DAFTAR PUSTAKA

Aberoumand, A., 2010, Isolation and Characteristics of Collagen from Fish Waste Material, *World Journal of Fish and Marine Sciences*, **2**(5): 471-474.

[AOAC] Association of Official Analytical Chemyst, 1995, *Official Method of Analysis of The Association of Official Analytical of Chemist*, published by The Association of Official Analytical Chemist, Arlington Virginia (US).

[AOAC] Association of Official Analytical Chemyst, 2005, *Official Method of Analysis of The Association of Official Analytical of Chemist*, published by The Association of Official Analytical Chemist, Arlington Virginia (US).

[AOAC] Association of Official Analytical Chemist. 2006. *Official Methods of Analysis*, Edisi ke-18, published by The Association of Official Analytical Chemist, Washington DC (US).

Amri dan Khairuman, 2008, *Buku Pintar Budidaya 15 Ikan Konsumsi*, Agromedia, Jakarta.

Apriyantono, A., Fardiaz, D., Puspitasari, N.L., Sedamawati, Y., Budianto, S., 1989, *Petunjuk Laboratorium Analisis Pangan*, Universitas Pangan dan Gizi, Institut Pertanian Bogor (IPB), Bogor.

Ariesta, C., 2014, *Ekstraksi dan karakterisasi kolagen dari kulit ikan cobia (Rachycentron canadum)*, Skripsi tidak diterbitkan, Departemen Teknologi Hasil Perairan, Institut Pertanian Bogor, Bogor.

Asyiraf, N., 2011, *Extraction of Collagen From Fish Waste and Determination of Its Physico-chemical Characteristic, Final Project*, Degree of Bachelor of Science (Hons.) Food Science and Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, Selangor.

Badan Pusat Statistik, 2017, *Produktifitas Perikanan Indonesia*, Kementrian Kelautan dan Perikanan Republik Indonesia, Jakarta.

[BSN] Badan Standardisasi Nasional, 2014, *Kolagen kasar dari sisik ikan- Syarat mutu dan pengolahan,* SNI 8076- 2014, Jakarta: Badan Standardisasi Nasional.

Bella, J., Eaton, M., dan Berman, H.M., 1994, Crystal and molecular structure of a collagen-like peptide at 1.9 A resolution, *Journal of Science.* **266**(12): 75-81.

Cahyono, B., 2011, *Untung Berlipat Budi Daya Tawes Sebagai Bahan Baku Keripik,* Lili Publisher, Yogyakarta.

Cardoso, V.S., Quelemes, P.V., Amorin, A., Primo, F.L., Gobo, G.G., Tedesco, A.C., Mafud, A.C., Mascarenhas, Y.P., Corrêa, J.R., Kuckelhaus, S.A *et al.*, 2014, Collagen Based Silver Nanoparticles for Biological Applications: Synthesis and Characterization, *Journal of Nanobiotechnology*, **12**(36): 1-9.

Chai, H. Y., Li, J. H., Huang, H. N., Li, T. L., Chan, Y. L., Shiau, C. Y., dan Wu, C. J., 2010, Effects of Sizes and Conformations of Fish-Scale Collagen Peptides on Facial Skin Qualities and Transdermal Penetration Efficiency, *Journal of Biomedicine and Biotechnology*, **1**(1): 1-9.

Cho, S.M., Gu, Y.S., Kim, S.B., 2005, Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins, *Food Hydrocolloids,* **19**(1): 221- 229.

Coates, J., 2000, *Interpretation of infrared spectra, a practical approach.* Di dalam: Meyers RA, editor. Encyclopedia of Analytical Chemistry, Chichester: John Wiley & Sons Ltd.

Devi, H.L.N.A., Suptijah, P., dan Nurilmala, M., 2017, Efektifitas Alkali dan Asam terhadap Mutu Kolagen dari Kulit Ikan Patin, *Jurnal Pengolahan Hasil Perikanan Indonesia*, **20**(2): 255–265.

Dincer, M.T., Agcay, O.Y., Sargin, H., dan Baryam, H., 2015, Functional Properties of Gelatin Recovered From Scales of Farmed Sea Bass (*Dicentrarchus labrax*), *Turkish Journal of Veterinary and Animal Sciences,* **39**(1): 102-109.

