APPENDICES

Appendix A Shrimp Identification

LEMBAGA ILMU PENGETAHUAN INDONESIA
(INDONESIAN INSTITUTE OF SCIENCES)
PUSAT PENELITIAN OSEANOGRAFI
(RESEARCH CENTER FOR OCEANOGRAPHY)
Jl. Pasir Putih I, Ancol Timur, Jakarta 14430, PO. Box : 4801/JKTF Jakarta 11048
Telepon : (021) 64713850, Fax. : (021) 64711948
Homepage : http://www.oseanografi.lipi.go.id, E-mail : humas@oseanografi.lipi.go.id

Nomor : B- 761/IPK.2/IF.01/II/2018 Jakarta, 26 Februari 2018
Lamp. : --
Perihal : Permohonan Identifikasi

Yth.
Ketua Program Studi Teknologi Pangan
Fakultas Sains dan Teknologi
Universitas Pelita Harapan
UPH Global Campus,Lippo Karawaci
Tangerang 15811

Menjawab surat tugas Saudara Nomor : 17/TP-FaST-UPH/I/2018 tanggal 12 Januari 2018 dalam rangka identifikasi limbah kulit dan kepala udang windu pada prinsipnya kami dapat membantu. Setelah dilakukan identifikasi oleh staf peneliti kami Sdr. Rianta Pratiwi, M.Sc (Laboratorium Crustacea) diperoleh hasil identifikasi seperti di bawah ini :

Kingdom	:	Animalia
Phylum	:	Arthopoda
Sub-Phylum	:	Crustacea
Class	:	Malacostraca
Sub-class	:	Eumalacostraca
Super-order	:	Eucarida
Order	:	Decapoda
Sub-order	:	Dendrobranchiata
Super-family	:	Penaeoidea
Family	:	Penaeidae
Genus	:	*Penaeus*
Species	:	*Penaeus monodon* Fabricius, 1798
Typetaxon of	:	*Penaeus* Fabricius, 1798
Parent	:	*Penaeus* Fabricius, 1798
Orig. name	:	Penaeus monodon Fabricius, 1798
Synonymised names	:	*Penaeus bubulus* Kubo, 1949 (synonym)
		Penaeus carinatus Dana, 1852
		Penaeus coeruleus Stebbing, 1905 (synonym)
		Penaeus durbani Stebbing, 1917
		Penaeus semisulcatus exsulcatus Hilgendorf, 1879
		Penaeus tahitensis Heller, 1862

Atas perhatian dan kerjasama Saudara kami ucapkan terima kasih.

Kepala Pusat Penelitian Oseanografi LIPI

Dr. Dirhamsyah, MA.

Appendix B Moisture Content Analysis

Table B-1. Moisture content of shrimp powder

Replication	Weight of Ev. Dish (g)	Initial weight of sample (g)	Final weight of sample + ev. Dish (g)	Final weight of sample (g)	Moisture Content (%db)	Moisture Content (%wb)
1	26.7558	5.0532	31.3399	4.5841	10.23	9.28
	18.7958	5.3488	23.6199	4.8241	10.88	9.81
2	26.2195	4.8580	30.5995	4.3800	10.91	9.84
	21.4712	3.0007	24.1780	2.7068	10.86	9.79
3	21.5880	3.0009	24.2775	2.6895	11.58	10.38
	18.3240	3.0006	21.0173	2.6933	11.41	10.24
				Average	10.98 ± 0.48	9.89 ± 0.39

Example Calculation:

Moisture content (%db)

$$= \frac{\text{Initial weight of sample - final weight of sample}}{\text{final weight of sample}} \text{x100}$$

$$= \frac{5.0532 - 4.5841}{4.5841} \text{x100}$$

= 10.23%

Table B-2. Moisture content of isolated chitin powder

Replication	Weight of Ev. Dish (g)	Initial weight of sample (g)	Final weight of sample + ev. Dish (g)	Final weight of sample (g)	Moisture Content (%db)	Moisture Content (%wb)
1	26.8994	2.4842	29.2664	2.3670	4.95	4.72
	20.6749	2.8147	23.3453	2.6704	5.40	5.13
2	28.2290	3.0601	31.1352	2.9062	5.30	5.03
	40.3555	3.0008	43.2343	2.8788	4.24	4.07
3	44.5486	3.0007	47.4221	2.8735	4.43	4.24
	39.5911	3.0006	42.4612	2.8701	4.55	4.35
				Average	4.81 ± 0.48	4.59 ± 0.44

Appendix C Ash Content Analysis

Table C-1 Ash content of shrimp powder

Replication	Weight of Crucible (g)	Weight of sample (g)	Weight of sample + Crucible after ashing (g)	Final weight of sample (g)	Ash Content (%)
1	22.2207	4.3307	24.5080	2.2873	52.82
	21.3858	4.8335	23.9237	2.5379	52.51
2	20.3079	4.5912	22.7756	2.4677	53.75
	27.9271	5.1236	30.2795	2.3524	45.91
3	23.2670	4.8636	25.5447	2.2777	46.83
	20.8424	4.7304	23.0408	2.1984	46.47
				Average	49.72 ± 3.66

Example Calculation:

Ash content

$$= \frac{24.5080 - 22.2207}{4.3307} x100$$

= 52.82%

Table C-2 Ash content of isolated chitin

Replication	Weight of Crucible (g)	Weight of sample (g)	Weight of sample + Crucible after ashing (g)	Final weight of sample (g)	Ash Content (%)
1	36.0517	5.0006	36.0740	0.0223	0.45
	36.2120	5.0007	36.2321	0.0201	0.40
2	40.9396	5.0005	40.9633	0.0237	0.47
	20.5231	5.0005	20.5470	0.0239	0.48
3	20.3007	5.0004	20.3231	0.0224	0.45
	20.8255	5.0001	20.8483	0.0228	0.47
				Average	0.45 ± 0.03

Appendix D Yield determination

Table D-1. Yield of isolated chitin

Replication	Initial weight of shrimp (g)	Weight of dry shrimp shell (g)	Weight of chitin powder (g)	Yield (%db)	Yield (%wb)
1	1,000	392	91.76	23.41	9.18
2	1,000	383	88.54	23.12	8.85
3	1,000	378	85.22	22.54	8.52
		Average		23.02 ± 0.44	8.85 ± 0.33

Example of calculation

Yield (%wb)

$$= \frac{\text{weight of chitin powder}}{\text{Initial weight of shrimp}} \text{x}100$$

$$= \frac{91.76}{1000} \text{x}100 = 9.18\%$$

Yield (%db)

$$= \frac{\text{weight of chitin powder}}{\text{weight of dry shrimp shell}} \text{x}100$$

$$= \frac{91.76}{392} \text{x}100 = 23.41\%$$

The picture of steps to isolate chitin from shrimp shell and head of *P. monodon* were presented below.

Figure D-1. Head of *P. monodon* from PT Lola Mina

Figure D-2. Shrimp shell after 2 days of drying

Figure D-3. Shrimp shell powder

Figure D-4. Demineralization process

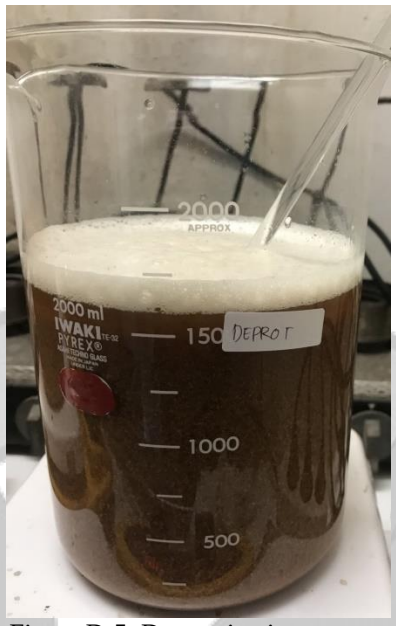


Figure D-5. Deproteination process


Figure D-6. Isolated chitin powder

Appendix E Degree of Deacetylation of Chitin Powder

LABORATORIUM PUSAT PENELITIAN FISIKA
PUSAT PENELITIAN FISIKA
LEMBAGA ILMU PENGETAHUAN INDONESIA
Komplek PUSPIPTEK Gd 440-442 Tangerang Selatan 15314
Tel. 021-7560570 Fax. 021-7560554

