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APPENDICES

APPENDIX A

Reagents for identification and amylase activity assay

1. Starch and reducing sugar content determination

1.1 7.8 N perchloric acid

70% v/v Perchloric acid (Density 1.70 g/ml) 32.92 ml

70% v/v Perchloric acid was diluted with double-distilled water and adjust the volume to 50 ml.

1.2 0.16 N Sodiumthiosulfate

Sodiumthiosulfate 3.97 g

Dissolve in 50 ml double-distilled water. The mixture is adjusted the volume to 100 ml with double-distilled water.

1.3 2 M NaOH

NaOH 8 g

Dissolve in 50 ml double-distilled water. The mixture is adjusted the volume to 100 ml with double-distilled water.

1.4 DNS (3, 5-dinitro-salicylic acid) reagent

Solution A

3, 5-dinitro-salicylic acid	1.5	g
2 M NaOH	50	ml
Sodium potassium tratrate ($NaKC_4H_4O_6.4H_2O$)	75	g

Dissolve and heat 3, 5-dinitro-salicylic acid in 2 M NaOH. The mixture is adjusted the volume to 125 ml with double-distilled water. The mixture is added with sodium potassium tratrate solution which dissolve in double-distilled water 80 ml and adjusted the volume to 125 ml. The mixture is adjusted the volume to 250 ml with double-distilled water. The mixture is kept in light brown bottle at 4 °C.

2. Protein determination

2.1 Bradford solution

Coomassie brilliant blue G250	100	mg
95% Ethanol	50	ml
85% Phosphoric acid	100	ml

Coomassie brilliant blue G250 is dissolve in 95% ethanol and mixed with 85% Phosphoric acid. The mixture is adjusted the volume to 1,000 ml with double-distilled water and filtered through Whatman No. 1 filter paper.

3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

3.1 30%T, 2.6%C Acrylamide/Bis

Acrylamide	87.6	g
N,N-bis-methylene-Acrylamide	2.4	g

Dissolve in 200 ml double-distilled water. The mixture is adjusted the volume to 300 ml with double-distilled water. When the substances are completely dissolved, filter solution through a 0.45 μm filter. The mixture is kept in light brown bottle at 4°C.

3.2 1.5 M Tris-HCl, pH 8.8

Tris-base	18.17	g

Dissolve in 80 ml double-distilled water and adjust pH to 8.8 with HCL, then adjust the volume to 100 ml with double-distilled water. Store at 4 °C.

3.3 0.5 M Tris-HCl, pH 6.8

Tris-base	6.057	g

Dissolve in 80 ml double-distilled water and adjust pH to 6.8 with HCL, then adjust the volume to 100 ml with double-distilled water. Store at 4 °C.

3.4 10% Sodium Dodecyl Sulphate (SDS)

SDS	10	g

Dissolve in 80 ml double-distilled water and adjust the volume to 100 ml with double-distilled water.

3.5 0.2% Bromophenol blue-Metanol solutions

Bromophenol blue	0.02	g

Dissolve in 10 ml methanol.

3.6 10% Ammonium Persulfate (APS)

APS	0.1	g

Dissolve in 1 ml double-distilled water.

3.7 2×Solubilizing Buffer (SB) without β-Mercaptoethanol

0.5 M Tris-HCl, pH 6.8	2.5	ml
Glycerol	2.0	ml
Bromophenol blue-methanol	0.1	ml
10% SDS	2.0	ml

They were mixed and adjust the volume to 10 ml with double-double-distilled water. Store at 4 °C.

3.8 2×Solubilizing Buffer (SB) with β-Mercaptoethanol

0.5 M Tris-HCl, pH 6.8	2.5	ml
β-Mercaptoethanol	1.0	ml
Glycerol	2.0	ml
Bromophenol blue-methanol	0.1	ml
10% SDS	2.0	ml

They were mixed and adjust the volume to 10 ml with double-distilled water. Store at 4 °C.