Fontaine-Vive, F., Merzel, F., Johnson, M.R., Kearley, G.J., 2009, Collagen and component polypeptides: low frequency and amide vibrations, *Chemical Physics,* **355**(1): 141-148.

Friess, W., 1998, Collagen-biomaterial for drug delivery, *European Journal of Pharmaceutics and Biopharmaceutics,* **45**(1): 113–136.

Harjanto, S., 2017, Perbandingan Pembacaan Absorbansi Menggunakan Spektronik 20D[+] dan Spectrometer UV-VIS T60U dalam Penentuan Kadar Protein dengan Larutan Standar BSA, *Jurnal Kimia Sains dan Aplikasi,* **20**(30): 114-116.

Hema, G.S., Shyni, K., Mathew, S., Anandan, R., Ninan, G., dan Lakshmanan, P.T., 2013, A Simple Method for Isolation of Fish Skin Collagen- Biochemical Characterization of Skin Collagen Extracted from Albacore Tuna (*Thunnus alalunga*), Dog Shark (*Scoliodon sorrakowah*), and Rohu (*Labeo rohita),* *Annals of Biological Research,* **4**(1):271-278.

Hinterwaldner, R., 1997, *The Science and Technology of Gelatin*, Academy Press, New York (US).

Hsiao, C.Y., Chou, C.H., Sun, H.W., dan Seah, J.N., 2004, Novel Collagen Production Method, United States Patent Application Publication, Patent Number 0253678 A1.

Jaswir, I., Monsur, H.A., Salleh, H.M., 2011, Nano-structural analysis of fish collagen extracts for new process development, *African Journal of Biotechnology,* **10**(81): 18847-18854.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Nagai, T., Tanaka, M., 2005, Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*), *Food Chemistry,* **93**(1): 475-484.

Kasim, S., 2013, *Ekstraksi Kolagen Tulang Rawan Ikan Pari (Himantura gerrardi) dan Kulit Ikan Tuna (Thunnus sp) Menggunakan Variasi Jenis Larutan Asam*, Majalah Farmasi dan Farmakologi, Fakultas Farmasi, Universitas Hasanuddin, Makassar.

Katili, A.S., 2009, Struktur dan Fungsi Kolagen, *Jurnal Pelangi Ilmu*, **2**(5): 19-29.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., Tanaka, M., 2005, Characterization of Acid-Soluble Collagen from Skin and Bone of Bigeye Snapper (Priacanthustayenus), *Food Chem*, **221**(1): 363-372.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Shahidi, F., 2010, Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (*Chiloscyllium punctatum*) and blacktip shark (*Carcharhinus limbatus*), *Food Science and Technology,* **43**(1): 792–800.

Kementerian Kelautan dan Perikanan (KKP), 2016, *Laporan Kinerja KKP Triwulan II* Tahun 2016.

Kementerian Kelautan dan Perikanan (KKP), 2018, *Data Rujukan Wilayah Kelautan Indonesia* Tahun 2018.

Komala, A.H., 2015, *Ekstraksi dan Karakterisasi Kolagen dari Kulit Ikan Tongkol (Euthynnus affinis)*, Skripsi tidak diterbitkan, Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor, Bogor.

Kong, J., Yu, S., 2007, Fourier transform infrared spectroscopic analysis of protein secondary structures, *Acta Biochim Biophys Sinica*, **39**(8): 549-559.

Kottelat, M., Whitten, J.A., Kartikasari, N.S., and Wirjoatmodjo, S., 1993, *Freshwater Fishes of Western Indonesia and Sulawes*i, Dalhousie University, Canada.

Kumar, M.H., Spandana, V., Poonam, T., 2011, Extraction and determination of collagen peptide and its clinical importance from tilapia fish scales

(*Oreochromis niloticus*), *International Research Journal of Pharmacy,* **2**(10): 97-99.

Lee, C.H., Singla, A., Lee, Y., 2001, Review: Biomedical Application of Collagen, *International Journal of Pharmacy,* **221**(1): 1–22.

Lehninger, A. L., 1982, *Dasar-dasar Biokimia, Jilid I*, Terjemahan oleh Maggy Thenawijaya, Erlangga, Jakarta.

Lehninger, Albert, L., 1993, *Dasar-Dasar Biokimia,* Terjemahan oleh Maggy Thenawijaya, Erlangga, Jakarta.

