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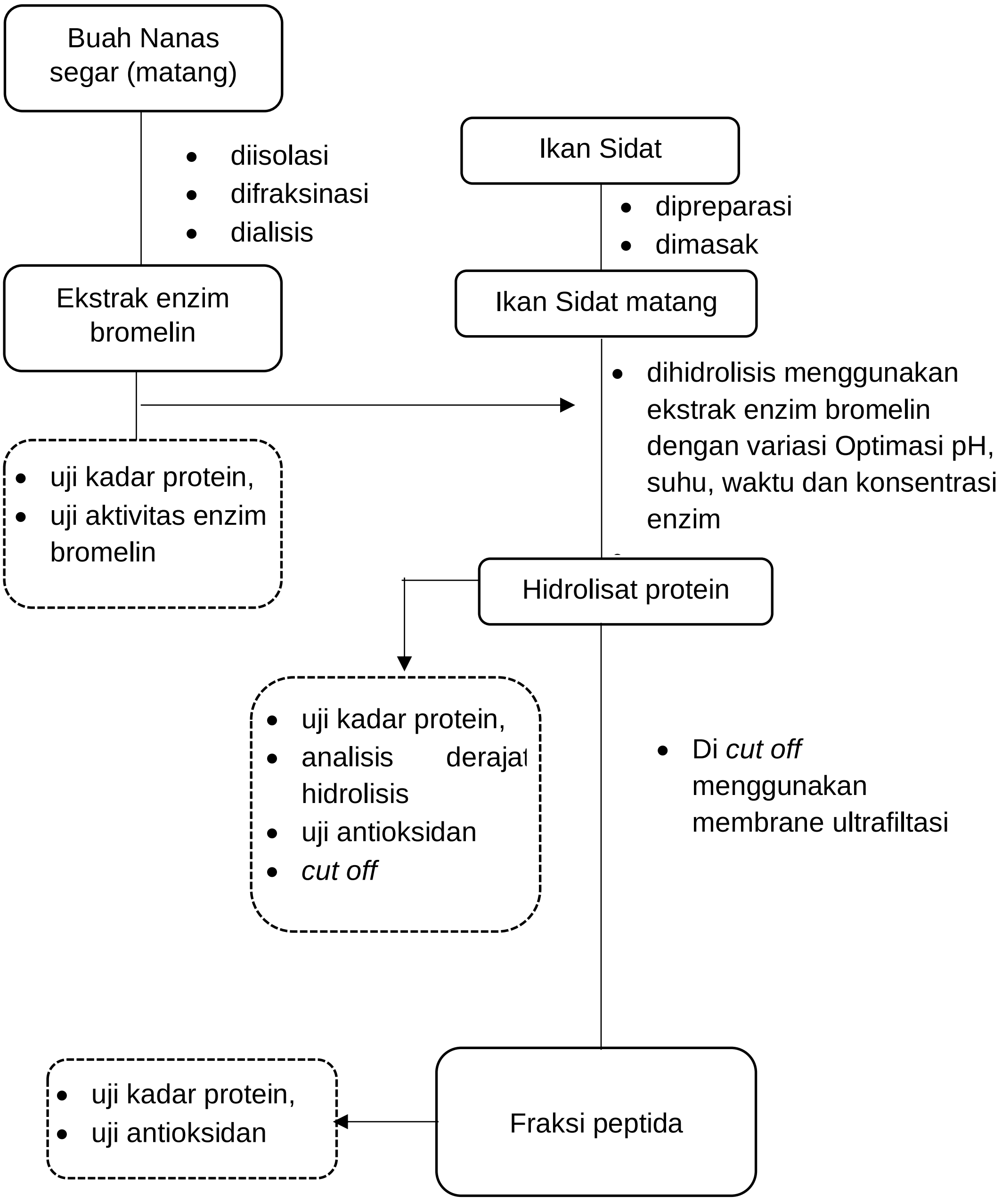
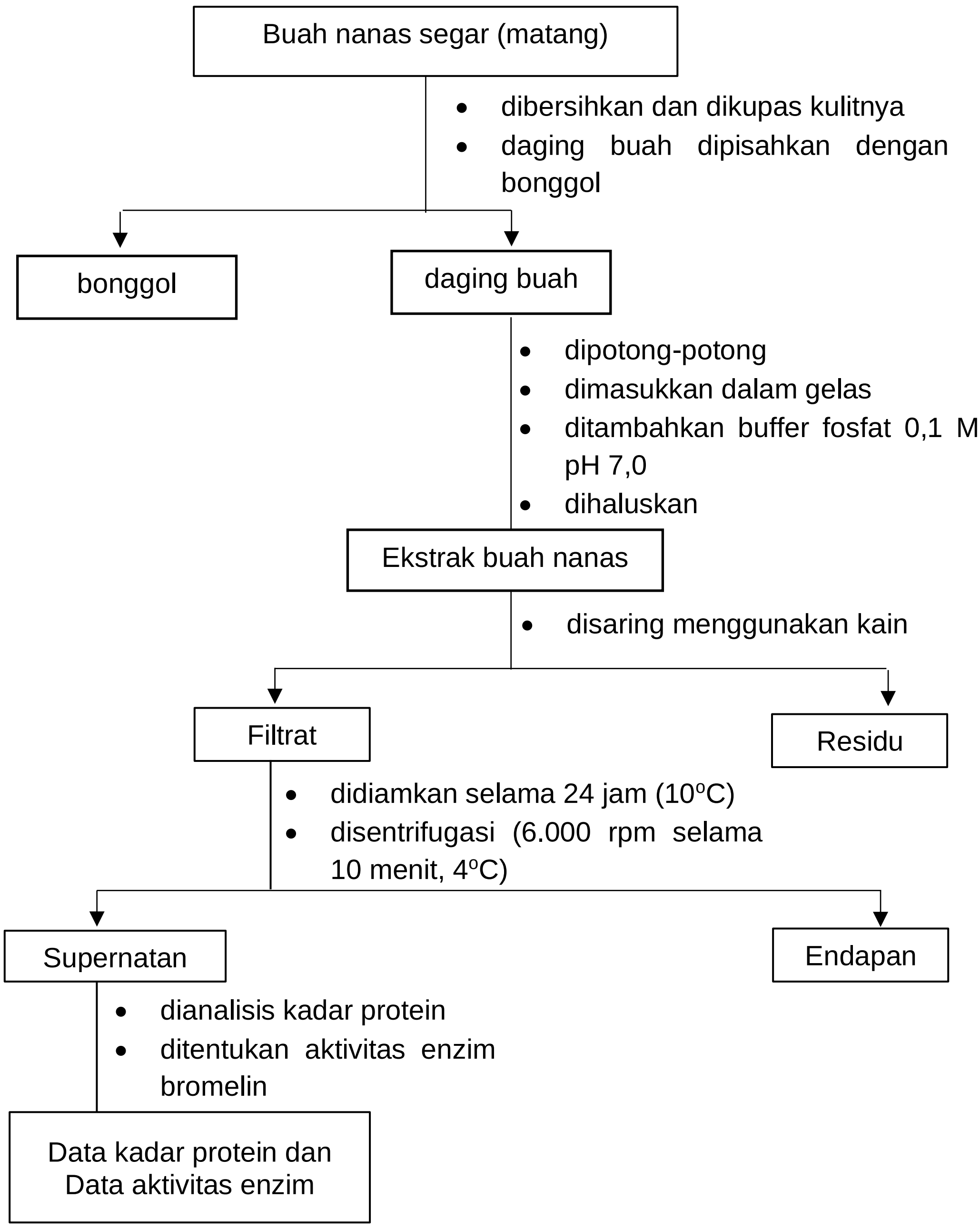
LAMPIRAN