3.9 5×Electrode Buffer, pH 8.3

Tris-base	15.0	g
Glycine	72.0	g
SDS	5.0	g

Dissolve in 800 ml double-distilled water and adjust the volume to 1,000 ml with double-distilled water. Store at 4 °C.

3.10 1×Electrode Buffer, pH 8.3

Dilute 80 ml of 5×Electrode buffer, pH 8.3 with double-distilled water to 400 ml.

3.11 0.1% Coomassie Brilliant Blue R-250

Coomassie Brilliant Blue R-250	0.1	g

Dissolve in methanol 40 ml then added with glacial acetic acid 10 ml and double-distilled water 50 ml.

3.12 Destain Solution

Methanol	400	ml
Acetic acid	100	ml

Dissolve in double-distilled water and adjust to 1,000 ml.

3.13 13% Separating Gel SDS-PAGE (1 gel)

Acrylamide/Bis (30%T)	1,734	µl
1.5 M Tris-HCl, pH 8.8	1,000	µl
Double-distilled water	1,200	µl
10% SDS	40	µl
10% APS	26	µl
TEMED	2.5	µl

The chemicals are mixed consecutively and poured into the gel chamber bewaring of air bubbles. Double-distilled water is carefully overlaid above the gel. The mixture is left for three hours for complete polymerization.

3.14 4% Separating Gel SDS-PAGE (1 gel)

Acrylamide/Bis (30%T, 2.6%C)	265	µl
0.5 M Tris-HCl, pH 6.8	500	µl
Double-distilled water	1,215	µl
10% SDS	20	µl
10% APS	12.5	µl
TEMED	6.25	µl

The chemicals are mixed consecutively and poured into the gel chamber bewaring of air bubbles. The comb is inserted and the gel was allowed to stand for one hour for complete polymerization.

3.15 Low molecular weight marker

Low Molecular Weight Marker is dissolved in with 2× Solubilizing buffer with β-mercaptoethanol 100 µl and double-distilled water 100 µl. The mixture is heated at 100 °C for 5 min. Store at 20 °C.

4. Zymographic method

4.1 1 % w/v of soluble starch

Starch	2	g

Dissolve in 40 ml double-distilled water and added to hot double-distilled water (72 °C) 160 ml. Stir for 30 min at 72 °C. Store at 4 °C.

4.2 13% separating gel containing 0.1% w/v of soluble starch as final concentration (1 gel)

Acrylamide/Bis (30%T)	1,734	µl
1.5 M Tris-HCl, pH 8.8	1,000	µl
Double double-distilled water	800	µl
1% Starch solution	400	µl
10% SDS	40	µl
10% APS	26	µl
TEMED	2.5	µl

The chemicals are mixed consecutively and poured into the gel chamber bewaring of air bubbles. Double-distilled water is carefully overlaid above the gel. The mixture is left for three hours for complete polymerization.

4.3 1% triton X-100 solution

Triton X-100	1	ml

Dissolve in double-distilled water 80 ml and adjust the volume to 100 ml with double-distilled water.

4.4 Activity solution (mixture of 0.2% starch with 10 mM Sodium acetate buffer pH 4.8, 2 mM $CaCl_2$)

1 M Sodium acetate buffer pH 4.8	1	ml
1% Soluble starch	20	ml
1 M $CaCl_2$	1	ml

Dissolve in double-distilled water 60 ml and adjust the volume to 100 ml with double-distilled water. Store at 4 °C.

4.5 Activity staining solution (10 mM Iodine, 14 mM KI and 1% Acetic acid)

Acetic acid	1	ml
Iodine	0.126	g
KI	0.232	g

Dissolve in double-distilled water 80 ml and adjust the volume to 100 ml with double-distilled water.

5. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

5.1 Rehydration Solution without IPG Buffer

Containing: 7 M Urea, 2 M Thiourea, 2% CHAPS, 0.002% bromophenol blue.

Urea	10.51	g
Thiourea	3.81	g
CHAPS	0.50	g
Bromophenol blue	50	µl

Dissolve in double-distilled water and adjust the volume to 25 ml. Store at 4°C. DTT and IPG buffer are added just prior to used: 1 mg of DTT and 1µl of IPG buffer per 400 µl of rehydration solution. Store at 20 °C.