Li, Z., Wang, B., Chi, C., Zhang, Q., Gong, Y., Tang, J., Luo, H., Ding, G., 2013, Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (*Scomberomorous niphonius*), *Food Hydrocolloids,* **31**(1): 103-113.

Liu, D., Wei, G,. Li, T., Hua, J., Lu, J., Regenstein, J.M., Zhou, P., 2015, Effects of alkaline *preatreatment* and acid extraction onditions on the acid sluble collagen from grass carp (*Ctenopharyngodon idella*) skin, *Food Chemistry,* **172**(1): 836-843.

Liu, D., Liang, L., Regenstein, J.M., Zhou, P., 2012, Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (*Hypophthalmichthys nobilis*), *Food Chemistry,* **133**(1): 1441-1448.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D., dan Darnell, J., 2000, *Molecular Cell Biology*, 4th Edition., W. H. Freeman, New York.

Mahardika, S., 2013, *Isolasi dan Karakterisasi Kolagen Nano partikel dari Kulit Ikan Cucut Bambu (Chiloscyllium Punctatum)*, Skripsi tidak diterbitkan, Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor, Bogor.

Mahboob, S., 2015, Isolation and Characterization of Collagen From Fish Waste Material- Skin, Scales and Fins of *Catla catla* and *Cirrhinus mrigala*, *Journal of Food Science and Technology*, **52**(7): 4296-4305.

Massod, Z., Yasmeen, R., Haider, M.S., Tarar, O.M., Zehra, L., dan Hossain, M. Y., 2015, Evaluation of Crude Portein and Amino Acid Contents From The Scales of Four Mullet Species (Mugilidae) Collected From Karachi Fish Harbour, Pakistan, *Indian Journal of Geo-Marine Science*, **44**(5): 1-9.

Matmaroh, K., Benjakul, S., Prodpran, T., Encarnacion, A.B., Kishimura, H., 2011, Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*), *Food Chemistry* **129**(1): 1179-1186.

Mayasari, D., 2016, *Ekstraksi Kolagen Optimun dari Kulit Ikan Tuna* (*Thunnus* sp.) Sebagai Antioksidan, Skripsi tidak diterbitkan, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.

Muyonga, J.H., Cole, C.G.B., Duodu, K.G., 2004, Characterisation of acids soluble collagen from skins of young and adult Nile perch (*Lates niloticus*), *Food Chemistry,* **85**(1)**:** 81-89.

Nagai, T, and Suzuki, N., 2000, Isolation of Collagen from Fish Waste Material Skin, Bone and Fins*, Journal of Food Chemistry,* **68**(3): 277–281.

Nagarajan, M., Benjakul, S., Prodpran, T., Songtipya, P., and Kishimura, H., 2012, Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures, *Food Hydrocolloids,* **29**(1): 389-397.

Nelson, S.J., 2006, *Fishes of the World*, Wiley, Canada.

Nollet, L., 1996, *Handbook of Food Analysis Amino*, Marcel Dekker, New York (US).

Nurhayati, Tazwir, dan Murniyati, 2013, Ekstraksi dan Karakterisasi Kolagen Larut Asam dari Kulit Ikan Nila (*Oreochromis niloticus*), *JPB Kelautan dan Perikanan*, **8**(1): 85-92.

Nurhidayah, B., Soekendarsi, E., Erviani, A.V., 2019, Kandungan Kolagen Sisik Ikan Bandeng (*Chanos-chanos*) dan Sisik Ikan Nila (*Oreochromis niloticus*), *Jurnal Biologi Makassar*, **4**(1): 39-47.

Nurilmala, M., A.M. Jacoeb, dan R.A Dzaky, 2017, Karakteristik Gelatin Kulit Ikan Tuna Sirip Kuning. *Jurnal Pengolahan Hasil Perikanan Indonesia*. **20**(2): 340 – 350.

Nur'aenah, N., 2013, *Ekstraksi dan karakterisasi kolagen dan nanopartikel kolagen dari kulit ikan pari (Pastinachus solocirostris) sebagai bahan baku kosmetik,* Tesis tidak diterbitkan, Sekolah Pascasarjana, Institut Pertanian Bogor, Bogor.