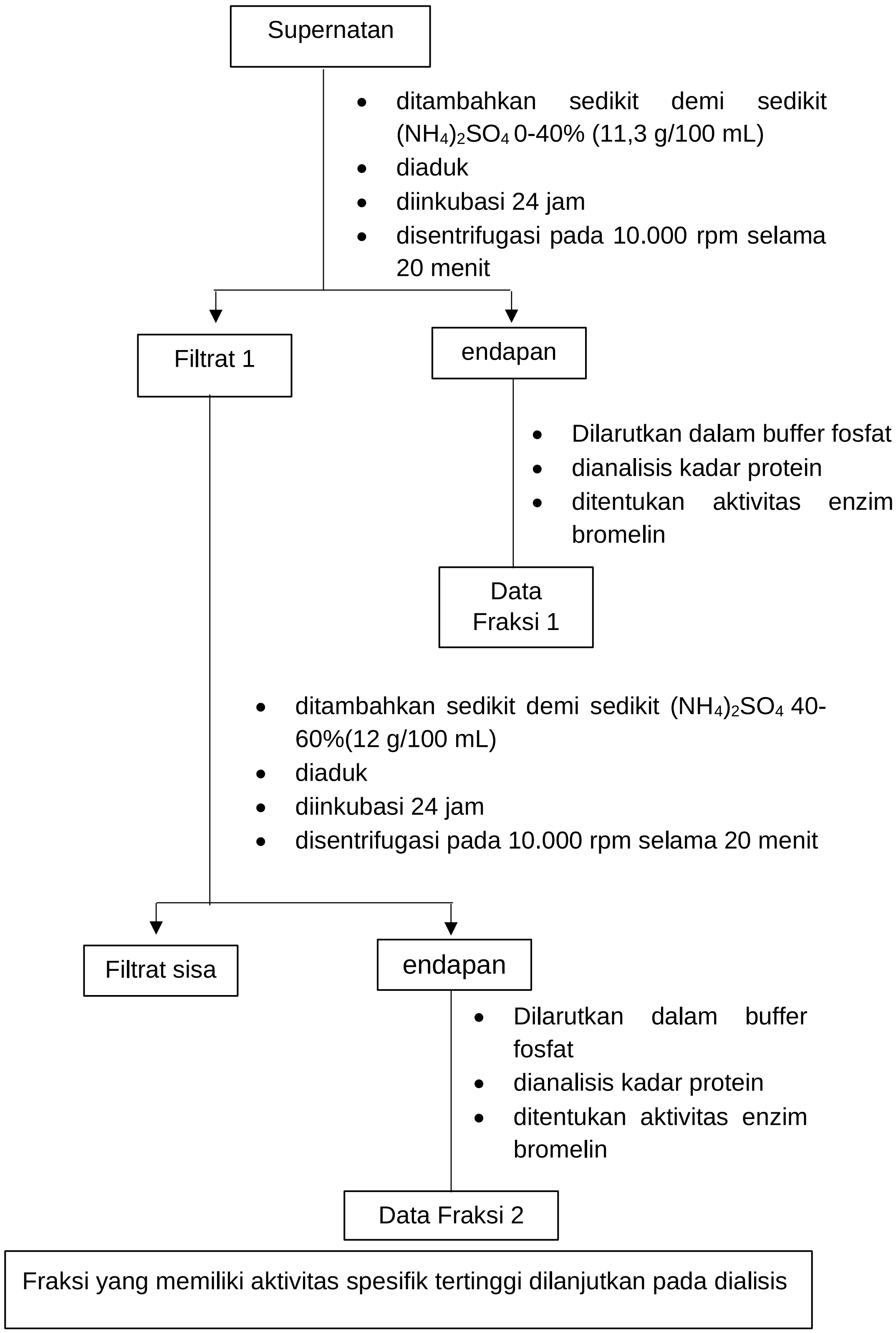
Lampiran 1: Tahapan Penelitian



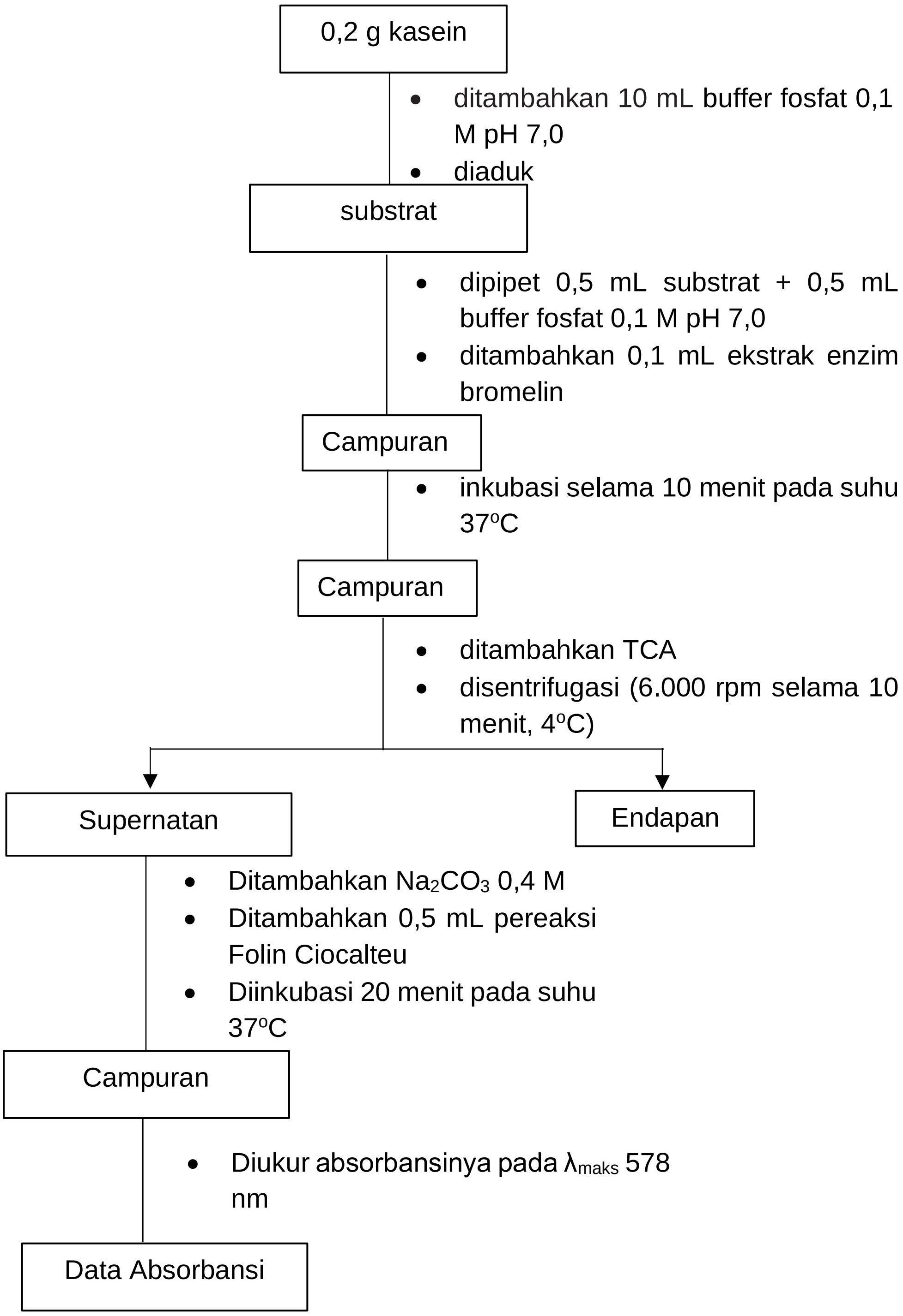
Lampiran 2: Skema Kerja Preparasi dan Isolasi Ekstrak Enzim Bromelin



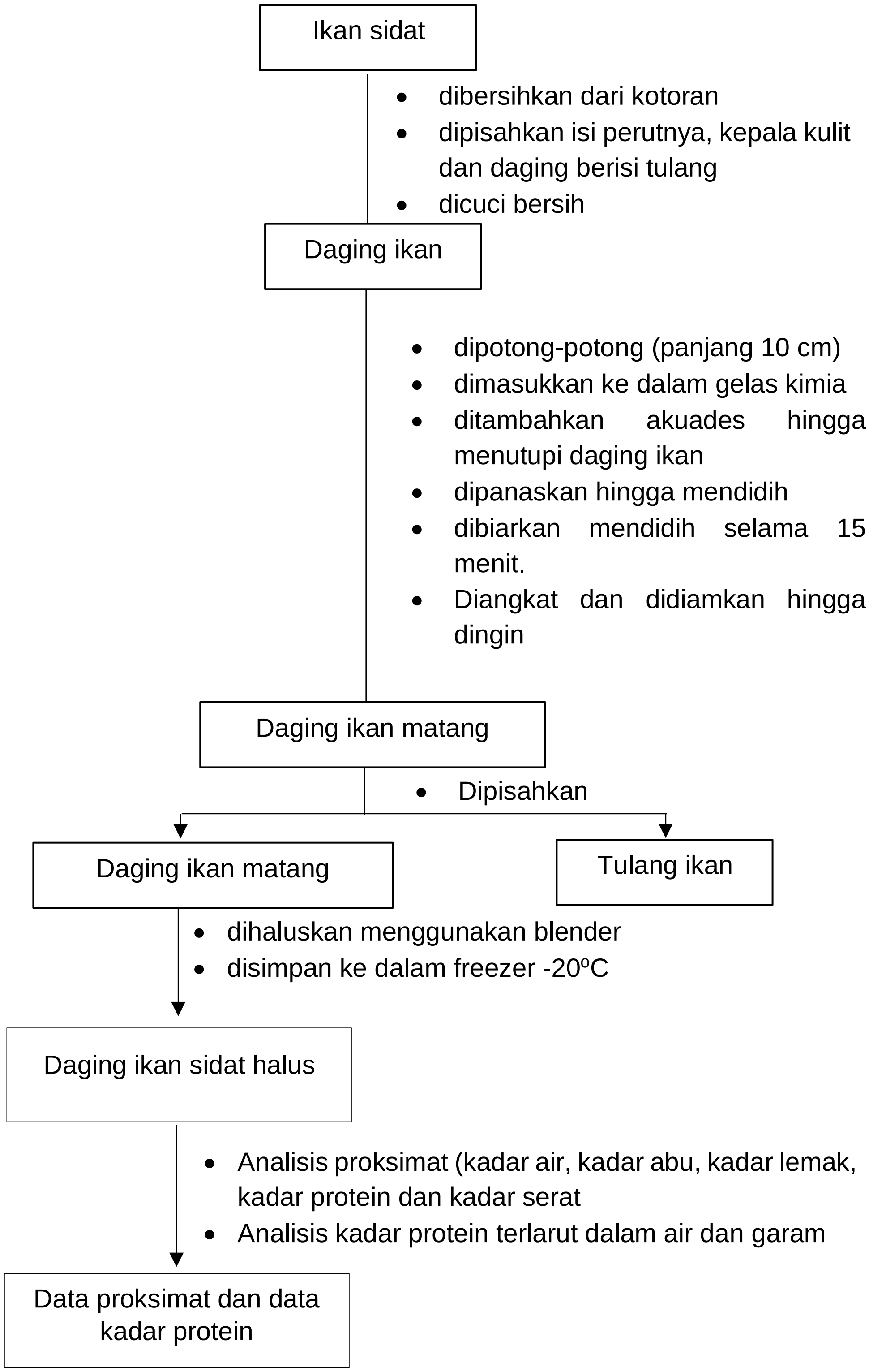
Lampiran 3: Skema Kerja Pemurnian Parsial Ekstrak Enzim Bromelin