5.2 Bromophenol blue solution

Bromophenol blue	100	mg
Tris-base	60	mg

Dissolve in 7 ml of double-distilled water. When the substances are completely dissolved, add double-distilled water to a final volumn of 10 ml.

5.3 SDS equilibration buffer

Containing: 75 mM Tris-HCl pH 8.8, 6 M Urea, 29.3% glycerol, 2% SDS and 0.002% bromophenol blue.

Tris-HCl, pH 8.8	10	ml
Urea	72.10	g
Glycerol	69	ml (84.20 g)
SDS	4.0	g
Bromophenol blue	400	µl

Dissolve in double-distilled water and adjust the volume to 200 ml with double-distilled water. Store at 4°C.

5.4 Agarose sealing slution

Agarose	0.50	g
SDS electrode buffer	100	ml
Bromophenol blue	200	µl

Heat in a microwave oven until the agarose is completely dissolved.

5.5 Fixing solution

Ethanol	500	ml
Phospholic acid	20	ml

Dissolve in double-distilled water and adjust the volume to 1,000 ml with double-distilled water.

5.6 Coomassie brilliant blue G 250 staining solution

Coomassie brilliant blue G 250	1	g
Phosphoric acid	20	ml
Ammonium sulphate	100	g
Methanol	250	ml

Dissolve in double-distilled water and adjust the volume to 1,200 ml with double-distilled water.

APPENDIX B

Identification by Mascot search

1. Step of Predict the protein by Mascot (Matrix science, 2010)

The ion spectra of peptides were searched against NCBI database using MS/MS Ion Search mode of MASCOT (http://www. matrixscience.com/search_form_select. html).

1. Go to the Mascot website (www.matrixscience.com)

This takes you to the Matrix Science homepage, which hosts Mascot, Click the "Mascot" link on the top of the form to go to the Mascot home page. Select the MS/MS Ion search Form. This search use spectra of peptide ion masses from a protein digest to predict the protein sequence. Fill in the fields as follows:

Your name: fill in with your name

Email: your real email address. Mascot will email your results to you if you get disconnected or time out.

Search title: A text string which will be printed at the top of results report pages.

Database: Select the sequence database to be searched. The databases available on the free, public Mascot server are:

Database	Comment
EST	EST divisions of EMBL, (Environmental_EST, Fungi_EST, Human_EST, Invertebrates_EST, Mammals_EST, Mus_EST, Plants_EST, Prokaryotes_EST, Rodents_EST, Vertebrates_EST)
MSDB	MSDB has not been updated since 2006 and should be considered obsolete
NCBInr	Comprehensive, non-identical protein database
SwissProt	High quality, curated protein database
contaminants	Common contaminants compiled by Max Planck Institute of Biochemistry, Martinsried

For a Sequence Query or an MS/MS Ions Search, on the free, public Mascot server, you must search one of the protein databases before searching an EST database. A search of a non-identical protein database takes only a fraction of the time of an EST search. If the protein database search fails to produce a positive match, the master results page will allow you to repeat the search against an EST database. Choose the NCBInr or SwissProt.

Taxonomy: The Taxonomy parameter allows searches to be limited to entries from particular species or groups of species. This can speed up a search, and ensures

that the hit list will only contain entries from the selected species. If the search data are marginal, and you are completely confident of the origin of the protein, this can help bring a weak match to the top of the list.

Allows the searches to be restricted to a specific species or group of species. Use "Green Plants".

Enzyme: the cleavage enzyme used to digest the protein into peptide fragments. Choose "Trypsin".

Missed cleavages: Setting the number of allowed missed cleavage sites to zero simulates a limit digest. If you are confident that your digest is perfect, with no partial fragments present, this will give maximum discrimination and the highest score. If experience shows that your digest mixtures usually include some partials, that is, peptides with missed cleavage sites, you should choose a setting of 1, or maybe 2, 3 missed cleavage sites. Don't specify a higher number without good reason, because each additional level of missed cleavages increases the number of calculated peptide masses to be matched against the experimental data. If the actual digest does not contain extended partials, this simply increases the number of random matches, and so reduces discrimination. Choose "3" allowed missed cleavage site.