Purnomo, E. 1991. *Penyamakan Kulit Kaki Ayam*. Kanisius.Yogyakarta

Putra, A.B.N., Sahubawa, L., dan Ekantari, N., 2013, Ekstraksi dan Karakterisasi Kolagen dari Kulit Ikan Nila Hitam (*Oreochromis niloticus*), *JPB Perikanan*, **8**(2): 171-180.

Potaros, T., Raksakulthai, N., Runglerdkreangkrai, J., Worawattanamateekul, W., 2009, Characteristics of collagen from nile tilapia (*Oreochromis niloticus*) skin isolated by two different methods, *Kasetsart Journal,* **43**(1): 584-593.

Pranoto, Y., Lee, C.M., and Park, H.J., 2007. Characterizations of fish gelatin films added with gellan and κ-carrageenan. *LWT - Food Science and Technology*, **40**(5): 766–774.

Sahubawa, L., dan Putra, A.B.N., 2011, Pengaruh Konsentrasi Asam Asetat dan Waktu Ekstraksi Terhadap Mutu kolagen Limbah Kulit Ikan Nila Hitam, *Jurnal Teknosains*, **1**(1): 16-25.

Sahrianna, 2019, *Isolasi dan Karakterisasi Kolagen dari Sisik Ikan Kakatua (Chlorurus Bleekeri)*, Skripsi tidak diterbitkan, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Hasanuddin, Makassar.

Sai, K.P., Babu, M., 2001, Studies on *Rana tigerina* skin collagen, *Comparative Biochemistry and Physiology Part B,* **128**(1): 81-90.

Setyowati, H., Setyani, W., 2015, Potensi Nano Kolagen Limbah Sisik Ikan Sebagai *Cosmeceutical, Jurnal Farmasi Sains dan Teknologi,* **12**(1): 30-40.

Shahiri, H.T., Maghsoudlou, Y., Motamedzadegan, A., Sadeghi, A. R. M., dan Rostamzad, H., 2012, Study on Some Properties of Acid-Soluble Collagens Isolated from Fish Skin and Bones of Rainbow Trout (Onchorhynchus mykiss), *International Food Research Journal*, **19**(1): 251-257.

Shah, V, Manekar, A., 2012, Isolation and characterization of collagen from the placenta of buffalo (*Bovidae bubalus bubalis*) for the biomaterial applications, *Trend in Life Science,* **1**(4): 26–32.

Shon, J., Ji-Hyun, E., Hwang, S.J., and Jong-Bang, E, 2011, Effect of processing conditions on functional properties of collagen powder from Skate (*Raja kenojei*) skins*, Food Sci Biotechnol,* **20**(1): 99-106.

Singh, P., Benjakul, S., Maqsood, S., Kishimura, H., 2011, Isolation and Characterization of Collagen Extracted From the Skin of Striped Catfish (*Pangasianodon hypophthalmus*), *The Journal of Food Chemistry,* **124**(1): 97-105.

Stephanie, T.A., Risfah, Y., Fitriyanti, J.S., Naimah, R., 2016, Isolasi Kolagen Dari Kulit dan Tulang Ikan Cakalang (*Katsuwonus pelamis*), *Journal of Pharmaceutical and Medicinal Sciences,* **1**(1):27-30.

Suptijah, P., Dini, I., Supriyono, E.W., 2018, Isolasi dan Karakterisasi Kolagen dari Kulit Ikan Patin (*Pangasius* Sp.), *Jurnal Sains Natural Universitas Nusa Bangsa*, **8**(1):8-23.

Taofiqurohman, A., Nurruhwati, I., dan Hasan, Z., 2007, *Studi Kebiasaan Makanan Ikan (Food Habit) Ikan Nilem (Osteochilus Hasselti) Di Tarogong Kabupaten*

Garut, Laporan Penelitian Peneliti Muda (Litmud) Unpad, Fakultas Perikanan Dan Ilmu Kelautan universitas Padjadjaran.

Tridhar, N. A., 2016, Perbandingan Produksi Kolagen dari Sisik dan Tulang Ikan Gurami (*Osphronemus gouramy*) Secara Kimia dan Enzimatis, Skripsi, Universitas Negeri Pasundan, Bandung.

