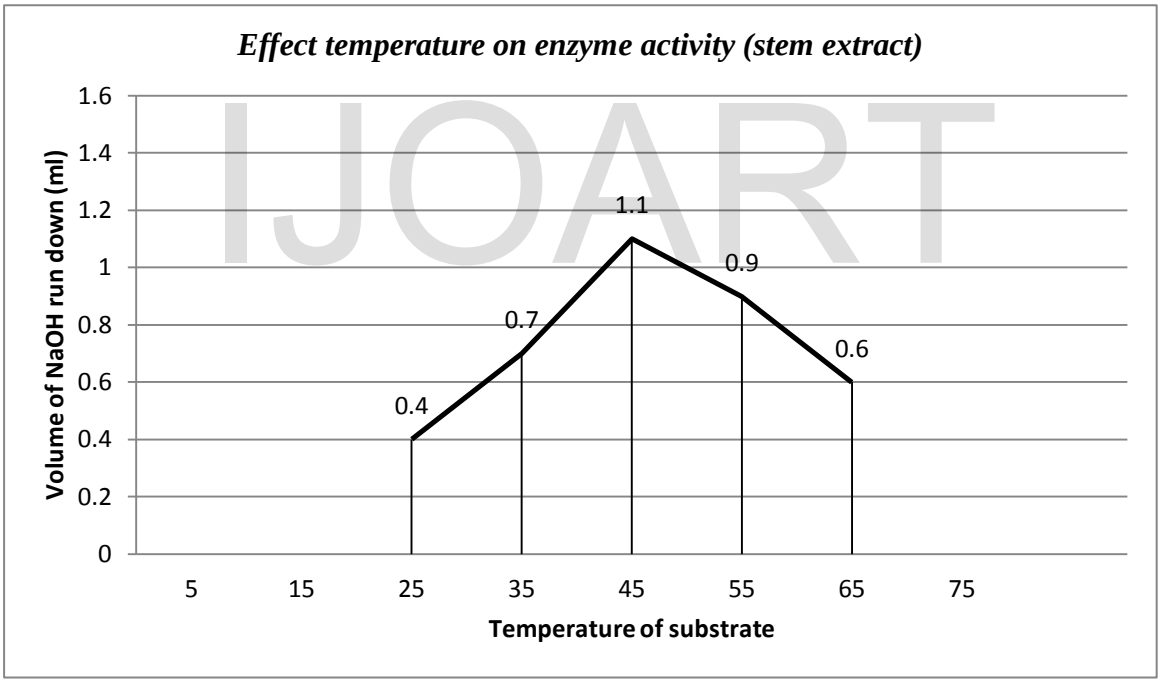
Sl.No	Temp. (^oC)	Gelatin Solution (ml)	Equilibriate at 45°C in water bath for 10 mins	Enzyme (ml) stem extract	Incubate in waterbath at 45°C for 20 min.	Vol. of H_2O_2 (ml)	Enzyme (ml) stem extract	Adjust both the test& blank to p^H 6.9	Vol. of Formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down (ml)	
Test	25(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		0.9	0.4
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.5	
Test	35(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.5	0.7
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.8	
Test	45(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		2.2	1.1
Blank		2.5ml		---		0.01ml	0.1ml		1ml		1.1	
Test	55(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.8	0.9
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.9	
Test	65(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.3	0.6
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.7	



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Effect of Temperature on Enzyme Activity (peel extract) :

Sl.No	Temp. (^oC)	Gelatin Solution (ml)	Equilibrate at respective temperature in water bath	Enzyme (ml) peel extract	Incubate at respective temp. in water bath for 20 min.	Vol. of H_2O_2 (ml)	Enzyme (ml) peel extract	Adjust both the test& blank to p^H 6.9	Vol. of Formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down (ml)	
Test	25(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		0.9	0.4
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.5	
Test	35(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.5	0.7
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.8	
Test	45(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		2.0	1.1
Blank		2.5ml		---		0.01ml	0.1ml		1ml		1.0	
Test	55(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.6	0.9
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.8	
Test	65(^oC)	2.5ml		0.1ml		0.01ml	----		1ml		1.1	0.6
Blank		2.5ml		---		0.01ml	0.1ml		1ml		0.6	

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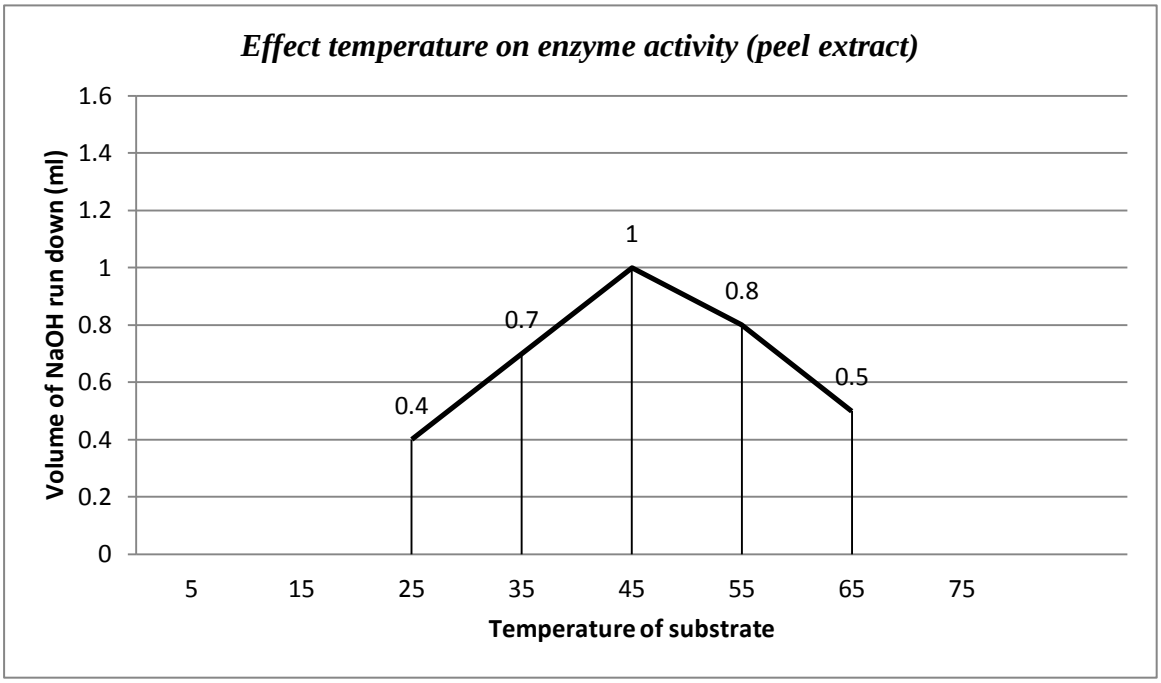