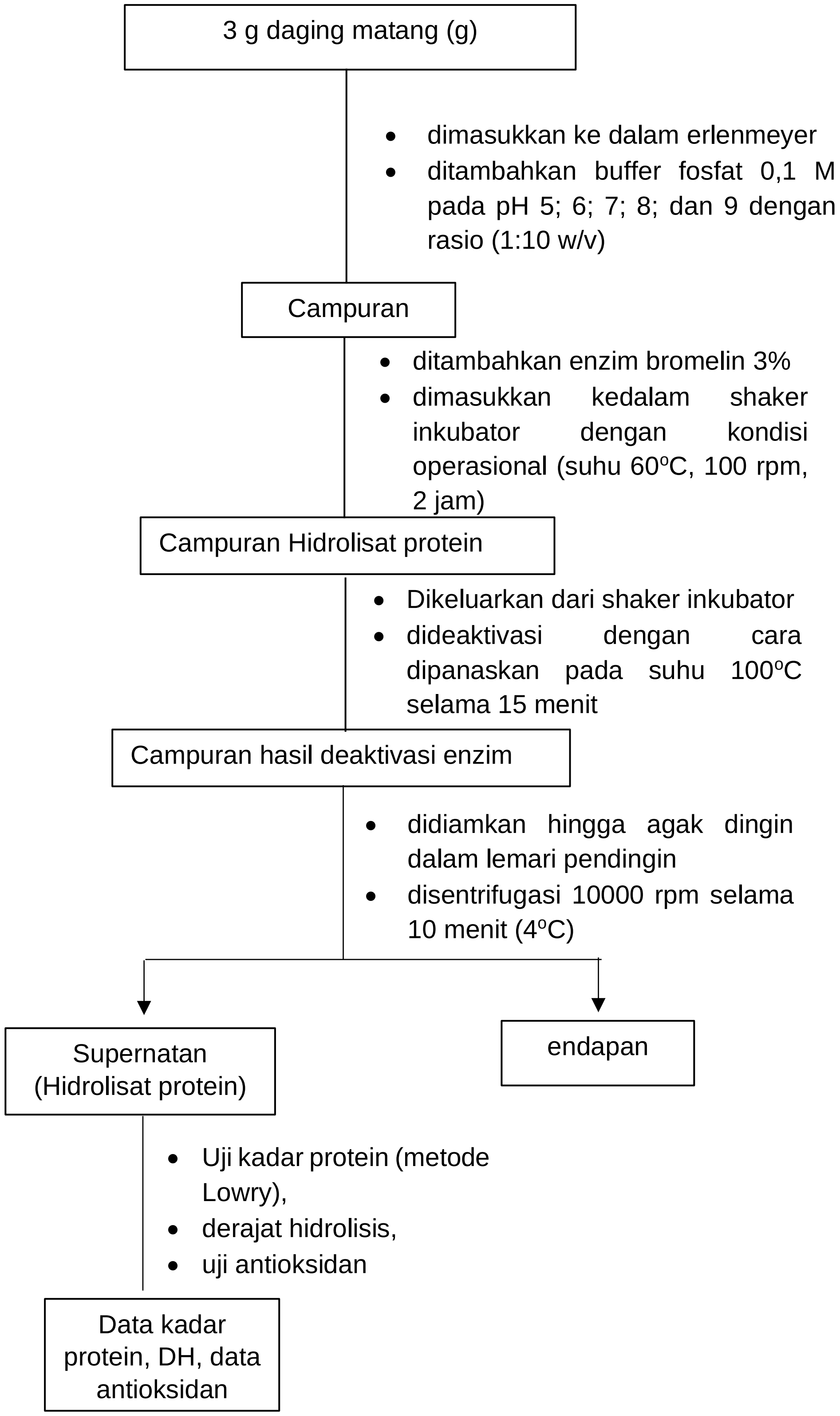
Lampiran 4: Skema Kerja Penentuan Aktivitas Bromelin Terhadap Kasein



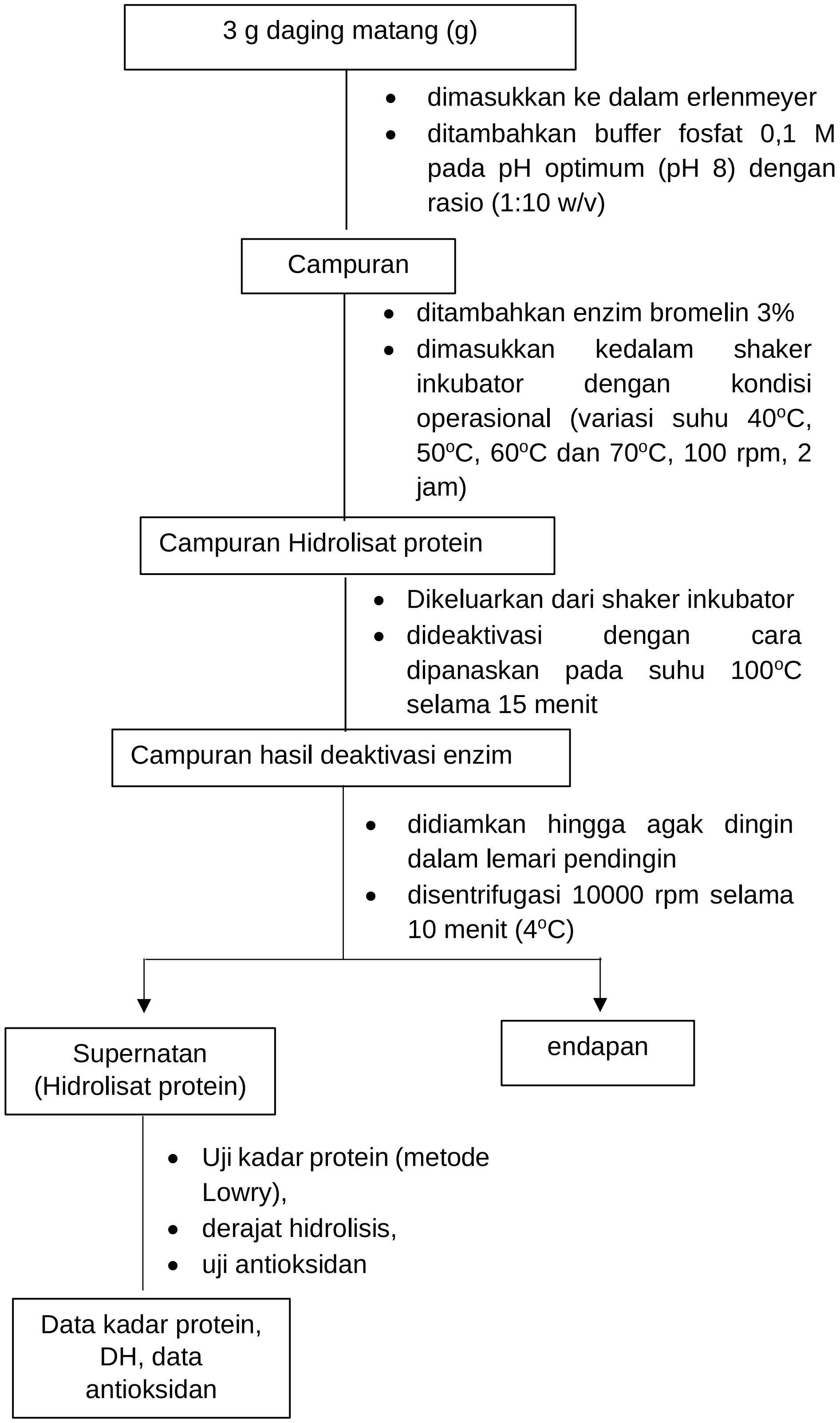
Lampiran 5: Skema Kerja Preparasi Ikan Sidat