LIPI

KAN

LAPORAN HASIL PENGUJIAN

No. Kendali: EK/2018/04/0358/FTIR/0064

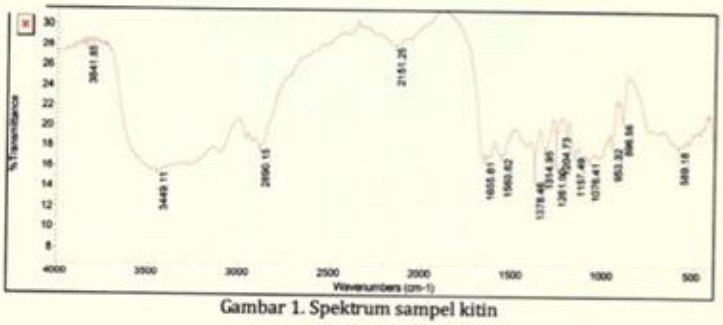
Pemakai Jasa *Customer*	: Gerardo K. Liguna	Pengujian *Tested for*	: Analisis kualitatif spektrum IR
Akamat Pemakai Jasa *Customer Address*	:UPH	Bahan *Material*	: kitin (1 Sampel)
Tanggal Penerimaan Sampel *Date of Sample Received*	: 12-04-2018	Metoda *Method*	: IK- 9
Tanggal Pengujian Sampel *Date of Sample Tested*	:13-04-2018		

Kondisi Pengukuran/Parameter Pengujian *Measurement Conditions/Testing Parameters*

Spektrum	:	Infra Red
Rentang Pengkuran	:	4000 – 400 cm^{-1}
Absolute threshold	:	94.985
Sensitivitas	:	50

Hasil Pengujian *Testing Results* :

Sampel 1 : Kitin



Gambar 1. Spektrum sampel kitin

ORIGINAL

EK/2018/04/0358/FTIR/0064 Hal 1 dari 3

LABORATORIUM PUSAT PENELITIAN FISIKA
PUSAT PENELITIAN FISIKA
LEMBAGA ILMU PENGETAHUAN INDONESIA
Kompleks PUSPIPTEK Gd 440-442 Tangerang Selatan 15314
Tel. 021-7560570 Fax. 021-7560554

LAPORAN HASIL PENGUJIAN

Tabel 1. Rincian puncak karakteristik sampel kitin

Position:	589.18	Intensity:	18.232
Position:	896.56	Intensity:	20.906
Position:	953.32	Intensity:	18.498
Position:	1076.41	Intensity:	16.952
Position:	1157.49	Intensity:	17.385
Position:	1204.73	Intensity:	19.391
Position:	1261.00	Intensity:	19.615
Position:	1314.95	Intensity:	18.050
Position:	1378.46	Intensity:	17.435
Position:	1560.62	Intensity:	17.105
Position:	1655.81	Intensity:	16.904
Position:	2151.25	Intensity:	28.186
Position:	2890.15	Intensity:	17.926
Position:	3449.11	Intensity:	15.592
Position:	3841.85	Intensity:	28.040

ORIGINAL

EK/2018/04/0358/FTIR/0064 Hal 2 dari 3

Example of calculation:
A1655 = -log (16,904/100) = 0.77
A3450 = -log (15,592/100) = 0.81

$$DD(\%)=100 \times \left(1-\frac{\left(\frac{0.77}{0.81}\right)}{1.33}\right)$$

$$= 28.08\%$$

E-2. Surat Pernyataan Hasil Analisis *Degree of Deacetylation*

SURAT PERNYATAAN

Saya yang bertanda tangan di bawah ini:

Nama : Gerardo Kevin Liguna

NIM : 00000009112

Program Studi : Teknologi Pangan

Dengan ini menyatakan bahwa analisis kualitatif spektrum IR yang dilakukan oleh Lembaga Ilmu Pengetahuan Indonesia (LIPI) merupakan sampel penelitian dari:

1. Gerardo Kevin Liguna (00000009112)
2. Nicholas Candra (0000006612)
3. Reinald Febryanto Pengalila (00000006538)

dan digunakan bersama-sama. Hasil analisis yang diterima dari LIPI ditunjukan ke saya a.n. Gerardo Kevin Liguna sebagai perwakilan dari ketiga nama diatas.

Demikian surat pernyataan ini dibuat dengan sebenar-benarnya tanpa paksaan agar dapat digunakan sebagaimana mestinya.

Yang membuat pernyataan

Gerardo Kevin Liguna

Appendix F Hemocytometer Spore Counting

Chamber	Square 1	Square 2	Square 3	Square 4	Total Spore	Average Total Spore	Spore/mL	Average
Upper	1160	985	1277	1250	4672	1168	1.168×10^7	1.168×10^7
Bellow	1265	1100	1201	1106	4672	1168	1.168×10^7	

Df = 1 (mix of spore suspension and methylene blue in ratio 1:1)

Example of calculation

Spores/mL = average number of spores from 4 large square x df x 10^4

Spores/mL = $1168 \times 1 \times 10^4$

= 1.168×10^7 Spores/mL

= 1.2×10^7 Spores/mL

Appendix G Chitinolytic Index

Replication	Diameter of purple zone (cm)	Diameter of colony (cm)	Chitinolytic Index	Average of Chitinolytic Index
1	7.60	7.95	0.96	0.96 ± 0.01
	7.64	7.95	0.96	
2	7.75	7.95	0.97	
	7.74	7.95	0.97	
3	7.65	7.95	0.96	
	7.61	7.95	0.96	

Example of calculation

$$\text{Chitinolytic Index} = \frac{\text{Diameter of purple zone}}{\text{Diameter of colony}}$$

$$\text{Chitinolytic Index} = \frac{7.60}{7.95} = 0.96$$

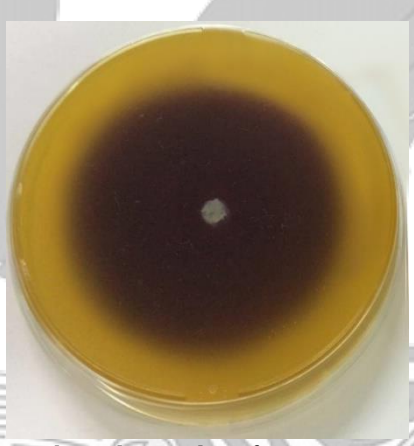


Figure G-1 Chitinolytic index of *Mucor circinelloides*

Appendix H Protein Content of Shrimp Powder and Isolated Chitin Powder

Table H-1 Standard Curve of BSA

Concentration of BSA (mg/mL)	Absorbance 595nm (A)	
	Replication	Average Abs (A)
	0.247	
0.2	0.277	0.265
	0.271	
	0.358	
0.4	0.387	0.371
	0.367	
	0.613	
0.6	0.634	0.632
	0.648	
	0.784	
0.8	0.753	0.78
	0.803	
	0.952	
1	0.978	0.954
	0.933	

From the data above, the standard curve can be formed, and the equation can be used to determine protein content of shrimp powder and isolated chitin powder.

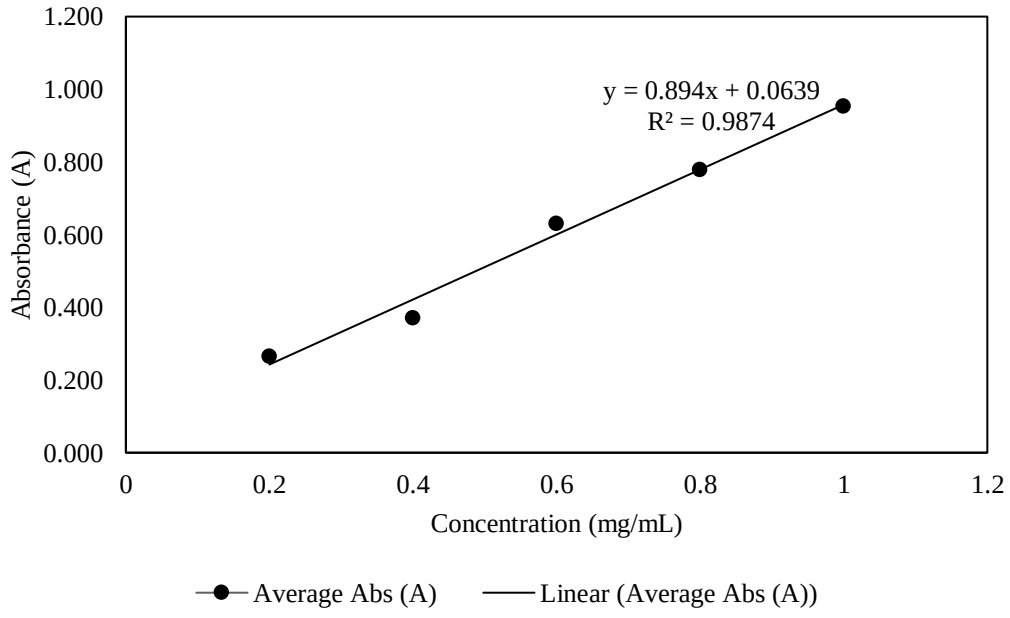


Figure C-1. Bradford Standard Curve

Table H-2 Protein content of shrimp shell powder

Replication	Absorbance (A)	X (mg/mL)	DF	Weight of Protein (g)	Weight of sample	Protein Content (%)	Average Duplo	Average Replication
1	0.855	0.8849	100	1.6592	5.0005	33.18	34.63	
	0.924	0.9622	100	1.8041	5.0001	36.08		
2	0.878	0.9107	100	1.7076	5.0002	34.15	33.65	34.84 ± 1.30
	0.854	0.8838	100	1.6571	5.0008	33.14		
3	0.937	0.9767	100	1.8313	5.0003	36.62	36.23	
	0.918	0.9555	100	1.7916	5.0000	35.83		