Triyono, B., 2005, *Perbedaan Tampilan Kolagen di Sekitar Luka Insisi pada Tikus Wistar yang Diberi Infiltrasi Penghilang Nyeri Levobupivakain dan yang Tidak Diberi Levobupivakain*, Tesis tidak diterbitkan, Program Magister Biomedik dan PPDS I, Universitas Diponegoro, Semarang.

Veeruraj, A., Arumugam, M., Balasubramanian, T.,, 2013, Isolation and characterization of thermostable collagen from the marine eel-fish (*Evenchelys macrura*), *Process Biochemistry,* **48**(1): 1592-1602.

Waters, 2012, *Acquity UPLC H-Class and H-Class Bio Amino Acid Analysis System Guide*, Waters Corporation, USA.

Winarno, 1992, *Kimia Pangan dan Gizi*, Pt Gramedia Pustaka Utama, Jakarta.

Wulandary, Suptijah, P., Tarman, K., 2015, Efektifitas Pretreatment dan Hidrolis Asam Asetat Terhadap Karakteristik Kolagen dari Kulit Ikan Gabus, *Jurnal IPB*, **18**(3): 287-302.

Yakimets, I., Wellner, N., Smith, A.C., Wilson, R.H., Farhat, I., Mitchell, J., 2005, Mechanical properties with respect to water content of gelatin films in glassy state, *Journal Polymer,* **46**(1): 12577-12585.

Yan, M.Y., Li, B.F., Zhao, X., Ren, G.Y., Zhuang, Y.L., Hou, H., Zhang, X.K., Chen, L., dan Fan, Y., 2008, Characterization of Acid-Soluble Collagen From The Skin of Walleye pollock (Theragra chalcogramma), *Food Chemistry*, **107**(1): 1581-1586.

Yumrawati, 2007, *Pengaruh Pemberian pakan Alami Berbeda Terhadap Pertumbuhan dan Kelulushidupan Larva Ikan Selais (Ompok hypophthalmus)*, Skripsi tidak diterbitkan, Fakultas Perikanan dan Ilmu Kelautan, Universitas Riau, Pekanbaru.

Zhou, P., and Regenstein, J.M, 2005, Effects of alkaline and acid pretreatments on alaska pollock skin gelatin extraction, *Journal Food Sci,* **70**(6): 392-396.

Lampiran 1. Bagan Alir Penelitian

| **Kulit Ikan Tawes** | → | Analisis Proksimat (Kadar air, abu dan protein) |

- Direndam dengan NaOH 1:10 b/v selama 12 jam

| Kulit 1 0,025 M | Kulit 2 0,05 M | Kulit 3 0,075 M | Kulit 4 0,1 M | **Larutan** hasil perendaman dengan NaOH |

Uji lowry

Kulit hasil perendaman terbaik

- Direndam dengan butil alkohol 10 % 1:10 b/v selama 24 jam

Kulit hasil perendaman dengan butil alkohol 10%

- Direndam dengan CH$_3$COOH 1:15 b/v selama 1,2,3,4 dan 5 jam

| Kulit A1 0,5 M | Kulit A2 0,75 M | Kulit A3 1 M |

Uji DP 1,2,3,4 dan 5 Jam

Kulit dengan DP terbaik

- Disaring dengan kain saring

| Endapan | **Filtrat** |

- Disalting Out dengan NaCl 2,6 M selama 24 jam
- Diendapkan dengan sentrifus pada kecepatan 8000 rpm, 4 ^{0}C, 20 menit

Endapan kolagen

- Dimasukkan ke dalam membran selofan
- Membram yang berisi kolagen direndam dengan CH$_3$COOH 0.1 M, diganti setiap 2 jam, (diulangi 12 kali dengan asam asetat yang baru)
- Membram yang berisi kolagen direndam di dalam akuades, diganti setiap 2 jam sampai pH 5 atau lebih.
- Dikeringkan dengan *freeze dryer,* 24 jam.