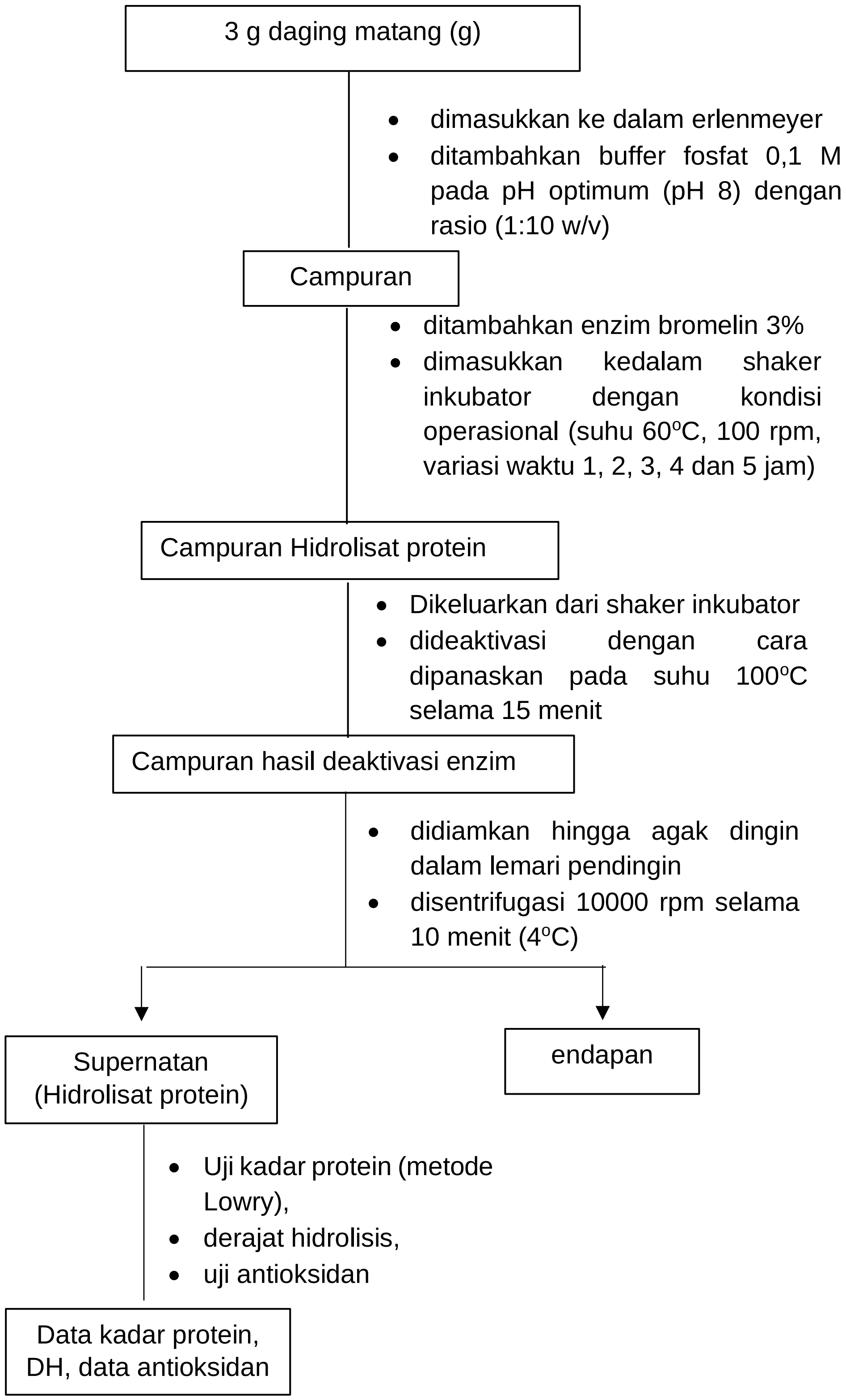
Lampiran 6: Skema Kerja Penentuan pH Optimum Hidrolisis Protein Ikan Sidat



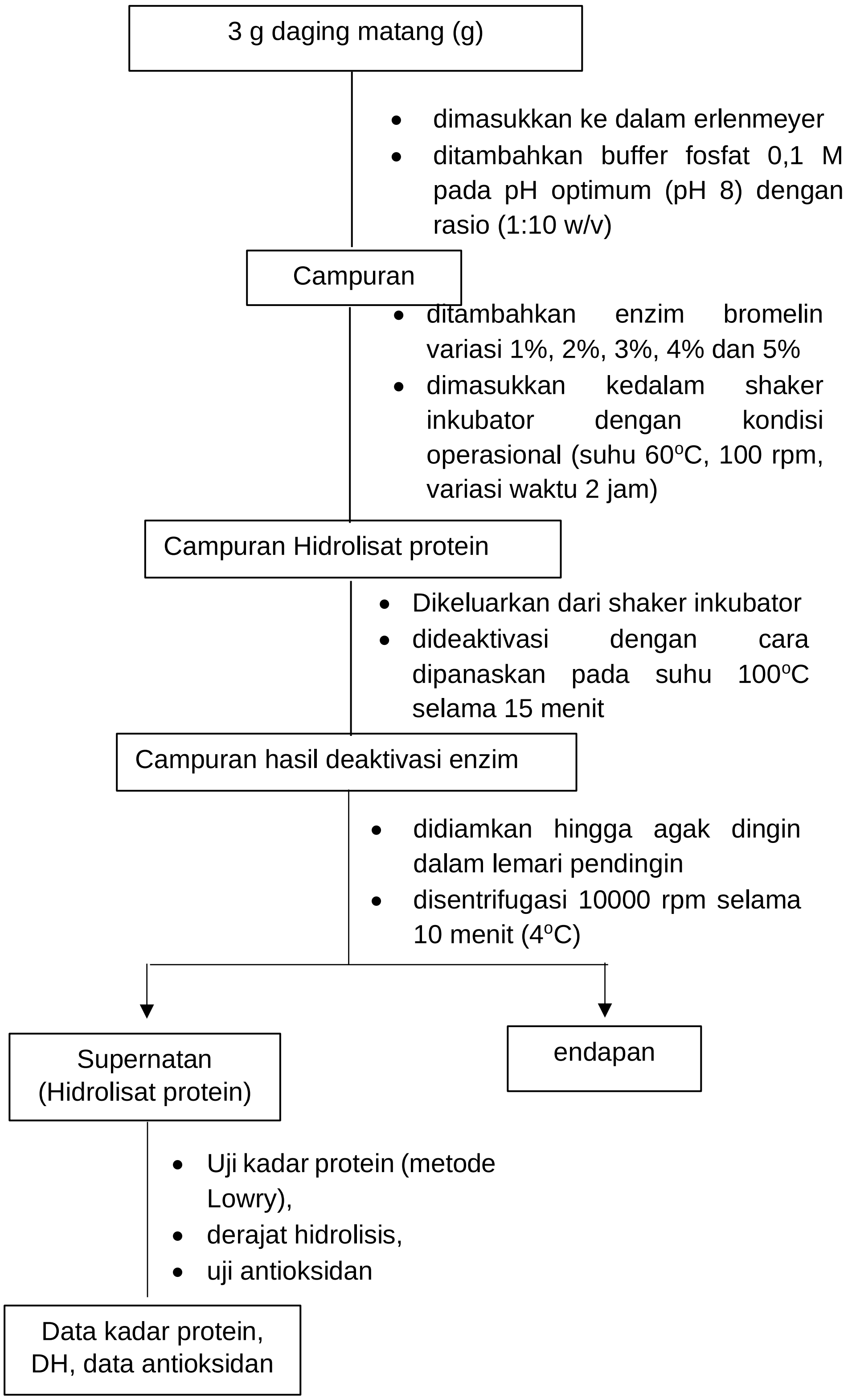
Lampiran 7: Skema Kerja Penentuan Suhu Optimum Hidrolisis Protein Ikan Sidat