Example Calculation

Y = 0.894x + 0.0639

0.855 = 0.894x + 0.0639

0.7911 = 0.894x

X = 0.8849 mg/mL x df

X = 0.8849 x 100

X = 88.49 mg/mL (sample taken in 18.75 mL)

Protein in sample = 88.49 mg/mL x 18.75 mL

= 1659.2 mg

= 1.6592 g

Protein content

$= \frac{1.6592}{5.0005} x100 = 33.18\%$

Table H-3 Protein content of isolated chitin powder

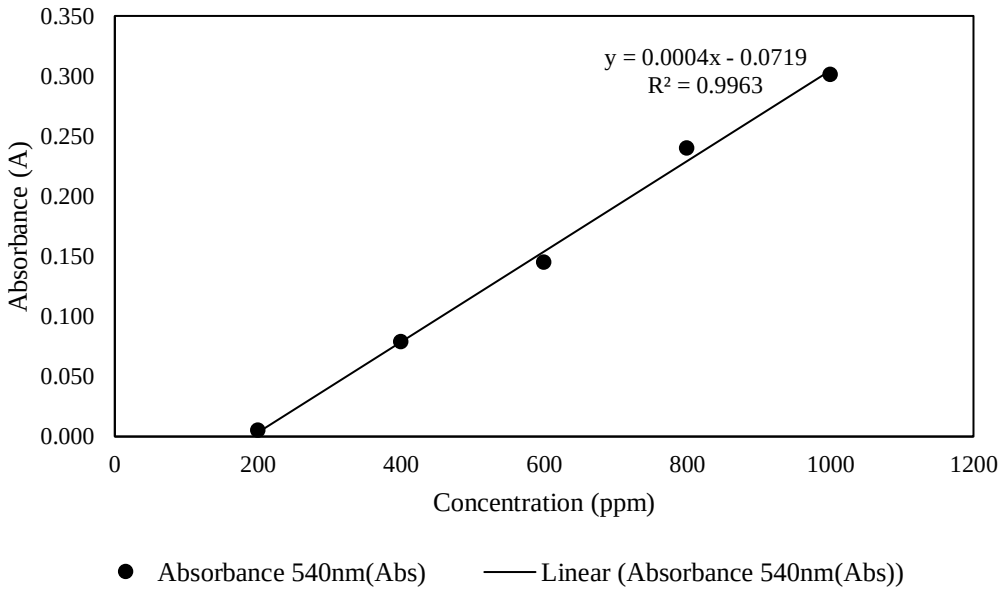
Replication	Absorbance (A)	X (mg/mL)	DF	Weight of Protein (g)	Weight of sample	Protein Content (%)	Average Duplo	Average Replication
1	0.237	0.1933	25	0.0906	5.0000	1.81	1.80	
	0.234	0.1899	25	0.0890	5.0002	1.78		
2	0.176	0.1250	25	0.0586	5.0007	1.17	1.30	1.64 ± 0.29
	0.201	0.1530	25	0.0717	5.0003	1.43		
3	0.254	0.2123	25	0.0995	5.0004	1.99	1.82	
	0.221	0.1754	25	0.0822	5.0001	1.64		

Appendix I N-Acetyl-Glucosamine Standard Curve

Table I-1. Absorbance of N-Acetyl-Glucosamine Standard

Concentration (ppm)	Absorbance 540nm(Abs)
200	0.005
400	0.079
600	0.145
800	0.240
1000	0.301

From the data above, the standard curve of N-Acetyl-Glucosamine can be formed, and the equation can be used to determine the amount of glucosamine in the result of first and second stage of research.



Appendix J Effect of pH and Temperature on Chitinase Enzyme Activity

Table J-1. Absorbance for optimum pH determination

pH	Replication	Absorbance (A)	Concentration	Activity (U/mL)	Average Duplo (U/mL)	Average Replication (U/mL)
3	1	0.201	437.50	1.98	2.01	2.03 ± 0.07
		0.202	450.00	2.03		
	2	0.203	462.50	2.09	2.12	
		0.204	475.00	2.15		
	3	0.201	437.50	1.98	1.98	
		0.201	437.50	1.98		
4	1	0.213	587.50	2.66	2.68	2.64 ± 0.06
		0.214	600.00	2.71		
	2	0.211	562.50	2.54	2.57	
		0.212	575.00	2.60		
	3	0.213	587.50	2.66	2.66	
		0.213	587.50	2.66		
5	1	0.219	662.50	3.00	2.97	2.96 ± 0.05
		0.218	650.00	2.94		
	2	0.218	650.00	2.94	2.97	
		0.219	662.50	3.00		
	3	0.217	637.50	2.88	2.94	
		0.219	662.50	3.00		
6	1	0.236	875.00	3.96	3.90	3.92 ± 0.05
		0.234	850.00	3.84		
	2	0.235	862.50	3.90	3.93	
		0.236	875.00	3.96		
	3	0.236	875.00	3.96	3.93	
		0.235	862.50	3.90		
7	1	0.238	900.00	4.07	4.07	4.04 ± 0.03
		0.238	900.00	4.07		
	2	0.237	887.50	4.01	4.04	
		0.238	900.00	4.07		
	3	0.237	887.50	4.01	4.01	
		0.237	887.50	4.01		
8	1	0.243	962.50	4.35	4.32	4.38 ± 0.06
		0.242	950.00	4.29		
	2	0.243	962.50	4.35	4.38	
		0.244	975.00	4.41		
	3	0.244	975.00	4.41	4.44	
		0.245	987.50	4.46		
9	1	0.233	837.50	3.79	3.76	3.74 ± 0.06
		0.232	825.00	3.73		
	2	0.231	812.50	3.67	3.73	
		0.233	837.50	3.79		
	3	0.233	837.50	3.79	3.73	
		0.231	812.50	3.67		

Control = 0.166A (594.75 ppm), df = 5

Example of calculation

Y = 0.0004x – 0.0719

0.201 = 0.0004x – 0.0719

0.2729 = 0.0004x

X = 682.25 ppm

Chitinase concentration = (Concentration – Control) x df

= (682.25 – 594.75) x 5

= 437.50 ppm

Chitinase Activity

$$= \frac{437.50}{221.2} = 1.98 \text{ U/mL}$$

Table J-2. Absorbance for optimum temperature determination

Temperature	Replication	Absorbance (A)	Concentration	Activity (U/mL)	Average Duplo (U/mL)	Activity Replication (U/mL)
30	1	0.246	1000.00	4.52	4.46	4.42 ± 0.07
		0.244	975.00	4.41		
	2	0.243	962.50	4.35	4.35	
		0.243	962.50	4.35		
	3	0.245	987.50	4.46	4.44	
		0.244	975.00	4.41		
40	1	0.250	1050.00	4.75	4.78	4.80 ± 0.04
		0.251	1062.50	4.80		
	2	0.252	1075.00	4.86	4.83	
		0.251	1062.50	4.80		
	3	0.251	1062.50	4.80	4.80	
		0.251	1062.50	4.80		
50	1	0.261	1187.50	5.37	5.37	5.42 ± 0.06
		0.261	1187.50	5.37		
	2	0.262	1200.00	5.42	5.45	
		0.263	1212.50	5.48		
	3	0.263	1212.50	5.48	5.42	
		0.261	1187.50	5.37		
60	1	0.243	962.50	4.35	4.32	4.38 ± 0.06
		0.242	950.00	4.29		
	2	0.243	962.50	4.35	4.38	
		0.244	975.00	4.41		
	3	0.244	975.00	4.41	4.44	
		0.245	987.50	4.46		
70	1	0.241	937.50	4.24	4.27	4.23 ± 0.08
		0.242	950.00	4.29		
	2	0.243	962.50	4.35	4.27	
		0.240	925.00	4.18		
	3	0.240	925.00	4.18	4.15	
		0.239	912.50	4.13		
80	1	0.236	875.00	3.96	3.90	3.92 ± 0.05
		0.234	850.00	3.84		
	2	0.235	862.50	3.90	3.93	
		0.236	875.00	3.96		
	3	0.236	875.00	3.96	3.93	
		0.235	862.50	3.90		