Fixed modifications: Mascot supports two types of modification. Fixed modifications are applied universally, to every instance of the specified residue(s) or terminus. There is no computational overhead associated with a fixed modification, it is simply equivalent to using a different mass for the modified residue(s) or terminus. This sample has been treated with iodoacetamide, so in the fixed modifications box, select "Carbamidomethyl (C)".

Variable Modifications: Variable modifications can be a very powerful means of finding a match, but there are also dangers to be aware of. Even a single variable modification will generate many possible additional peptides to be tested. More than one variable modification causes the number of arrangements to increase geometrically. This means that a search can take dramatically longer than the same search with fixed modifications. More importantly, testing all possible arrangements of modifications generates many more random matches, so that discrimination can be sharply reduced. Select "Oxidation (M)".

Peptide Charge

Used to specify the precursor peptide charge state in a sequence query or an MS/MS ions search. The peptide mass value supplied in an MS/MS data file is usually an observed m/z value. The charge state field is used to calculate the relative molecular mass (M_r) of the precursor from the observed m/z *unless* the data file explicitly specifies a different charge state. Choose 1+ , 2+ and 3+

Monoisotopic or Average:

Specify whether the experimental mass values are average or monoisotopic. If you are unsure which to choose, refer to the mass accuracy help page. the monoisotopic mass is the mass determined using the masses of the most abundant isotopes. The average mass is the abundance weighted mass of all isotopic components. Modern mass spectrometers can readily resolve the various isotope peaks of peptides and proteins, so choose Monoisotopic.

Data file: Enter the path to a data file containing mass data. Data for MS/MS ion searches must be supplied as an ASCII file.

Instrument: Instrument for analysis of this data

Report top: the number of hits to return. Select 50.

APPENDIX C

Reagents for purification and enzyme characterization

1. 0.1 M NaOH

NaOH	4	g

Dissolve in double-distilled water and adjust the volume to 1,000 ml.

2. 2.5 % Glucose solution

Glucose	5	g

Dissolve in double-distilled water and adjust the volume to 500 ml.

3. 0.1 M Acetate buffer pH 4.0 containing 0.5 M NaCl

$NaC_2H_3O_2.\ 3H_2O$	2.27	g
NaCl	5.84	g

Dissolve in 100 ml double-distilled water and adjust pH to 4.0 with 1 M Acetic acid, then adjust the volume to 200 ml with double-distilled water.

4. 0.1 M Tris-HCl buffer pH 8.0 containing 0.5 M NaCl

Tris-base	2.42	g
NaCl	5.84	g

Dissolve in 100 ml double-distilled water and adjust pH to 8.0 with 1 M HCl, then adjust the volume to 200 ml with double-distilled water.

5. 1 M Sodium acetate buffer, pH 6.0

$NaC_2H_3O_2.\ 3H_2O$	27.22	g

Dissolve in 100 ml double-distilled water and adjust pH to 6.0 with 1 M Acetic acid, then adjust the volume to 200 ml with double-distilled water.

6. 2 M $CaCl_2$

$CaCl_2.\ 2H_2O$	29.40	g

Dissolve in double-distilled water and adjust the volume to 100 ml.

7. 2 M NaCl

NaCl	11.69	g

Dissolve in double-distilled water and adjust the volume to 100 ml.

8. 50 mM Acetate buffer, pH 6.0 containing 5 mM $CaCl_2$

1 M Acetate buffer pH 6.0	10	ml
2 M $CaCl_2$	0.5	ml

They were mixed and adjust the volume to 200 ml with double-distilled water.

9. 20 mM Acetate buffer, pH 6.0 containing 25 mM $CaCl_2$ and 0.5 M NaCl (Washing buffer)

1 M Acetate buffer pH 6.0	4	ml
2 M $CaCl_2$	2.5	ml
2 M NaCl	50	ml

They were mixed and adjust the volume to 200 ml with double-distilled water.