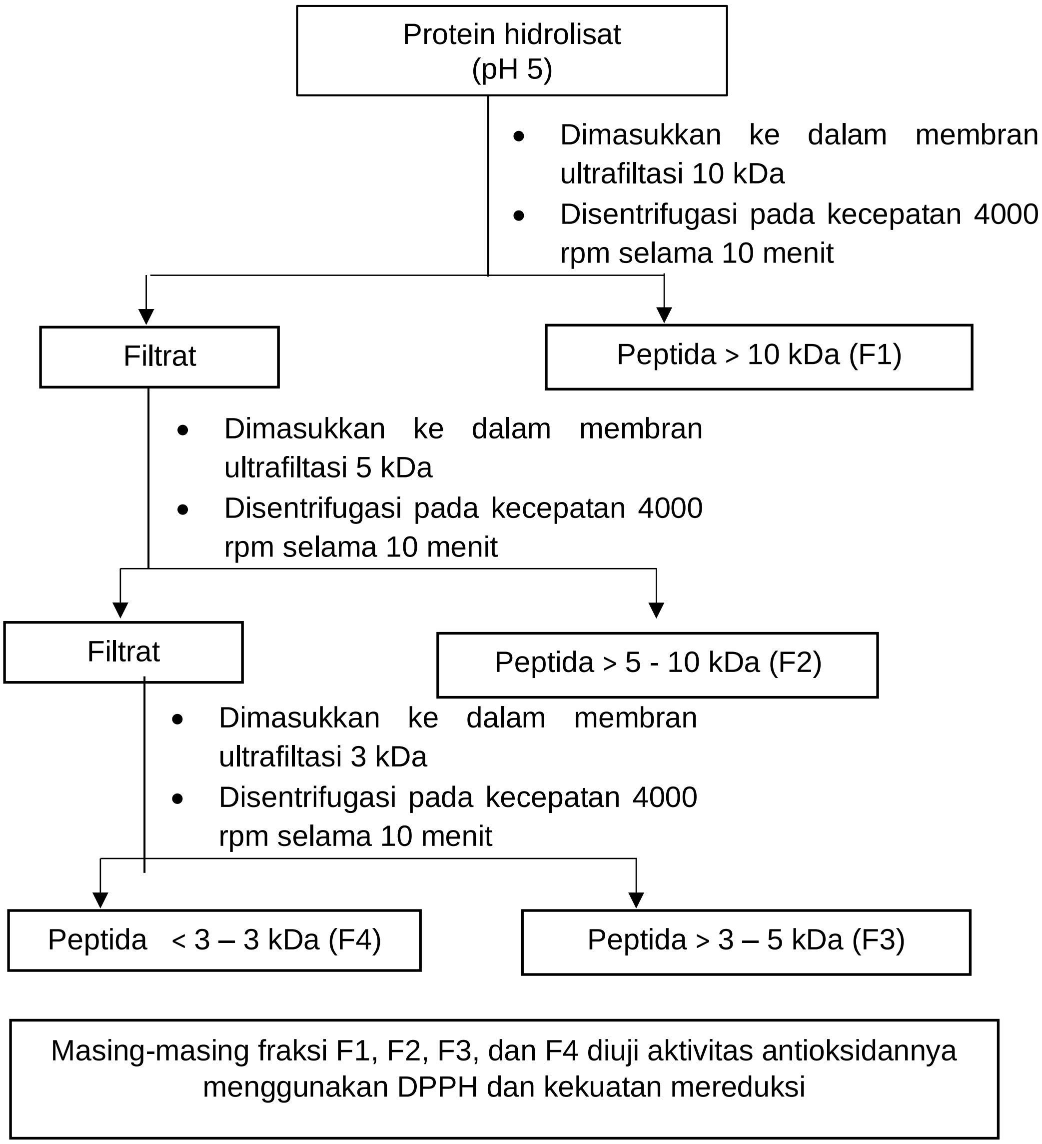
Lampiran 8: Skema Kerja Penentuan Waktu Hidrolisis Optimum Protein Ikan Sidat



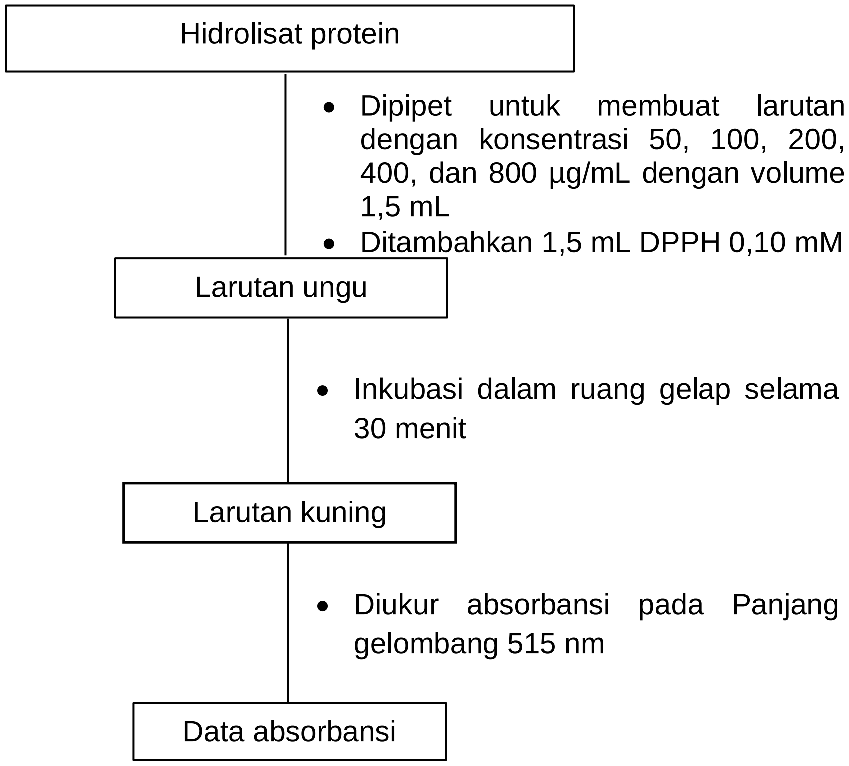
Lampiran 9: Skema Kerja Optimum Konsentrasi Enzim pada Hidrolisis Protein Ikan Sidat



Lampiran 10: Skema Kerja Ultrafiltrasi Hidrolisat Protein Ikan Sidat



Lampiran 11: Skema Kerja Penentuan Aktivitas Antioksidan Hidrolisat Protein dan peptida dengan Metode DPPH



Lampiran 12: Skema Kerja Pengujian Kekuatan Reduksi Fe^{3+} Hidrolisat Protein dan Peptida

1,5 mL hidrolisat protein/peptida

- Ditambahkan 1,5 mL buffer fosfat pH 6,6
- Ditambahkan 1,5 mL $K_3[Fe(CN)_6]$

Larutan kuning

- Diinkubasi selama 20 menit pada suhu 50^{o}C
- Ditambahkan 1,5 mL TCA 10%

Larutan kuning

- Disentrifugasi pada kecepatan 3500xg selama 10 menit

Supernatant

Endapan

- Dipipet 1,5 mL
- Ditambahkan 1,5 mL aquades
- Ditambahkan 300 µL $FeCl_3$ 1%
- Diinkubasi selama 10 menit

Campuran berwarna biru

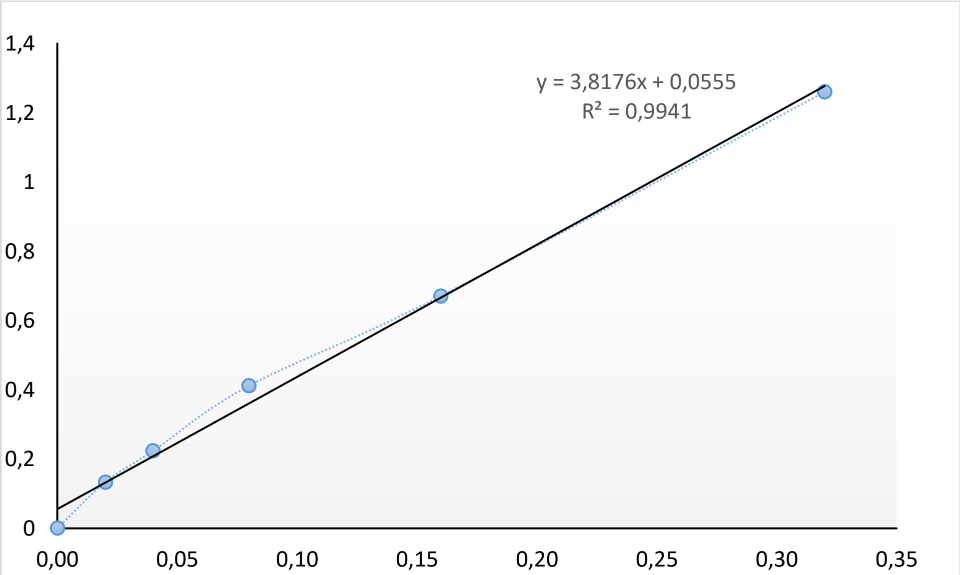
- Diukur absorbansinya pada Panjang gelombang 700 nm