Kolagen Kering → Analisis Rendamen

- **Dikarakterisasi**

kadar air (41.103 AOAC 2006)

kadar abu (41.103 AOAC 2006)

kadar protein (AOAC 2005)

pH (Apriyantono, dkk., 1989)

gugus fungsi (FTIR) (Muyonga, dkk., 2004)

komposisi asam amino (UPLC) (Nollet, 1992)

Lampiran 2. Perhitungan Kadar Air

a) **Kadar air kulit Ikan Tawes**

$$\text{Kadar air } (\%) = \frac{D - ((A + C) - A)}{D} \times 100\%$$

Contoh perhitungan

$$\text{Kadar air } (\%) = \frac{2,0045 - (48,8122 - 48,0745)}{2,0045} \times 100\%$$

$$= 63,19 \%$$

No.	Bobot kosong cawan (g) A	Bobot cawan + sampel (g) B	Bobot konstan (g) C	Bobot awal sampel (g) D	Kadar air (%)
1	48,0745	50,0790	48,8122	2,0045	63,19
2	48,9937	50,9759	49,6909	1,9822	64,82
Rata – rata					64,005

b) **Kadar air kolagen Kulit Ikan Tawes**

No.	Bobot kosong cawan (g) A	Bobot cawan + sampel (g) B	Bobot konstan (g) C	Bobot awal sampel (g) D	Kadar air (%)
1	20,8700	21,0701	21,0419	0,2001	14,00
2	28,9522	29,1532	29,1176	0,2010	17,61
Rata – rata					15,81

Lampiran 3. Perhitungan Kadar Abu

a) Kadar abu kulit ikan Tawes

$$\text{Kadar Abu (\%)} = \frac{C - A}{(B-A) \times (1-(\text{kadar air}/100))} \times 100\%$$

Contoh perhitungan

$$\text{Kadar Abu (\%)} = \frac{26,8397 - 26,8295}{(31,7237-26,8295) \times (1-(64,005/100))} \times 100\%$$

$$= 0,33 \%$$

No.	Bobot kosong cawan (g) A	Bobot cawan + sampel (g) B	Bobot konstan C	Kadar abu (%)
1	26,8295	31,7237	26,8397	0,33
2	26,9550	31,9717	26,9652	0,32
Rata – rata				0,3221

b) Kadar abu kolagen kulit ikan tawes

No.	Bobot kosong cawan (g) A	Bobot cawan + sampel (g) B	Bobot konstan C	Kadar abu (%)
1	20,8700	21,0701	20,8702	0,12
2	28,9522	29,1532	28,9525	0,17
Rata – rata				0,15

Lampiran 4. Perhitungan Kadar Protein

a) Protein kulit ikan tawes

$$\text{Protein (\%)} = \frac{(V1-V2) \times N \times 14{,}007 \times FK}{W \times 1000} \times 100\%$$

Contoh perhitungan

$$\text{Protein (\%)} = \frac{(56{,}5-0) \times 10{,}8 \times 14{,}007 \times 6{,}25}{1{,}0052 \times 1000} \times 100\%$$

$$= 53{,}1428 \%$$

No.	Volume HCl (V_1)	Normalitas HCl standar	Berat sampel (g) (W)	Kadar protein (%)
1	56,5	10,8	1,0052	53,1428
2	55,0	10,8	1,0001	51,9957
Rata – rata				52,5693

b) Protein kolagen kulit ikan tawes

No.	Volume HCl (V_1)	Normalitas HCl standar	Berat sampel (g) W	Kadar protein (%)
1	38,4	10,8	0,49	74,09

Lampiran 5. Perhitungan rendamen Kolagen

$$\text{Rendamen Kolagen (\%)} = \frac{\text{Berat kolagen kering (g)}}{\text{Berat bahan baku kulit (g)}} \times 100\%$$

$$\text{Rendamen Kolagen (\%)} = \frac{2,4130 \text{ gram}}{84,8343 \text{ gram}} \times 100\%$$

Rendamen kolagen (%)   = 2,84 %

Lampiran 6. Perhitungan Derajat Pengembangan (DP)