10. Dialysis bag preparation

10 mM $NaHCO_3$ (0.84 g dissolve with DDW and adjust to 1,000 ml)

10 mM Na_2EDTA (3.72 g dissolve with DDW and adjust to 1,000 ml)

20% Ethanol (40 ml Ethanol dilute and adjust to 200 ml with DDW)

Dialysis bag activation, dialysis bag was cut and wash with double-distilled water. Then, boiling dialysis bag in 10 mM $NaHCO_3$, 80 °C, 30 min and stir. Dialysis bag was wash with double-distilled water. Soak dialysis bag in 10 mM Na_2EDTA, 30 min. Final step, boiling in double-distilled water, 80 °C, 30 min and storage in 20% Ethanol, 4 °C.

11. 0.5 M NaOH

NaOH	20.0	g

Dissolve in double-distilled water and adjust the volume to 1,000 ml.

12. 0.5 M HCl

HCl (37% v/v, density 1.186)	41.52	ml

Dissolve in double-distilled water and adjust the volume to 1,000 ml.

13. 1 M Potassium phosphate buffer, pH 7.5

K_2HPO_4	17.41	g

Dissolve in double-distilled water 50 ml and adjust pH to 7.5 with 1M phosphoric acid then adjust the volume to 100 ml with distilled water.

14. 0.5 M NaCl

NaCl	29.25	g

Dissolve in double-distilled water and adjust the volume to 1,000 ml.

15. 1.5 % w/v of soluble starch

Starch	1.5	g

Dissolve in 20 ml double-distilled water and added to hot double-distilled water (72 °C) 80 ml. Stir for 30 min at 72 °C. Store at 4 °C.

16. 1% Soluble starch solution in 0.1 M phosphate buffer pH 7.0 containing 20 mM NaCl and 2mM $CaCl_2$

1.5 % w/v of soluble starch	66.67	ml
2 M $CaCl_2$	0.1	ml
2 M NaCl	1	ml
1 M phosphate buffer pH 7.0	10	ml

They were mixed and adjust the volume to 100 ml with double-distilled water. Stir for 30 min at 72 °C. Store at 4 °C.

17. 2 M NaOH

NaOH	40.0	g

Dissolve in double-distilled water and adjust the volume to 500 ml.

18. 1 M Sodium acetate buffer, pH 4.0

$NaC_2H_3O_2.\ 3H_2O$ 27.22 g

Dissolve in 100 ml double-distilled water and adjust pH to 4.0 with 1 M Acetic acid, then adjust the volume to 200 ml with double-distilled water.

19. 1 M Sodium acetate buffer, pH 5.0

$NaC_2H_3O_2.\ 3H_2O$ 27.22 g

Dissolve in 100 ml double-distilled water and adjust pH to 5.0 with 1 M Acetic acid, then adjust the volume to 200 ml with double-distilled water.

20. 1 M Sodium acetate buffer, pH 6.0

$NaC_2H_3O_2.\ 3H_2O$ 27.22 g

Dissolve in 100 ml double-distilled water and adjust pH to 6.0 with 1 M Acetic acid, then adjust the volume to 200 ml with double-distilled water.

21. 1 M Potassium phosphate buffer, pH 6

K_2HPO_4 17.41 g

Dissolve in double-distilled water 50 ml and adjust pH to 6.0 with 1M phosphoric acid then adjust the volume to 100 ml with distilled water.

22. 1 M Potassium phosphate buffer, pH 7.0

K_2HPO_4 17.41 g

Dissolve in double-distilled water 50 ml and adjust pH to 7.0 with 1M phosphoric acid then adjust the volume to 100 ml with distilled water.

23. 1 M Potassium phosphate buffer, pH 8.0

K_2HPO_4 17.41 g

Dissolve in double-distilled water 50 ml and adjust pH to 8.0 with 1M phosphoric acid then adjust the volume to 100 ml with distilled water.