Data absorbansi

Digunakan asam askorbat (1-5µg/mL) sebagai antioksidan pembanding dan kurva standar asam askorbat dan variasi konsentrasi hidrolisat protein >5-10 kDa (50 -250 µg/mL) sebagai kurva standar untuk penentuan EC_{50}

Lampiran 13. Penentuan Kadar Protein

1. Pembuatan kurva standar



2. Data penentuan kadar protein ekstrak kasar, fraksi 1, fraksi 2, filtrat sisa dan dialisat

Fraksi	Absorbansi Simplo	Absorbansi Duplo	Rata-rata	a	b	fp	Kadar Protein (mg/mL)
Ekstrak Kasar (EK)	0,482	0,482	0,482	3,8176	0,0555	50	5,586
F1	0,758	0,825	0,7915	3,8176	0,0555	50	9,640
F2	0,325	0,354	0,3395	3,8176	0,0555	50	3,720
Dialisat F2	0,139	0,135	0,137	3,8176	0,0555	50	1,067

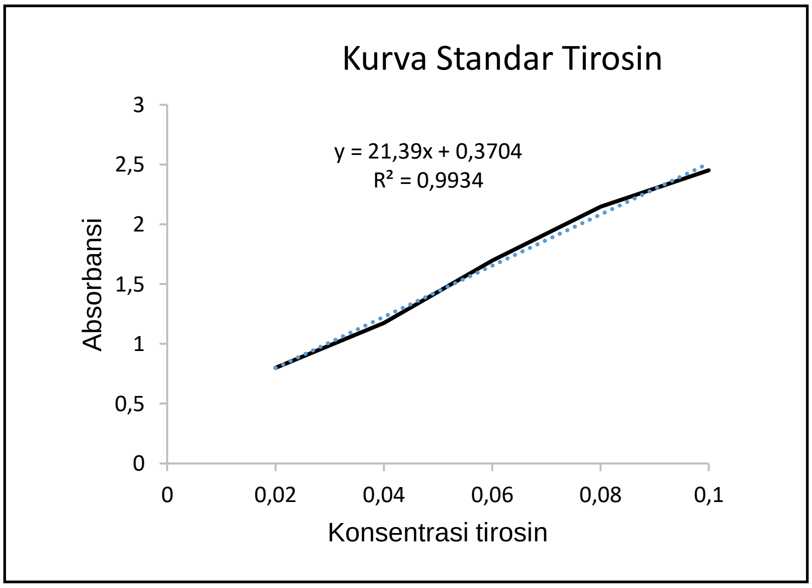
Contoh perhitungan kadar protein:

Kadar protein = $\frac{\text{Absorbansi rata–rata} - 0{,}0555}{3{,}8176} \text{ x fp}$

Kadar protein EK = $\frac{0{,}482 - 0{,}0555}{3{,}8176} \text{ x } 50 = 5{,}586$

Lampiran 14. Penentuan Aktivitas Katalitik Enzim Bromelin

1. Pembuatan Kurva Standar Tirosin



Data aktivitas

Fraksi	Abs	Kadar tirosin yang dilepaskan (μg/mL)	Kadar protein (mg/mL)	Aktivitas Enzim (U/mL)	Aktivitas spesifik (U/mg)	Kemurnian
EK	1,235	40,421	5,586	113,178	20,261	1,00
0-40% (F1)	1,384	47,387	9,640	132,683	13,764	0,68
40-60% (F2)	1,275	42,291	3,720	118,414	31,835	1,57
Dialisat F2	1,535	54,446	1,067	152,449	142,819	7,05

Contoh perhitungan aktivitas enzim, aktivitas spesifik dan tingkat kemurnian:

- Aktivitas Enzim = $\frac{(\text{Total } \frac{\mu g}{mL} \text{tyrosine dilepas} \times \text{total volume reaksi})}{(\text{Volume enzim} \times \text{waktu reaksi} \times \text{volume analisis})}$

Aktivitas Enzim = $\frac{(47{,}387 \times 2{,}1 \text{ ml})}{(0{,}1 \text{ mL} \times 10 \text{ menit} \times 0{,}75 \text{ ml})}$ = 132,68

- Aktivitas spesifik enzim = $\frac{\text{Aktivitas enzim}}{\text{kadar protein}}$

$$= \frac{132{,}68\ \text{U/mL}}{9{,}640\ \text{mg/mL}} = 13{,}764\ \text{U/mg}$$

- Tingkat kemurnian = $\frac{\text{Aktivitas spesifik enzim setelah fraksinasi}}{\text{Aktivitas spesifik enzim ekstrak kasar}}$

$$= \frac{13{,}764\ \text{U/mg}}{20{,}261\ \text{U/mg}} = 0{,}68$$

Lampiran 15. Data Hasil Analisis Proksimat pada Sampel Ikan Sidat *A.marmorata*

LABORATORIUM BIOTEKNOLOGI TERPADU PETERNAKAN
FAKULTAS PETERNAKAN
UNIVERSITAS HASANUDDIN
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Email: labbioternakfapetuh@gmail.com

No.Dok.: FSPO-LBTK-UH-12.2

SERTIFIKAT HASIL UJI

No.: 008/T/LBTK-UH/I/2022

Informasi Pelanggan

Nama Perusahaan/Pelanggan : Jumardi
Alamat Lengkap : Pascasarjana Universitas Hasanuddin
No. Telp./faks./e-mail : 085342617902
Personel Penghubung : 081241981874

Informasi Sampel

No. Identitas Laboratorium : 008/LBTK-RK/I-2022
Uraian/Matriks Sampel : -
Kondisi Saat Diterima : Baik
Tanggal Diterima : 18/1/2022
Tanggal Pengujian : 20/1/2022
Tujuan Pengujian : -

Informasi Hasil Pengujian

No	Kode Sampel	PARAMETER UJI				
		Kadar Air (%) (AOAC 930.15)	Kadar Abu (% BK) (AOAC 942.05)	Kadar Protein Kasar (% BK) (AOAC 984.13)	Kadar Lemak Kasar (% BK) (AOAC 920.39)	Kadar Serat Kasar (% BK) (AOAC 962.09)
1	Ikan	73,52	1,94	27,00	19,79	0,11

Makassar, 7 Februari 2022
Devisi Teknis,

Dr. Ir. Syahriani Syahrir, M.Si.
NIP.: 196511121990032001

Ket: 1. Kadar air ditetapkan sesuai sampel uji; 2. Selain kadar air, parameter ditetapkan berdasarkan 100% BK; 3. Lembaran sertifikat hasil uji ini tertelusur; 4. Hasil hanya berhubungan dengan contoh yang diuji dan laporan ini tidak boleh digandakan

Lampiran 16. Data Hasil Analisis Asam-Asam Amino pada Sampel Ikan Sidat *A. marmorata*

RESULT OF ANALYSIS

Laporan Hasil Pengujian : SIG.LHP.VII.2021.084174

No.	Parameter	Unit	Result		Limit Of Detection	Method
			Simplo	Duplo		
1	L-Metionin	mg / kg	3931.70	3930.48	-	18-12-38/MU/SMM-SIG (LC MS/MS)
2	L-Sistin	mg / kg	5242.79	5240.48	-	18-12-38/MU/SMM-SIG (LC MS/MS)
3	L-Histidin	mg / kg	7865.17	7898.91	-	18-5-17/MU/SMM-SIG (UPLC)
4	L-Threonin	mg / kg	9672.58	9712.21	-	18-5-17/MU/SMM-SIG (UPLC)
5	L-Prolin	mg / kg	6596.73	6626.59	-	18-5-17/MU/SMM-SIG (UPLC)
6	L-Tirosin	mg / kg	6917.11	6953.73	-	18-5-17/MU/SMM-SIG (UPLC)
7	L-Leusin	mg / kg	15493.54	15583.70	-	18-5-17/MU/SMM-SIG (UPLC)
8	L-Asam Aspartat	mg / kg	14223.50	14282.49	-	18-5-17/MU/SMM-SIG (UPLC)
9	L-Lisin	mg / kg	14940.74	15049.09	-	18-5-17/MU/SMM-SIG (UPLC)
10	Glisin	mg / kg	10370.35	10447.09	-	18-5-17/MU/SMM-SIG (UPLC)

No.	Parameter	Unit	Result		Limit Of Detection	Method
			Simplo	Duplo		
11	L-Arginin	mg / kg	12836.29	12910.10	-	18-5-17/MU/SMM-SIG (UPLC)
12	L-Alanin	mg / kg	10164.87	10223.26	-	18-5-17/MU/SMM-SIG (UPLC)
13	L-Valin	mg / kg	9650.25	9688.92	-	18-5-17/MU/SMM-SIG (UPLC)
14	L-Isoleusin	mg / kg	9003.04	9036.41	-	18-5-17/MU/SMM-SIG (UPLC)
15	L-Fenilalanin	mg / kg	9460.69	9494.26	-	18-5-17/MU/SMM-SIG (UPLC)
16	L-Asam glutamat	mg / kg	23857.76	23980.31	-	18-5-17/MU/SMM-SIG (UPLC)
17	L-Serin	mg / kg	8324.97	8340.97	-	18-5-17/MU/SMM-SIG (UPLC)