Control = 0.166A (594.75 ppm), df = 5

Appendix K Effect of Substrate Concentration and Fermentation Time toward N-Acetyl-Glucosamine Production

Table K-1. Absorbance of N-Acetyl-glucosamine production

Time (Hours)	Replication	Absorbance (A)			
		Substrate Concentration (%)			
		0.5	1	1.5	2
2	1	0.334	0.341	0.353	0.349
		0.332	0.342	0.353	0.348
	2	0.339	0.345	0.355	0.350
		0.338	0.348	0.352	0.351
	3	0.335	0.344	0.354	0.349
		0.331	0.341	0.355	0.348
4	1	0.303	0.310	0.295	0.290
		0.299	0.311	0.296	0.291
	2	0.301	0.313	0.295	0.291
		0.298	0.311	0.296	0.292
	3	0.302	0.312	0.297	0.293
		0.301	0.311	0.294	0.294
6	1	0.284	0.294	0.281	0.280
		0.279	0.294	0.282	0.279
	2	0.282	0.296	0.284	0.281
		0.281	0.297	0.285	0.278
	3	0.279	0.298	0.282	0.281
		0.282	0.294	0.281	0.280
24	1	0.253	0.266	0.255	0.235
		0.255	0.268	0.253	0.236
	2	0.249	0.265	0.256	0.238
		0.251	0.266	0.252	0.237
	3	0.251	0.267	0.252	0.239
		0.252	0.268	0.250	0.238

Control = 0.178A (624.75 ppm), df = 5

Example of calculation

Y = 0.0004x – 0.0719

0.334 = 0.0004x – 0.0719

0.4059 = 0.0004x

X = 1,014.75 ppm

NAG concentration = (Concentration – Control) x df

= (1,014.75 – 624.75) x 5

= 1,950 ppm

Table K-2 Concentration of N-acetyl-glucosamine

Time (Hours)	Replication	Concentration (ppm)				Average Con Duplo (ppm)				Average Con Replication (ppm)			
		Substrate Concentration (%)				Substrate Concentration (%)				Substrate Concentration (%)			
		0.5	1	1.5	2	0.5	1	1.5	2	0.5	1	1.5	2
2	1	1950.00	2037.50	2187.50	2137.50	1937.50	2043.75	2187.50	2131.25	1960.42	2068.75	2195.83	2139.58
		1925.00	2050.00	2187.50	2125.00								
	2	2012.50	2087.50	2212.50	2150.00	2006.25	2106.25	2193.75	2156.25				
		2000.00	2125.00	2175.00	2162.50								
	3	1962.50	2075.00	2200.00	2137.50	1937.50	2056.25	2206.25	2131.25				
		1912.50	2037.50	2212.50	2125.00								
4	1	1562.50	1650.00	1462.50	1400.00	1537.50	1656.25	1468.75	1406.25	1533.33	1666.67	1468.75	1422.92
		1512.50	1662.50	1475.00	1412.50								
	2	1537.50	1687.50	1462.50	1412.50	1518.75	1675.00	1468.75	1418.75				
		1500.00	1662.50	1475.00	1425.00								
	3	1550.00	1675.00	1487.50	1437.50	1543.75	1668.75	1468.75	1443.75				
		1537.50	1662.50	1450.00	1450.00								
6	1	1325.00	1450.00	1287.50	1275.00	1293.75	1450.00	1293.75	1268.75	1289.58	1468.75	1306.25	1272.92
		1262.50	1450.00	1300.00	1262.50								
	2	1300.00	1475.00	1325.00	1287.50	1293.75	1481.25	1331.25	1268.75				
		1287.50	1487.50	1337.50	1250.00								
	3	1262.50	1500.00	1300.00	1287.50	1281.25	1475.00	1293.75	1281.25				
		1300.00	1450.00	1287.50	1275.00								
24	1	937.50	1100.00	962.50	712.50	950.00	1112.50	950.00	718.75	922.92	1108.33	937.50	739.58
		962.50	1125.00	937.50	725.00								
	2	887.50	1087.50	975.00	750.00	900.00	1093.75	950.00	743.75				
		912.50	1100.00	925.00	737.50								
	3	912.50	1112.50	925.00	762.50	918.75	1118.75	912.50	756.25				
		925.00	1125.00	900.00	750.00								

Control = 0.178A (624.75 ppm), df = 5

Appendix L Statistical Analysis of Second Stage

Table L-1. Statistical Result of N-Acetyl-glucosamine production in Varying Substrate Concentration and Fermentation Time

Descriptive Statistics

Dependent Variable: NAG_Concentration

Substrate_Concentration	Fermentation_Time	Mean	Std. Deviation	N
0.5%	2Hours	1960.4167	39.69283	3
	4Hours	1533.3333	13.01041	3
	6Hours	1289.5833	7.21688	3
	24Hours	922.9167	25.25907	3
	Total	1426.5625	394.43145	12
1%	2Hours	2068.7500	33.07189	3
	4Hours	1666.6667	9.54703	3
	6Hours	1468.7500	16.53595	3
	24Hours	1108.3333	13.01041	3
	Total	1578.1250	362.68119	12
1.5%	2Hours	2195.8333	9.54703	3
	4Hours	1468.7500	.00000	3
	6Hours	1306.2500	21.65064	3
	24Hours	937.5000	21.65064	3
	Total	1477.0833	477.97612	12
2%	2Hours	2139.5833	14.43376	3
	4Hours	1422.9167	19.09407	3
	6Hours	1272.9167	7.21688	3
	24Hours	739.5833	19.09407	3
	Total	1393.7500	522.31456	12
Total	2Hours	2091.1458	94.69067	12
	4Hours	1522.9167	96.46940	12
	6Hours	1334.3750	82.89420	12
	24Hours	927.0833	137.40527	12
	Total	1468.8802	435.29171	48

Table L-2 Test of Between-Subjects Effects

Dependent Variable: NAG_Concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8893345.540[a]	15	592889.703	1560.049	.000
Intercept	103565235.189	1	103565235.189	272507.411	.000
Substrate_Concentration	233244.629	3	77748.210	204.576	.000
Fermentation_Time	8421239.421	3	2807079.807	7386.166	.000
Substrate_Concentration * Fermentation_Time	238861.491	9	26540.166	69.834	.000
Error	12161.458	32	380.046		
Total	112470742.188	48			
Corrected Total	8905506.999	47			

a. R Squared = .999 (Adjusted R Squared = .998)

From statistical result there is a significant difference between fermentation time and substrate concentration ($p<0.05$)

Appendix M Statistical Interaction between Substrate Concentration and Fermentation Time

NAG_Concentration

Duncan[a]

Interaction	N	Subset for alpha = 0.05											
		1	2	3	4	5	6	7	8	9	10	11	12
2%*24Hours	3	739.5833											
0.5%*24Hours	3		922.9167										
1.5%*24Hours	3		937.5000										
1%*24Hours	3			1108.3333									
2%*6Hours	3				1272.9167								
0.5%*6Hours	3				1289.5833								
1.5%*6Hours	3				1306.2500								
2%*4Hours	3					1422.9167							
1%*6Hours	3						1468.7500						
1.5%*4Hours	3						1468.7500						
0.5%*4Hours	3							1533.3333					
1%*4Hours	3								1666.6667				
0.5%*2Hours	3									1960.4167			
1%*2Hours	3										2068.7500		
2%*2Hours	3											2139.5833	
1.5%*2Hours	3												2195.8333
Sig.		1.000	.366	1.000	.055	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

From table above, the result show there is interaction between substrate concentration and fermentation time even though there are some interaction were not giving significant difference of NAG concentration produced ($p > 0.05$)

OPTIMUM CONDITION FOR N-ACETYL-GLUCOSAMINE PRODUCTION FROM TIGER SHRIMP (*Penaeus monodon)* SHELL USING EXTRACELLULAR CRUDE CHITINASE ENZYME FROM *Mucor circinelloides*

Reinald Febryanto Pengalila[1)*], Yuniwaty Halim[2)]

[1)]Undergraduate Student of Food Technology Study Program, Universitas Pelita Harapan
[2)]Lecturer of Food Technology Study Program, Universitas Pelita Harapan

ABSTRACT

N-Acetyl-glucosamine can be derived from chitin in shrimp shell waste, and can be used in the treatment for ostheoarthritis, knee pain and back pain. This research was conducted to determine the optimum condition (pH, temperature, substrate concentration and fermentation period) for N-acetyl-glucosamine production using extracellular crude chitinase enzyme from *Mucor circinelloides*. Optimum chitinase activity was determined by determining the optimum pH (3, 4, 5, 6, 7, 8 and 9) and optimum temperature was determined by varying from 30, 40, 50, 60, 70 and 80°C at optimum pH. The second stage were done by varying the substrate concentration (0.5, 1, 1.5 and 2%) and fermentation period (2, 4, 6, 24h). Result showed the optimum pH was 8 with enzyme activity 4.38 ±0.06 U/mL, for optimum temperature was 50°C with enzyme activity 5.42 ±0.06 U/mL. The fermentation condition was optimum with 1.5% of substrate concentration and 2 hours of fermentation. The concentration of N-acetyl-glucosamine produced from the fermentation was 2,195.83 ±15.14 ppm.