24. 1 M Tris-HCl, pH 8.0

Tris-base 12.12 g

Dissolve in 80 ml double-distilled water and adjust pH to 8.0 with 1 M hydrochloric acid, then adjust the volume to 100 ml with double-distilled water.

25. 1 M Tris-HCl, pH 9.0

Tris-base 12.12 g

Dissolve in 80 ml double-distilled water and adjust pH to 9.0 with 1 M hydrochloric acid, then adjust the volume to 100 ml with double-distilled water.

26. 1 M Tris-HCl, pH 10.0

Tris-base 12.12 g

Dissolve in 80 ml double-distilled water and adjust pH to 10.0 with 1 M hydrochloric acid, then adjust the volume to 100 ml with double-distilled water.

27. 1 M $CaCl_2$

$CaCl_2.2H_2O$ 14.70 g

Dissolve in double-distilled water and adjust the volume to 100 ml.

28. 1 M $MnCl_2$

$MnCl_2.4H_2O$ 19.79 g

Dissolve in double-distilled water and adjust the volume to 100 ml.

29. 1 M $ZnCl_2$

$ZnCl_2$ 13.63 g

Dissolve in double-distilled water and adjust the volume to 100 ml.

30. 1 M $CoCl_2.6H_2O$

$CoCl_2.6H_2O$ 23.79 g

Dissolve in double-distilled water and adjust the volume to 100 ml.

31. 1 M NaCl

NaCl	5.84	g

Dissolve in double-distilled water and adjust the volume to 100 ml.

32. 1 M KCl

KCl	7.46	g

Dissolve in double-distilled water and adjust the volume to 100 ml.

33. 1 M EDTA.2H_2O

$MgCl_2 \cdot 6H_2O$	37.22	g

Dissolve in double-distilled water and adjust the volume to 100 ml.

34.Silver Staining

Gels are stained according to the following protocol:

Step	Solutions	Amount	Time
Fixation	Ethanol	25 ml	30 min
	Glacial acetic acid	6.25 ml	
	Double distilled water	31 ml	
Sensitizing	Ethanol	18.75 ml	30 min
	Glutardialdehyde	313 µl	
	Sodium thiosulphate	2.5 ml	
	Sodium acetate	4.25 g	
	Double distilled water	41 ml	
Washing	Double distilled water		3×5 min
Silver reaction	Silver nitrate solution	6.25 ml	20 min
	Formaldehyde	25 µl	
	Double distilled water	56 ml	
Washing	Double distilled water		2×1 min
Developing	Sodium carbonate	1.56 g	2-5 min
	Formaldehyde	13 µl	
	Double distilled water	62.5 ml	
Stop	EDTA-Na_2	0.912 g	
	Double distilled water	62.5 ml	

APPENDIX D

The data and calculation

Table A1 Absorbance at 540 nm of glucose as standard solution by DNS method for analyze amylase activity

Glucose amount (µg/ml)	Absorbance 540 nm			$\bar{X}$	SD
	1	2	3		
200	0.205	0.2	0.193	0.199	0.006028
400	0.467	0.477	0.465	0.470	0.006429
600	0.954	0.752	0.728	0.811	0.124134
800	1.011	0.986	1.025	1.007	0.019757
1000	1.293	1.299	1.283	1.292	0.008083