Bogor, 08 Juli 2021
PT. Saraswanti Indo Genetech

SARASWANTI INDO GENETECH INDONESIA BOGOR

Dwi Yulianto Laksono, S.Si
GM Laboratorium

Lampiran 17. Data Perhitungan Derajat Hidrolisis

1. Kontrol

	Kadar Protein Terlarut (mg/mL)	**Kadar Protein dalam TCA (mg/mL)**	**DH (%)**
Kontrol	6,5	0,1768	2,72

2. Pengaruh pH

pH	**Kadar Protein Terlarut (mg/mL)**	**Kadar Protein dalam TCA (mg/mL)**	**DH (%)**
5	6,50	2,796	43,0
6	6,50	1,631	25,1
7	6,50	3,602	55,4
8	6,50	3,949	60,8
9	6,50	3,556	54,7

3. Pengaruh suhu

Suhu (^oC)	**Kadar Protein Terlarut (mg/mL)**	**Kadar Protein dalam TCA (mg/mL)**	**DH (%)**
40	6,50	2,397	36,9
50	6,50	2,528	38,9
60	6,50	2,986	45,9
70	6,50	1,015	15,6

4. Pengaruh waktu

Waktu (Jam)	**Kadar Protein Terlarut (mg/mL)**	**Kadar Protein dalam TCA (mg/mL)**	**DH (%)**
1	6,50	2,4819	38,2
2	6,50	3,3660	51,8
3	6,50	2,6522	40,8
4	6,50	2,1545	33,1
5	6,50	2,3444	36,1

5. Pengaruh konsentrasi enzim

Konsentrasi Enzim (%)	Kadar Protein Terlarut (mg/mL)	Kadar Protein Dalam TCA (mg/mL)	DH (%)
1%	6,50	1,6110	24,8
2%	6,50	2,7177	41,8
3%	6,50	3,9816	61,3
4%	6,50	3,5952	55,3
5%	6,50	3,4577	53,2

Contoh perhitungan derajat hidrolisis (DH)

$$\text{DH} = \frac{\text{Protein terlarut dalam TCA}}{\text{Protein total terlarut dalam sampel}} \text{ x } 100\%$$

$$\text{DH} = \frac{0{,}1768 \text{ mg/mL}}{6{,}500 \text{ mg/mL}} \text{ x } 100\% = 2{,}72\%$$

Lampiran 18. Pengujian Antioksidan dengan DPPH

Hidrolisat protein

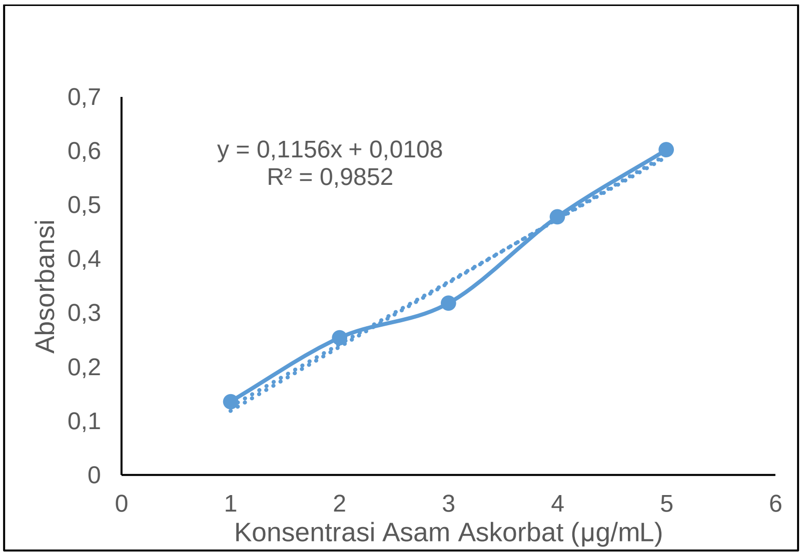
Data hasil uji antioksidan dari 20 sampel hidrolisat protein

No	Sampel	DPPH	
		Abs	% inhibisi
1	Blanko	0,654	-
2	Kontrol	0,577	11,8
3	pH 5	0,130	80,1
4	pH 6	0,368	43,7
5	pH 7	0,474	27,5
6	pH 8	0,455	30,4
7	pH 9	0,514	21,4
8	40^{o}C	0,575	12,1
9	50 ^{o}C	0,535	18,2
10	60 ^{o}C	0,582	11,0
11	70 ^{o}C	0,415	36,5
12	1 h	0,519	20,6
13	2 h	0,430	34,3
14	3 h	0,534	18,3
15	4 h	0,462	29,4
16	5 h	0,441	32,6
17	1%	0,569	13,0
18	2%	0,561	14,2
19	3%	0,505	22,8
20	4%	0,445	32,0
21	5%	0,434	33,6

Lampiran 19. Kekuatan Mereduksi Fe^{3+} dari Hidrolisat Protein

Kurva Standar Asam askorbat

Asam Askorbat		
No	**Conc (µg/mL)**	**Abs**
1	1	0,136
2	2	0,254
3	3	0,318
4	4	0,478
5	5	0,602



Lanjutan lampiran 19

Data hasil kekuatan mereduksi dari 20 sampel hidrolisat protein

No	Sampel	Power Reduksi	
		Abs	FRAP (mg AA/g HP)
1	Blanko	0	0,00
2	Kontrol	0,12	0,91
3	pH 5	1,1	9,08
4	pH 6	0,5	4,11
5	pH 7	0,2	1,75
6	pH 8	0,4	3,19
7	pH 9	0,8	6,86
8	40ºC	0,3	0,94
9	50 ºC	0,3	2,71
10	60 ºC	0,3	2,59
11	70 ºC	0,4	2,53
12	1 h	0,4	3,54
13	2 h	0,5	0,94
14	3 h	0,2	3,33
15	4 h	0,2	3,97
16	5 h	0,4	1,94
17	1%	0,6	2,05
18	2%	0,15	3,07
19	3%	0,3	0,94
20	4%	0,3	4,70
21	5%	0,3	1,19

Contoh perhitungan nilai FRAP :

Dari persamaan regresi pada kurva standar asam askorbat diperoleh persamaan:

y = 0,1156x + 0,0108

x = $\frac{y-0,0108}{0,1156}$

Nilai Y = nilai absorbansi hidrolisat protein

x = $\frac{1,1-0,0108}{0,1156} = 9,08$

nilai FRAP = 3,38 mg asam askorbat/ g hidrolisat protein

Lampiran 20. Kadar Protein Hasil Ultrafiltrasi

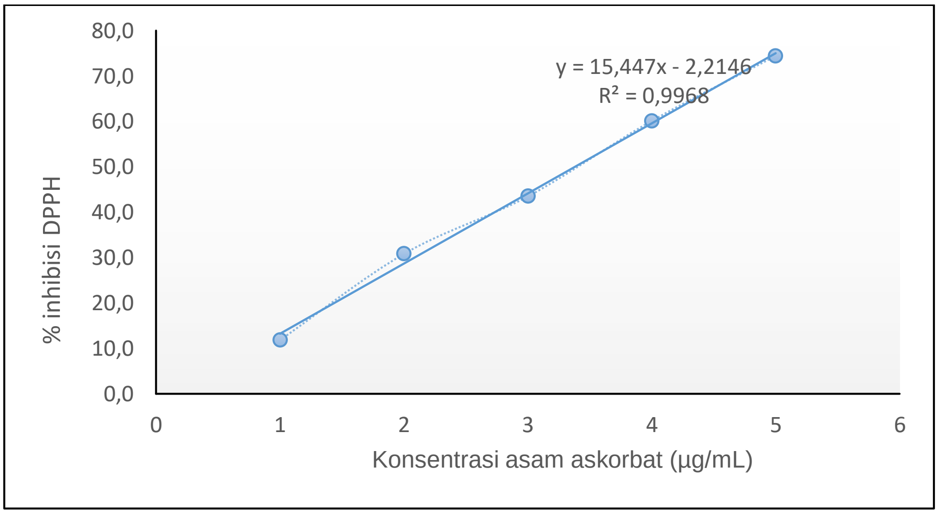
Kadar Protein fraksi hidrolisat protein hasil ultrafiltrasi