Effect of Temperature on Enzyme Activity (pulp extract) :

Sl.No	Temp. (^oC)	Gelatin Solution (ml)	Equilibrate at respective temperature in water bath	Enzyme (ml) pulp extract	Incubate at respective temp. in water bath for 20 min.	Vol. of H_2O_2 (ml)	Enzyme (ml) pulp extract	Adjust both the test& blank to p^H 6.9	Vol. of Formaldehyde (ml)	Titrate to p^H 7.8 with 0.05N NaOH	Amt. of NaOH run down (ml)	
Test Blank	25(^oC)	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		0.9 0.3	0.6
Test Blank	35(^oC)	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		2.0 1.2	0.8
Test Blank	45(^oC)	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		2.7 1.4	1.3

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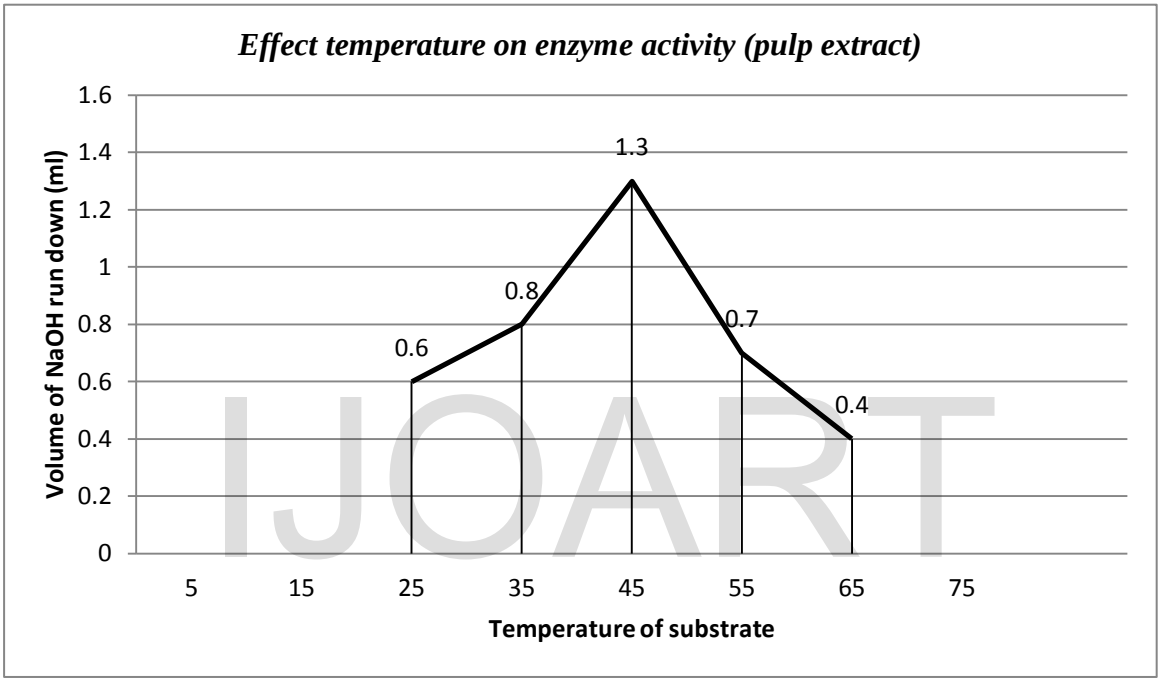
Test Blank	55(^{o}C)	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		1.7 1.0	0.7
Test Blank	65(^{o}C)	2.5ml 2.5ml		0.1ml ---		0.01ml 0.01ml	---- 0.1ml		1ml 1ml		1.3 0.9	0.4



Conclusion: Bromelain extracted from peel, pulp & stem part of pineapple by using buffer and purified by ammonium sulphate precipitation, dialysis followed by ion exchange chromatography. The purified bromelain from different parts were gone for enzyme kinetics study to get the specific pH, temperature and substrate concentration for the enzyme.

Then peel, pulp & stem bromelain were checked for the enzyme activity, specific activity, enzyme yield, protein concentration in each step of purification. Finally the enzyme activity, enzyme yield and specific activity was more in peel part then other parts (stem & pulp) but the protein concentration was more in the stem part.

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