Keywords: Chitin, chitinase enzyme, *Mucor circinelloides*, N-acetyl-glucosamine, tiger shrimp shell.

BACKGROUND

Shrimp is one of main commodities in fishery industry. Every year, the industry produces huge waste that could be an environmental hazard. About 75% of the total weight of shrimp was discarded as by-products.

Chitin is one of the product that comes from crustacean shell waste. Chitin is a linear polysaccharide and the second most abundant natural polymer after cellulose. Chitin and its derivatives have been used in many applications like pharmaceuticals, textile, food and cosmetics (Junianto, *et al.*, 2013).

Glucosamine can be obtained by direct breakdown of chitin from the shrimp waste using the fermentation process, chemical hydrolysis, enzyme process or any various combinations of these methods (Cahyono, *et al.*, 2014). Production of glucosamine by chemical hydrolysis has an environmental issue. Fermentation process has several disadvantages like long process

*Corresponding author: Faculty of Science and Technology Universitas Pelita Harapan
E-mail: reinald.pangalila@gmail.com

time, high capacity utilisation, high risk of contamination, difficulties in morphology, and challenges for upscaling and reproducibility. However for enzymatic hydrolysis has advantage that the selected enzyme can favour the production of monomer product over oligomer, high chance of reproducibility and potential for side reactions of contamination are minimized (Hodgins, 2001; McNeil, 2013). *Mucor circinelloides* is one of the fungi that can convert chitin into N-acetyl-glucosamine (Veronica, 2018). Fungi can secrete a wide variety of enzymes. However, the activity of enzyme produces from bacteria and fungi are affected by pH, temperature and substrate concentration (Scanlon, *et al.*, 2018). The objectives of this research are to determine optimum pH, temperature, substrate concentration and fermentation time of crude chitinase enzyme from *Mucor circinelloides.*

RESEARCH METHODOLOGY

Materials and Equipment

The materials used in this research were the shrimp shells of *Penaeus mondon* (60% of head shell, 40% of body and tail shell) obtained from PT Lola Mina located at Muara Baru, *Mucor circinelloides* culture obtained from fungi isolation that was done from previous study (Veronica, 2018), NaOH 3.5%, HCl 1M, Potato Dextrose Agar, Potato Dextrose Broth, distilled water, 5N NaOH, concentrated HCl (37%), absolute ethanol, $MgSO_4.7H_2O$, $Na_2HPO4.2H_2O$, phosphate, and citrate buffer. The materials used in analysis methods are N-Acetyl-glucosamine standard (Sigma Aldrich), phosphate buffer (0.1 M pH 8) and DNS solution.

The equipment used in this research were analytical balance, dry blender (Panasonic), cabinet dryer (Wangdi W.), petri dish, micropipette, Bunsen burner, incubator (Memmert), incubator shaker, autoclave (Hirayama), centrifuge (Hettich), evaporating dish, test tube, hemocytometer, inoculating loop, microscope, Mohr pipette, bulb pump, crucible, desiccator, magnetic stirrer and glasswares. The equipment used in analysis are oven (Memmert), UV-VIS spectrophotometer (GENESYS 2000), Quartz cuvette (Helma Analytics) and furnace (Thermolyne).

Preliminary Research

The preliminary research consisted of isolated chitin preparation, preparation of colloidal chitin, media preparation, stock and starter culture preparation, spore count, morphological characterization of culture and extraction of enzyme.

Preparation of Isolated Chitin (Arif, et al., 2013)

The shell of *Penaeus monodon* shrimp were washed with clean water and sun-dried for 1-3 days and then blender it to make it into shrimp powder. Demineralization was done by using HCl 1M (1:10) (w/v) at 75°C for 2 hours. Deproteination was done by using NaOH 3.5% (1:10) (w/v) at 80°C for 2 hours (Arif, *et al*., 2013). Isolated chitin was tested for yield analysis, moisture content, ash content, protein content and degree of deacetylation.

Preparation of Colloidal Chitin (Setia, 2015)

Ten grams of chitin added to HCl 37% with ratio 1:14 (w/v) and stirred for 2 hours until the chitin was totally dissolved. After that the mixture added with 500 mL of ethanol and filtered. The precipitation was added with 5N of NaOH until neutral pH and centrifuged (3500 rpm, 10 minutes). The solid part is the colloidal chitin that has been produced (Setia, 2015).

Media Preparation

Potato Dextrose Agar and Broth were made and prepared according to the instruction in the container of the media. PDA was used as the culture stock, and for PDB was used for starter and the media fermentation for enzyme production. PDB in the enzyme production were added 0.5% of $MgSO_4$, 0.5% of Na_2HPO_4, and 0.5% colloidal chitin to produce chitinase enzyme.

Stock and Starter Culture Preparation (Haedar, et al., 2017)

For stock culture, one ose of stock culture was taken and streak to the slant agar. The fungi was incubated in room temperature for 2 days. After incubation, the agar and fungi were stored at 4°C in the fridge as stock culture.

For starter culture, the fungi were taken using inoculating loop and transferred to 10 ml of PDB and incubated for 2 days, after incubation the culture used for starter culture (Haedar, *et al*., 2017).

Morphological Characterization of Culture (Heritage, et al., 1996)

Fungal was removed using sterile needle or tooth pick and placed to microscope slide, then add a drop methylene (1:5) and closed it using cover glass. The microscope slide was placed under microscope and observed (Heritage, *et al.*, 1996*)*.

Spore Counting (Saxena, et al., 2015)

One ml of *M. circinelloides* from PDB were taken and mixed with 1 ml of methylene blue (1:5), then incubated it for 10 minutes and 60µl of mixture were taken and placed into hemocytometer chamber and

counted under microscope (Saxena, *et al*., 2015).

Extraction of Extracellular Enzyme of *Mucor circinelloides* (Jenifer, et al., 2014)

One ose of inoculum was transferred to 10 mL of PDB and incubate for 48 hours at room temperature. 3 mL of inoculum medium was transferred to 300 of Potato Dextrose Broth (production medium) in Erlenmeyer flask then fortified with colloidal chitin, $MgSO_4$, and Na_2HPO_4 and incubate for 48 hours in a shaker. After incubation, the medium was centrifuged (3500 rpm, 10 minutes). The supernatant is the crude chitinase enzyme (Jenifer, *et al*., 2014).

First Stage Research

The first stage of this research was to determine the optimum pH and temperature of crude chitinase enzyme from *Mucor circinelloides*.

Determination of Optimum pH of Extracellular Enzyme (Wasli, et al., 2006)

Media containing 0.5% (w/v) of colloidal chitin was adjusted the pH into 3, 4, 5, 6, 7, 8, and 9 using citrate, phosphate buffer, or glycine buffer (Wasli, *et al*., 2004). Then the media inoculated with 1ml of crude chitinase enzyme and incubated for an hour. After incubation, the solution was centrifuged (3000 rpm, 10 minutes) to obtain the supernatant. Chitinase activity was measured using DNS method (Rahmansyah and Sudiana, 2003). The optimum pH was determined by the with highest value of enzyme activity.

Determination of Optimum Temperature of Extracellular Enzyme (Wasli, et al., 2006)

Media containing 0.5% of (w/v) colloidal chitin was adjusted to optimum pH using citrate or phosphate buffer and then sterilized using autoclave. Then the inoculated media was added with 1 ml of crude chitinase enzyme from *Mucor circinellodes* and incubated at 30°C, 40°C, 50°C, 60°C, 70°C and 80°C for one hour (Wasli, *et al*., 2006). After incubation, the solution was centrifuged (3000 rpm, 10 minutes) to obtain the supernatant. Chitinase activity was measured using DNS method (Rahmansyah and Sudiana, 2003). The optimum temperature was determined by the with highest value of enzyme activity.

Second Stage Research

The second stage of this research was to determine the optimum substrate concentration and fermentation time of crude chitinase enzyme from *Mucor circinelloides*.