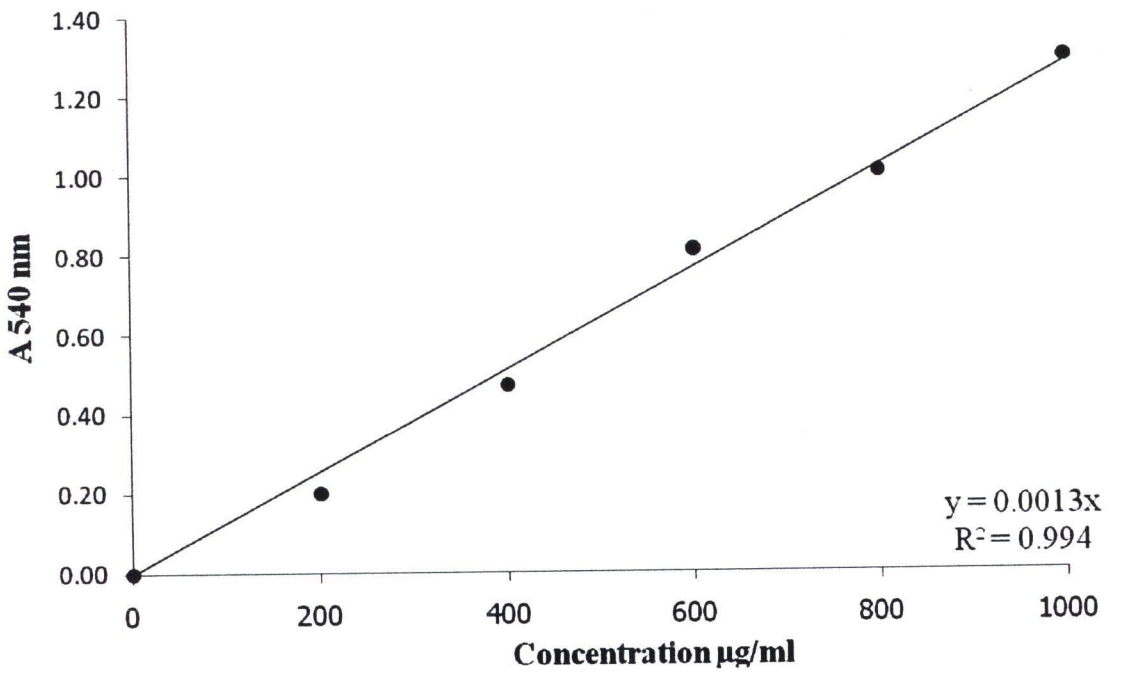

Figure A3 Glucose calibration curve

2. Determination of amylase activity

One unit of enzyme was defined as the amount of enzyme that produced 1 μmol of reduced sugar per minute under standard assay conditions.

Unit/ml = A × B / C × D× E

A = Amount of reducing sugar (μg/ml)

B = Volume of reaction (ml)

C = Molecular weight of glucose standard (g/mol)

D = Time of reaction (min)

E = Volume of enzyme (ml)

Unit/ml = A × 1.5 / 180 × 15 × 0.25
= A × 1.5 / 675

2.1 Calculation of specific activity

$$\frac{\text{Total activity}}{\text{Total protein}}$$

2.2 Calculation of purification fold

$$\frac{\text{Specific activity}}{\text{Specific activity of crude}}$$

2.3 Calculation of yield (%)

$$\frac{\text{Total activity} \times 100}{\text{Total activity of crude}}$$

Table A2 The amylase activity was determined in different pH from 4 to 10 by DNS method at 37 °C.

Buffer	Control					Test					Test-control	Reducing sugar µg/ml	Activity (Unit/ml)	% Relative activity
	1	2	3	$\bar{X}$	SD	1	2	3	$\bar{X}$	SD				
Sodium acetate														
pH 4	0.080	0.083	0.084	0.082	0.002	0.092	0.102	0.098	0.097	0.005	0.015	230.769	0.513	5.97
pH 5	0.096	0.099	0.101	0.099	0.003	0.151	0.153	0.159	0.154	0.004	0.056	856.410	1.903	22.15
pH 6	0.106	0.108	0.104	0.106	0.002	0.347	0.348	0.342	0.346	0.003	0.240	3687.179	8.194	95.35
Potassium phosphate														
pH 6	0.118	0.116	0.122	0.119	0.015	0.365	0.360	0.368	0.364	0.004	0.246	3780.000	8.400	97.75
pH 7	0.123	0.123	0.125	0.124	0.003	0.378	0.373	0.374	0.375	0.003	0.251	3866.667	8.593	100.00
pH 8	0.135	0.140	0.138	0.138	0.002	0.355	0.350	0.357	0.354	0.004	0.216	3328.205	7.396	86.07
Tris-HCl														
pH 8	0.131	0.135	0.138	0.135	0.001	0.345	0.340	0.342	0.342	0.003	0.208	3194.872	7.100	82.62
pH 9	0.144	0.140	0.138	0.141	0.011	0.228	0.229	0.224	0.227	0.003	0.086	1328.205	2.952	34.35
pH 10	0.100	0.102	0.104	0.102	0.005	0.123	0.133	0.125	0.127	0.005	0.025	384.615	0.855	9.95