Protein	Abs		Rata-Rata	a	b	Conc mg/mL I
	I	II				
>10 kDa	0,351	0,365	0,358	3,8176	0,0555	3,962
10-5 kDa	0,462	0,469	0,466	3,8176	0,0555	5,370
5-3 kDa	0,373	0,365	0,369	3,8176	0,0555	4,107
<3 kDa	0,288	0,278	0,283	3,8176	0,0555	2,980

Lampiran 21. Pengujian Antioksidan Dengan DPPH Dan Kekuatan Mereduksi Fe^{3+} dari Peptida Hasil Ultrafiltrasi

1. Kurva Standar asam askorbat

No	**Konsentrasi (µg/mL)**	**Abs**	**Abs**	**% inhibisi**
1	Kontrol	0,727	0,727	-
2	1	0,638	0,641	11,8
3	2	0,515	0,503	30,8
4	3	0,401	0,411	43,5
5	4	0,291	0,290	60,1
6	5	0,179	0,186	74,4



Contoh perhitungan persen inhibisi:

Perhitungan % inhibisi $= \frac{\text{Abs Blanko} - \text{Abs sampel}}{\text{Abs Blanko}} \; x \; 100\%$

Perhitungan % inhibisi = $\frac{0{,}727 - 0{,}641}{0{,}727} \; x \; 100\%$ = 11,8%

Contoh perhitungan IC_{50}:

Dari persamaan regresi pada kurva standar DPPH diperoleh persamaan:

y = 15,447x – 2,2146

x = $\frac{y + 2{,}2146}{15{,}447}$

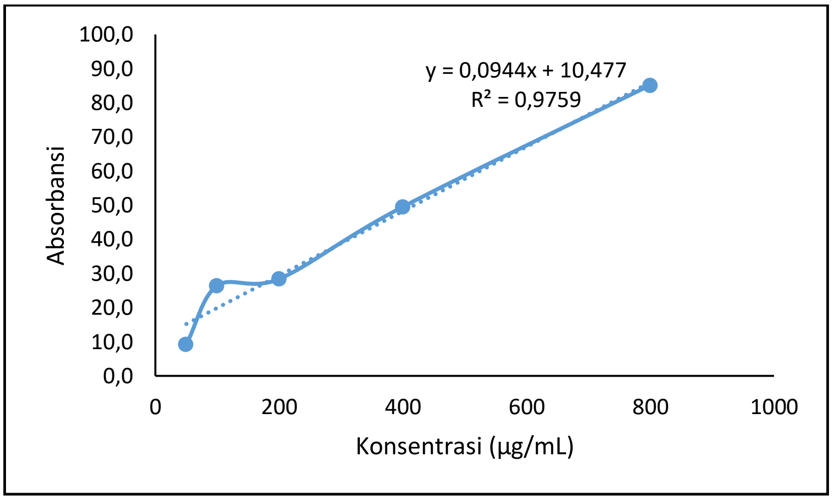
Nilai Y = 50

x = $\frac{50 + 2{,}2146}{15{,}447} = 3{,}38$

nilai IC_{50} = 3,38 µg/mL atau 0,0034 mg/mL

2. Kurva Standar DPPH untuk sampel 10 kDa

No	Conc (µg/mL)	Abs	Abs	Abs rata-rata	% inhibisi
1	Kontrol	0,603	0,637	0,620	-
2	50	0,570	0,556	0,563	9,2
3	100	0,450	0,462	0,456	26,5
4	200	0,447	0,441	0,444	28,4
5	400	0,311	0,315	0,313	49,5
6	800	0,094	0,090	0,092	85,2



Contoh perhitungan persen inhibisi:

Perhitungan % inhibisi $= \frac{\text{Abs Blanko} - \text{Abs sampel}}{\text{Abs Blanko}} \; x \; 100\%$

Perhitungan % inhibisi = $\frac{0{,}631 - 0{,}466}{0{,}631} \; x \; 100\%$ = 26,1%

Contoh perhitungan IC_{50}:

Dari persamaan regresi pada kurva standar DPPH diperoleh persamaan:

y = 0,0944x + 10,477

x = $\frac{y - 10{,}477}{0{,}0944}$

Nilai Y = 50

x = $\frac{50 - 10{,}477}{0{,}0944} = 418$

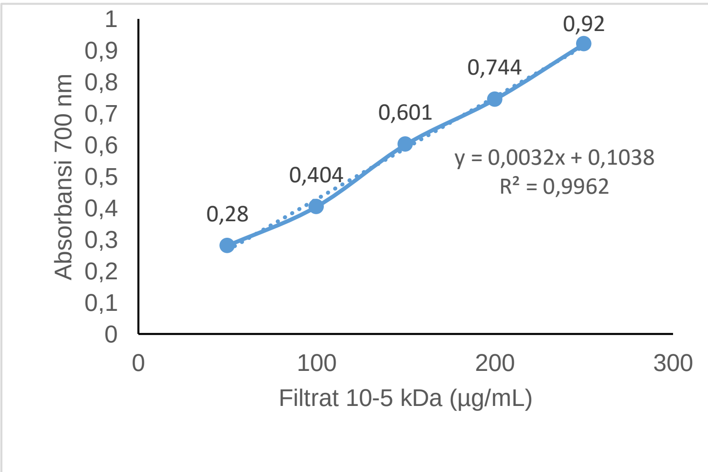
nilai IC_{50} = 418 µg/mL atau 0,418 mg/mL

3. Aktivitas antioksidan terhadap DPPH

No	Sampel	DPPH		
		Abs	% inhibisi	IC_{50} (mg/mL)
1	Kontrol	0,631	-	-
2	> 10 kDa	0,485	23,1	-
3	10-5 kDa	0,409	35,2	0,418
4	5-3 kDa	0,465	26,3	-
5	<3 kDa	0,466	26,1	-
6	Asam askorbat			0,0034

4. Kekuatan mereduksi Fe^{3+} dari fraksi hidrolisat

No	Sampel	Metode FRAP		
		Abs	Nilai FRAP	EC_{50} (mg/mL)
1	Kontrol	0,12	0,91	-
2	> 10 kDa	0,65	8,1	-
3	10-5 kDa	0,95	7,9	15,59
4	5-3 kDa	0,92	7,6	-
5	<3 kDa	0,89	5,6	-



Dari persamaan regresi pada kurva standar DPPH diperoleh persamaan:

y = 0,0032x + 0,1038

$$x = \frac{y - 0{,}1038}{0{,}0032}$$

Nilai Y = 50

$$x = \frac{50-0,1038}{0,0032} = 15592,56$$

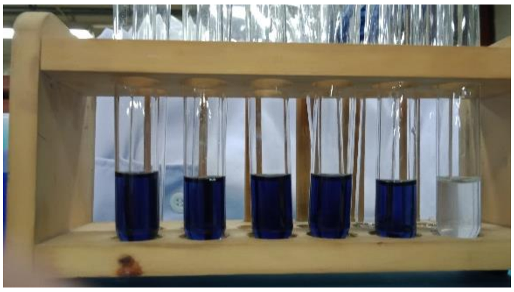
nilai IC_{50} = 15592, 56 µg/mL atau 15, 59 mg/mL

Lampiran 22. Dokumentasi Penelitian

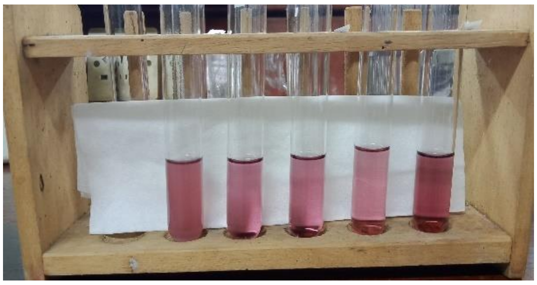
Isolasi Enzim bromelin dari buah nanas

Sampel Ikan sidat (*Anguilla marmorata*)

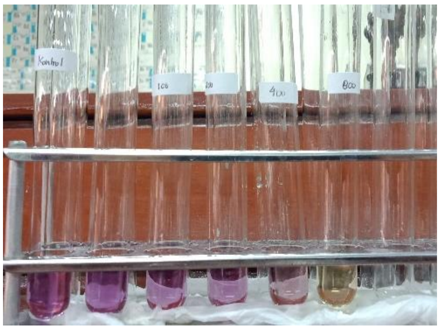
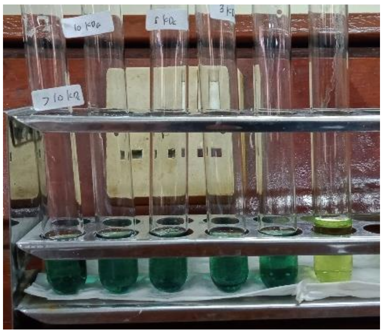
Hidrolisis protein ikan sidat menggunakan enzim bromelin

Pengujian protein

Pengujian antioksidan DPPH dan kekuatan mereduksi hidrolisat protein

Pengujian antioksidan DPPH dan kekuatan mereduksi hasil ultrafiltrasi