Determination of Optimum Substrate Concentration and Fermentation Time (Wasli, et al., 2006)

1 ml of buffer with adjusted pH was added into media that contains 0.5%, 1%, 1.5% and 2% (w/v) of chitin then sterilized using autoclave. Then the media is added with 1 ml of crude chitinase enzyme and incubated at optimum temperature for 2h, 4h, 6h and 24h (Jamialahmadi, *et al*., 2011). After incubation, the solution was centrifuged (3000 rpm, 10 minutes) to obtain the supernatant. N-Acetyl-Glucosamine was measured using DNS method (Rahmansyah and Sudiana, 2003). The optimum substrate concentration and fermentation time was determined by statistical analyses using SPSS.

RESULTS AND DISCUSSIONS

Shrimp Shell Powder Analysis

Chemical composition that were analyzed are moisture content, ash content and protein content. The result of analysis can be seen at Table 1.

Table 1. Chemical composition of shrimp shell powder

Parameter	Content (%)
Moisture content (wb)	9.74 ± 0.34
Ash Content (wb)	49.72 ± 3.66
Protein content (wb)	34.84 ± 1.30

Moisture content of shrimp shell powder in this experiment is 9.74%, this result has lower value compared to result from Sanusi (2004) and Percot *et al*. (2003) experiment stated moisture content of shrimp shell powder is around 13.29% and 11.3%. The result of moisture content from this experiment showed that sun drying for 2 days was effective and could give lower value of moisture content.

Ash content of shrimp shell powder from this experiment is 49.72%, this value is higher than the result from Hossain and Iqbal (2014) and Percot *et al*. (2003) which has ash content around 32.27% and 35.49%. Higher value of ash content in this research might due to the difference of shrimps were used in the experiment.

Protein content of shrimp shell powder from this research was 34.84%. The result obtained is in the range of theoretical value (20-40%) stated by Antonino, *et al*. (2017), but the protein content in this experiment has higher value than protein content from Percot, *et al*. (2003) which has value around 15-20%. Bradford method was used to measure protein content in this experiment while Percot, *et al*. (2003) used Lowry method to measure protein content of the shrimp shell powder. Bradford method relies on interaction between basic amino acid residues with Coomassie brilliant blue in acidic matrix, for Lowry method there are

two reaction process, the reduction of Cu^{2+} to Cu^{+} and reduction of Folin-Ciocalteu reagent when it binds with protein. However, Lowry method can give false indication of protein content, due to the ability of phenol to reduce Folin-Ciocalteu reagent (Redmile-Gordon, *et al*., 2013).

Isolated Chitin Analysis

The analyses conducted on isolated chitin were moisture content, ash content, protein content, degree of deacetylation, and yield determination. The result of analyses shown at Table 2.

Table 2. Chemical composition of isolated chitin

Parameter	Content (%)
Moisture content (wb)	4.59 ± 0.44
Ash Content (wb)	0.45 ± 0.03
Protein content (wb)	1.74 ± 0.07
Yield (db)	23.02 ± 0.44
Degree f deacetylation	28.08

he moisture content of isolated chitin after demineralization and deproteination is 4.59%, this result is lower than the result that is reported by Hossain and Iqbal (2014) with moisture content of 8.50%. According to Khan *et al*. (2002) chitin should have moisture content below 10% because chitin has hygroscopic behavior. The decreasing moisture content in this research due to the drying process after demineralization and deproteination process.

The ash content of isolated chitin after demineralization and deproteination is 0.45%, this result is comparable to result from Hossain and Iqbal (2014) that have ash content around 0.36%. Hossain and Iqbal (2014) during the demineralization process used 4% of HCl at 28°C for 16 hours, in this experiment the demineralization process was done with 1M of HCl at 70°C for 2 hours. Low value of ash content indicates the effectiveness of demineralization process.

The protein content of isolated chitin after demineralization and deproteination is 1.74%. Protein content from Islam *et al.* (2016) research is 3.5% this result is higher than the result in this research might due to the difference of temperature use, in this experiment temperature used was 80°C and in Islam *et al.* (2016) research temperature used was 70°C. Protein content of chitin from Percot *et al.* (2003) was around 0.25%, it is lower than the result obtained from this research because in Percot *et al.* (2003) research the deproteination process was done with NaOH 1M at temperature close to 70°C with time up to 24 hours. Low value of protein content indicates the effectiveness of deproteination process.

The yield of isolated chitin after demineralization and deproteination is 23.02%, this result is higher than Ramadhan *et al*. (2010) and Hossain and Iqbal (2014) result around 18.40% and 17.36%. This result showed that during each process of isolation

the component like mineral, pigment, and protein were removed.

Degree of deacetylation was determined with baseline method using Fourier Transform Infra-Red (FTIR). Degree of deacetylation is the percentage of acetyl bond removed from the chitin structure during the isolation process (Sanusi, 2004). Higher degree of deacetylation indicates the lower acetyl bond which made the interaction between ion and hydrogen bond stronger. Degree of deacetylation was measured to determine the number of chitin that was transformed to chitosan (Puspawati and Simpen, 2010). Degree of deacetylation (DD) of isolated chitin in this researched is 28.08%. According to Terbojevich and Muzzarelli (2000), DD around 40-100% is a chitosan. Khan, *et al*. (2002) also stated that DD 75% or more generally recognized as chitosan. It shown that with 3.5% of NaOH used in this research, chitin still can maintain the covalent bond of acetyl and acetamide and not transformed to amine group.

Morphology of *Mucor circinelloides*

M. circinelloides was observed microscopically. The microscopic morphology (Figure 1) shows that multipolar budding and each cell harbors more than one nucleus, while the mycelium is aseptate and it has nuclei distributed in the hyphae. The microscopic observation is the same with Iwen, *et al.* (2006) where there were sporagium, sporangiospore and aseptate mycelium observed from it, therefore the fungi used in this experiment was assured as *Mucor circinelloides*. *Mucor circinelloides* known as pathogenic fungi. The pathogenicity of Mucor is largely believed to be due to endocellular excretions and productions of substilisins, chitinase, proteinases, and antioxidant proteins (Hameed, *et al*., 2017). Therefore, *M. circinelloides* can be used to produced chitinase enzyme for production of N-Acetyl-glucosamine. *M. circinelloides* can be growth easily because, it can grown with optimum temperature at 30°C with incubation time 48 hours (Tang, *et al*., 2015).

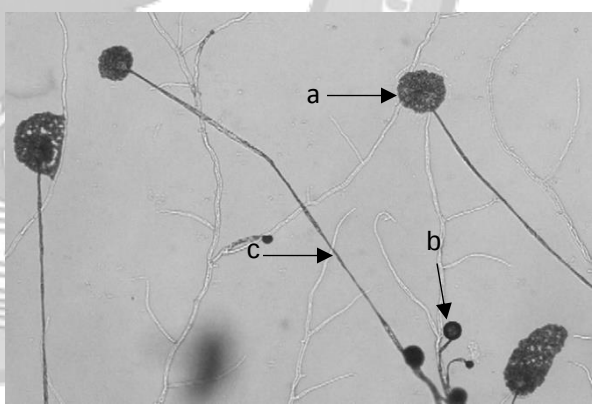


Figure 1 Microscopic observation of *Mucor circinelloides* (100x). (a) sporangium, (b) sporangiospore, (c) aseptate mycelium.

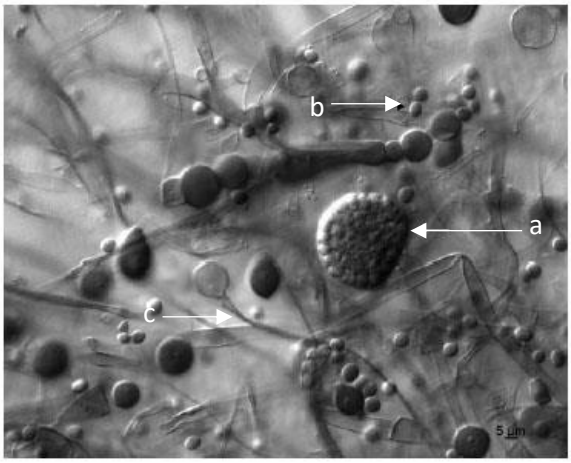


Figure 2 Microscopic observation of *Mucor circinelloides* using Nomarski optic with Bar, 5 µm. (a) sporangium, (b) sporangiospore, (c) aseptate mycelium.

Spore Counting

Spore counting was used to determine the amount of starter that was used for the fermentation process. The starter used for fermentation is 48 hours because according to Tang, *et al.* (2015) in 48 hours *M. circinelloides* is already on stationary phase. According to Jenifer *et al.* (2014) 2 mL of starter were used for 100 mL of production media because the number of spores is $8.0x10^6$ spores/mL, the average spores of *M. circinelloides* counted is $1.2x10^7$ spores/mL, hence 1 mL of starter culture were used for fermentation.