Table A3 The amylase activity was determined in different temperature from 30 to 100 °C by DNS method at pH 7.0.

Temperature (°C)	Control					Test					Test-control	Reducing sugar µg/ml	Activity (Unit/ml)	Relative activity
	1	2	3	$\bar{X}$	SD	1	2	3	$\bar{X}$	SD				
30	0.156	0.154	0.158	0.156	0.002	0.338	0.349	0.345	0.344	0.006	0.188	2892.307	6.427	73.05
37	0.152	0.150	0.145	0.149	0.004	0.373	0.377	0.375	0.375	0.002	0.226	3476.923	7.726	87.82
40	0.138	0.140	0.136	0.138	0.002	0.385	0.406	0.395	0.395	0.011	0.257	3958.974	8.798	100.00
50	0.147	0.146	0.146	0.146	0.001	0.394	0.398	0.395	0.396	0.002	0.249	3835.897	8.524	96.89
60	0.146	0.145	0.146	0.146	0.001	0.398	0.394	0.378	0.390	0.011	0.244	3758.974	8.353	94.95
70	0.126	0.126	0.127	0.126	0.001	0.396	0.331	0.350	0.359	0.033	0.233	3579.487	7.954	90.41
80	0.107	0.113	0.130	0.117	0.012	0.279	0.279	0.259	0.265	0.012	0.148	2282.051	5.071	57.64
90	0.126	0.126	0.128	0.127	0.001	0.252	0.252	0.254	0.253	0.001	0.126	1938.461	4.308	48.96

APPENDIX F

Research Publications

RESEARCH PUBLICATIONS

Poster presentation and proceeding

Saijai Posoongnoen, Withan Teajaroen, Raksmont Ubonbal, Nunthawun Uawonggul, Sompong Thammasirirak, Jureerut Daduang, Sakda Daduang. Amylase activity and proteomic patterns in Monthong Durian (*Durio zibethinus* Murr. cv. Monthong). **The 36 th Congress on Science and Technology of Thailand (STT 36)**, 2010, October 26-28, Bangkok, Thailand.

Poster presentation

Saijai Posoongnoen, Withan Teajaroen, Wuttichai chanmoontree, Raksmont Ubonba, Nunthawun Uawonggul, Sompong Thammasirirak, Jureerut Daduang and Sakda Daduang. Study of amylase activity in durian (*Durio zibethinus* Murr. cv. Monthong). **The 35th Congress on Science and Technology of Thailand (STT 35)**, 2009, October 15-17, Chonburi, Thailand.

Saijai Posoongnoen, Raksmont Ubonbal, Muchalin Meunchan, Sophida Sukprasert, Sompong Thammasirirak, Jureerut Daduang and Sakda Daduang. Study of amylase activity in Monthong durian (*Durio zibethinus* Murr. cv. Monthong). **The 6 th Conference on Science and Technology for Youths** , 2011, March 18-19, Bangkok, Thailand.

Saijai Posoongnoen, Raksmont Ubonbal, Muchalin Meunchan, Withan Teajaroen, Sophida Sukprasert, Sompong Thammasirirak, Jureerut Daduang and Sakda Daduang. Partial purification of amylase from Monthong durian (*Durio zibethinus* Murr. cv. Monthong). **The Third International Conference on Science and Technology for Sustainable Developmentof the Greater Mekong Sub-region (3rd STGMS) The Second International Conference on Applied Science (2nd ICAS)**, 2011, March 24-25, Souphanouvong University, Luang Prabang, LAO People's Democratic Republic.

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