Chitinolytic Index

Chitinolytic index of *Mucor circinelloides* in this experiment is around 0.96 ± 0.01 after 3 days of incubation (Figure 2). Bromocresol purple (BCP) was used as the indicator of the chitinolytic index and colloidal chitin were added to media as the substrate. When *Mucor circinelloides* break down colloidal chitin to N-Acetyl-glucosamine, the color of the media from yellow will turn into purple because of the change in pH. The change of color from yellow to purple is caused by the change of the pH from acidic to basic. Larger value of diameter of purple and lower the value of diameter of the colony zone indicate higher value of chitinolytic index of the fungi (Pandey, *et al.*, 2014).

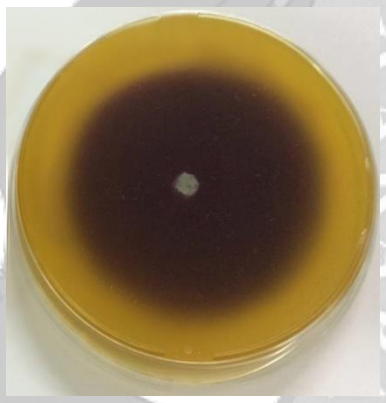


Figure 3 Chitinolytic index of *Mucor circinelloides*

First Stage Research

The first stage of this research was conducted to determine the optimum pH and optimum temperature of crude chitinase enzyme produced by *M. circinelloides*.

Effect of pH on Enzyme Activity

For determining the optimum pH of chitinase, the media were fortified with 0.5 % colloidal chitin and adjusted the pH from 3 until 9 using buffer. Chitinase enzyme from *M. circinelloides* has optimum pH at 8 with

4.38 ± 0.06 U/ml activity per hour. The optimum pH 8 also shown by the chitinase enzyme from *Alcaligenes faecalis* (Annamalai, 2011). According to Dahiya, *et al.* (2005) most of chitinase active at acidic pH. The difference of chitinase enzyme also based on the strain of bacteria or fungi used for chitinase production.

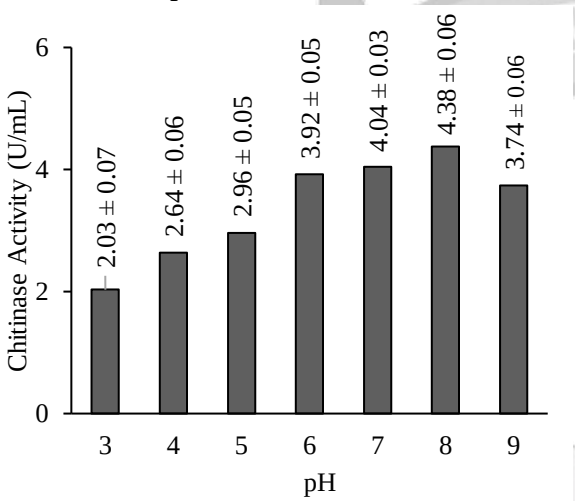


Figure 4 Effect of pH on enzyme activity

The decreasing activity of enzyme after reached optimum pH caused by tertiary structure changed when the hydrophobic groups contact with water decreased the solubility of enzyme and it could not react with the substrate thus lowering the activity of enzyme (Suryadi, 2013). Enzymes are sensitive to pH due to the enzyme reaction involves a hydrogen ion (H^+) as substrate or product, one or more substrate undergoes ionization thereby altering the enzyme-substrate binding affinity or Vmax, enzyme denature at extreme pH, also one or more enzyme active site groups ionizes at different pH. Enzyme binding to H^+ result in enzyme activation or inhibition (Apenten, 2004). The decreasing activity of enzyme after reached optimum pH caused by tertiary structure changed when the hydrophobic groups contact with water decreased the solubility of enzyme and it could not react with the substrate thus lowering the activity of enzyme (Suryadi, 2013).

Effect of Temperature on Enzyme Activity

For optimum temperature, the media that have been inoculated with crude chitinase enzyme were incubated at 30-80°C. Chitinase enzyme from *M. circinelloides* has optimum temperature at 50°C with enzyme activity around 5.42 ± 0.06 U/mL. Optimum temperature at 50°C also shown by chitinase enzyme from *Beauveria bassiana*, and isolate B1211 with activity respectively 0.486 U/mL and 0.71 U/mL (Suryadi *et al*., 2013; Hardi *et al.*, 2017). If temperature is increased beyond the optimum temperature, the enzyme activity will start to decrease due to denaturation of enzyme (Hardi *et al.*, 2017). Denaturation process caused the enzyme structure changed and lower the hydrogen bond that would have effect to the active site of enzyme to bind with substrate. Increase of temperature also made the reaction rate

increase and the reaction of substrate and enzyme will be faster (Mulyani, *et al*., 2009)

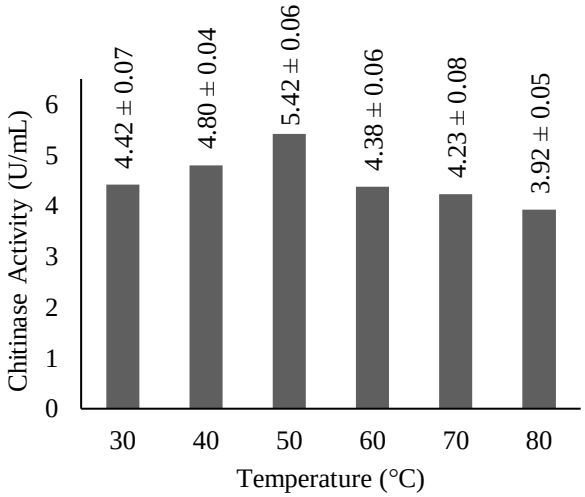


Figure 5 Effect of temperature on enzyme activity

Second Stage Research

Effect of Fermentation Time and Substrate Concentration to N-Acetyl-Glucosamine Production

According to Cahyani (2013), fermentation time and substrate concentration have effect to the production of N-Acetyl-Glucosamine. Scanlon, *et al.* (2018) also stated that, the increase of substrate concentration makes the product of fermentation linearly increases, and then at certain concentration of substrate the product will not increase anymore when all the enzyme are used. This behavior is known as Michaelis-Menten behavior.

N-Acetyl-glucosamine production has the highest concentration at 1.5% of substrate with 2 hours of fermentation. The fermentation result (2,195.83 ± 15.14 ppm) in this research is higher than Herdyastuti and Cahyaningrum (2017) research which have 1,360 ppm. The decline number of N-Acetyl-glucosamine production could be caused by the enzyme undergo denaturation process or there are inhibition occurs during the process of fermentation. According to Herdyastuti and Cahyaningrum 2017) GlcNAc and chitooligosaccharide produced through hydrolysis of chitin by chitinase in high concentration could

cause feedback inhibition due to the excess of GlcNAc. Suzuki, *et al.* (2006), also stated that several compounds like allosamidin and compound in addition of contained could inhibit the production of GlcNAc. Allosamidin is a unique pseudotrisaccharide structure mimic to chitin and inhibits all family 18 chitinases, which hydrolyze chitin and widely present in nature (Suzuki, *et al*., 2006).

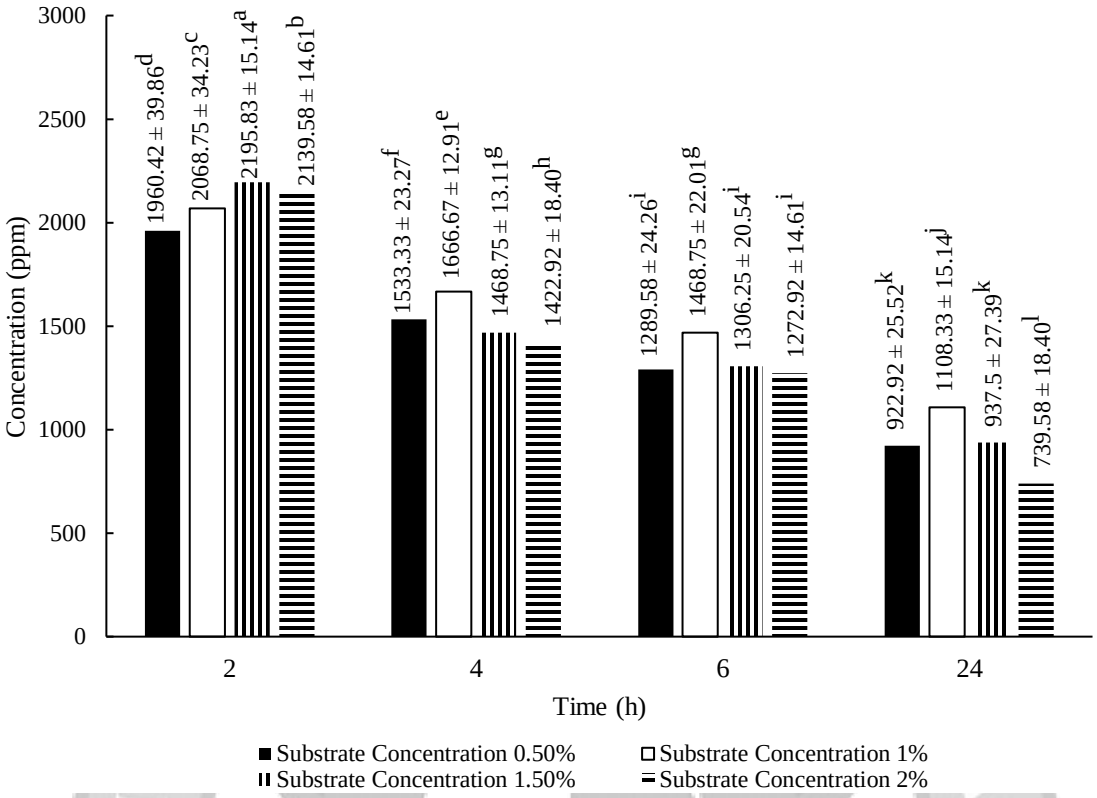


Figure 6 Effect of fermentation time and substrate concentration to N-Acetyl-glucosamine production
Note: Different notations indicate a significant difference ($p<0.05$)

CONCLUSIONS AND SUGGESTIONS

Conclusions

Isolated chitin powder produced from *P. monodon* have moisture content (wb) of 4.59 ± 0.44%, ash content of 0.45 ± 0.03%, protein content of 1.74 ± 0.07%, degree of deacetylation around 28.08%, and yield 23.02 ± 0.44%.

The optimum pH and temperature for the extracellular crude chitinase from *M. circinelloides* are 8 and 50°C. There is an interaction between fermentation time and substrate concentration also toward the production of N-Acetyl-glucosamine using extracellular crude chitinase from *M. circinelloides*. The highest N-Acetyl-glucosamine produced was 2,195.83 ± 15.14 ppm with substrate concentration of 1.5% and 2 hours of fermentation.

Suggestions

Enzyme activity assay has to be performed after separating chitinase enzyme from the media fermentation. The production of enzyme also has to be done in larger scale to obtain larger yield of chitinase enzyme.

Quantification of N-acetyl-glucosamine has to be done by HPLC or LC-MS because spectrophotometry will be

resulting unstable value of N-acetyl-glucosamine.

BIBLIOGRAPHY

Annamalai, N., Mayavan V. Rajeswari, S. V., Thangavel B. "Purification and Characterization of Chitinase from *Alcaligenes faecalis* AUO2 by Utilizing Marine Waste and Its Antioxidant Activity." Springer, 2011.

Arif, A. R., Ischaidar, Hasnah N., and Seniwati D. "Isolasi Kitin dari Limbah Udang Putih (*Penaeus merguiensis*) Secara Enzimatis." *Peran Sains dan Teknologi Dalam Mendukung Ketahanan Pangan dan Energi Nasional*, 2013: 10-16.

Cahyono, E., Pipih S., and Ietje W. "Development of A Pressurized Hydrolysis Method for Producing Glucosamine." *Asian Journal of Agriculture and Food Sciences*, 2014.

Dahiya, N., Tewari R., Tiwari R. P., Hoondal G. "Production of Antifungal Chitinase from *Enterobacter* sp.NRG4 and Its Application in Protoplast Production. *World J Microbiol Biothechnol*, 2005.

Haedar, N., Hasnah N., Fahruddin, and Wilda A. "Produksi dan Karakterisasi Enzim Kitinase dari Bakteri Kitinolitik Asal Kerang *Anadara granosa*." *Jurnal Alam dan Lingkungan* 8, 2017: 19-28.

Hardi, J., Ruslan, Abd. Rahman R., and Silva. "Karakterisasi Enzim Kitinase dari Isolat Bakteri Termofilik B1211 Asal Air Panas Bora." *Kovalen Jurnal Riset Kimia*, 2017.

Herdyastuti, N., and S. E. Cahyaningrum. "Analysis of N-Acetyl-Glucosamine from Enzymatic Degradation of Amorphorus Chitin." *Rasayan J. Chem.*, 2017.

Heritage, J., E. G. V. Evans, and R. A. Killington. "Introductory Microbiology". Cambridge University Press, 1996.

Hossain M. S. and A. Iqbal. "Production and Characterization of Chitosan from Shrimp Waste." *J. Bangladesh Agril. Univ.*, 2014: 153-160.

Iwen, P. C., Lynne S., Rhonda K. N., and Alison G. F.. "Mucor circinelloides Was Identified by Molecular Methods as a Cause." *Journal of Clinical Microbiology*, 2006.

Islam, S. Z., Murshida K., and Noswad A. "Production of Chitin and Chitosan from Shrimp Shell Waste." *Bangladesh Journal of Scientific and Industrial Research*, 2016.

Khan, T. A., K. K. Peh, and H. S. Ching. "Reporting Degree of Deacetylation of Chitosan: The Influence of Analytical Method." 2002.

Jenifer, S., J. Jeyasree, Kezia D. L., and K. M. "Purification and Characterization of Chitinase from Trichoderma Viride N9 and Its Antifungal Activity Against Phytopathogenic Fungi." *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014.

Junianto, Budiasih W., and Siswa S. "Selection of Methods for Microbiological Extraction of Chitin from Shrimp Shells." *Microbiology Indonesia*, 2013.

Pandey, S., Mohammad S., Mukesh S., Anuradha S., Vipul K., Shyyam J. "Chitinolytic Assay for Trichoderma species Isolated from Different Geographical Location of Uttar Pradesh." *African Journal of Biotechnology*, 2014.

Percot, A., Christophe V., and Alain D. "Characterization of Shrimp Shell Deproteination." *Biomicromolecules*, 2003: 1380-1385.

Percot, A., Christope V., and Alain D. "Optimization of Chitin Extraction from Shrimp Shells." *Biomacromolecules*, 2003: 12-18.

Rahmansyah, M. dan Sudiana, I. M. "Optimasi Analisis Amilase dan Glukanase yang Diekstrak dari Miselium Pleurotus Ostreatus dengan Asam 3,5 Dinitrosalisilat." Berk. Penel. Hayati 9. 2003: 7-12.

Redmile-Gordon, M. A., E. Armenise, R. P.White, P. R. Hirsch and K. W. T. Goulding. "A Comparison of Two Colorimetric Assays, based on Lowry and Bradford Techniques, to Estimate Total Protein in Soil Extract." *Soil Biol Biochem*, 2013.

Sanusi, M. "Transformasi Kitin dari Hasil Isolasi Limbah Industri Udang Beku menjadi Kitosan." *Mar. Chim Acta* 5 (2) 2004: 28-32.

Saxena, J., M. Baunthiyal, and I. Ravi. 2015. "Laboratory Manual of Microbiology, Biochemistry and Molecular Biology". Scientific Publishers.

Scanlon, M. G., A. W. Henrich, and J. R. Whitaker. "Factors affecting enzyme activity in food processing." *Proteins in Food Processing*, 2018.

Setia, I. N. "Chinolytic Assay and Identification of Bacteria Isolated from Shrimp Waste Based on 16S rDNA Sequences." *Advances in Microbiology 5*, 2015: 541-548.

Suryadi, Y., Tri P. P., I. Made S., Dwi N. S., Nuni L., and Eman K. "Pemurnian Parsial dan Karakterisasi Kitinase Asal Jamur Entomopatogen Beauveria bassiana Isolat BB200109." *Jurnal AgroBiogen 9*, 2013: 77-84.

Suzuki, S., Eiyu N., Tsuyoshi O., Ryu K., Hiromichi N., and Shohei S. "Chitinase Inhibitor Allosamidin Is a Signal Molecule for Chitinase Production in Its Producing Streptomyces." *The Journal of Antibiotics*, 2006.

Terbojevich, M., and RAA Muzzarelli. "Chitosan." *University of Ancona*, 2000.

Veronica. "Isolasi dan Identifikasi Kapang Penghidrolisis Kitin yang Diisolasi dari Kulit Udang Windu (*Penaeus monodon*)." 2018.

Wasli, A. S., Madihah MD S., and Rosli Md I. "Production and Characterization of Crude Chitinase Enzyme from *Trichoderma virens*." *Petroleum and Natural Resources Process*, 2006.