$$DP\ (\%) = \frac{(B-A)}{(A)} \times 100\%$$

Contoh perhitungan

$$DP\ (\%) = \frac{16,8118 - 3,0688}{3,0688} \times 100\%$$

DP (%) = 447,8297 %

Waktu	[NaOH] (M)	C (g)	D (g)	A (g)	E (g)	B (g)	DP (%)
1 jam	0.50	63.5431	66.6119	3.0688	80.3549	16.8118	447.8297
	0.75	63.1662	66.2126	3.0464	83.2677	20.1015	559.8444
	1.00	61.8862	64.8939	3.0077	88.5680	26.6818	787.1164
2 jam	0.50	64.2459	67.2492	3.0033	83.6817	19.4358	547.1481
	0.75	63.5411	66.5848	3.0437	89.6697	26.1286	758.4485
	1.00	63.4603	66.4874	3.0271	93.1983	29.7380	882.3923
3 jam	0.50	35.8168	38.8528	3.0360	58.6157	22.7989	650.9519
	0.75	30.6844	33.6894	3.0050	58.4672	27.7828	824.5524
	1.00	30.6640	33.6661	3.0021	64.8790	34.2150	1039.702
4 jam	0.50	55.7748	58.8198	3.0450	79.2087	23.4339	669.5862
	0.75	61.8884	64.9235	3.0351	93.9098	32.0214	955.0361
	1.00	35.7655	38.8548	3.0893	74.3522	38.5867	**1149.043**
5 jam	0.50	64.2636	67.3335	3.0699	89.1801	24.9165	711.6388
	0.75	63.6564	66.7645	3.1081	99.5863	35.9299	1056.008
	1.00	63.4739	66.4888	3.0149	99.7519	36.2780	1103.290

Keterangan :

A = bobot kulit sebelum perendaman
B = bobot kulit setelah perendaman
C = bobot gelas kosong
D = bobot gelas + kulit sebelum perendaman
E = bobot gelas + kulit setelah perendaman
DP = derajat pengembangan

Lampiran 7. Data Uji Lowry Larutan NaOH Hasil Perendaman

1. Absorbansi larutan standar BSA

[BSA] (mg/mL)	A (λ = 698 nm)
0.1	0.513
0.2	0.848
0.3	1.066
0.4	1.301
0.5	1.481

Kurva Standar BSA

$y = 2.389x + 0.3251$
$R^2 = 0.9877$

[BSA] (mg/mL)

A (λ = 698 nm)
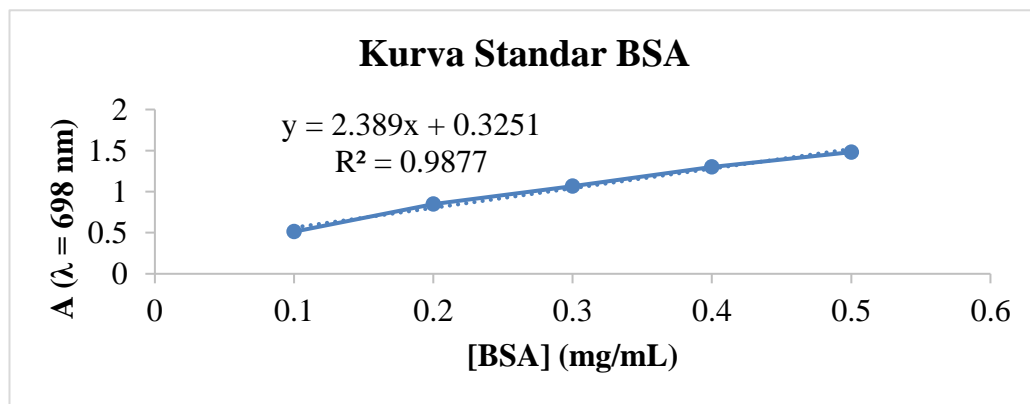
2. Contoh perhitungan kadar protein terlarut

$$\text{Kadar protein} = \frac{y - 0,3251}{2,389} \text{ x FP}$$

$$\text{Kadar protein} = \frac{0,616 - 0,3251}{2,389} \text{ x } 50 \quad = \text{ } 6,09 \text{ mg/mL}$$

3. Konsentrasi protein dalam larutan NaOH sisa perendaman kulit

Sampel	Pengulangan	A (λ = 698 nm)	[Protein] (mg/mL)	[Protein] Rerata (mg/mL)
0.025 M	Simplo	0.616	6.09	6.87
	Duplo	0.691	7.66	
0.050 M	Simplo	0.713	8.12	8.41
	Duplo	0.741	8.70	
0.075 M	Simplo	0.758	9.06	10.49
	Duplo	0.895	11.93	
0.100 M	Simplo	0.736	8.60	9.45
	Duplo	0.817	10.30	

Lampiran 8. Spektrum infra merah kolagen kulit ikan tawes
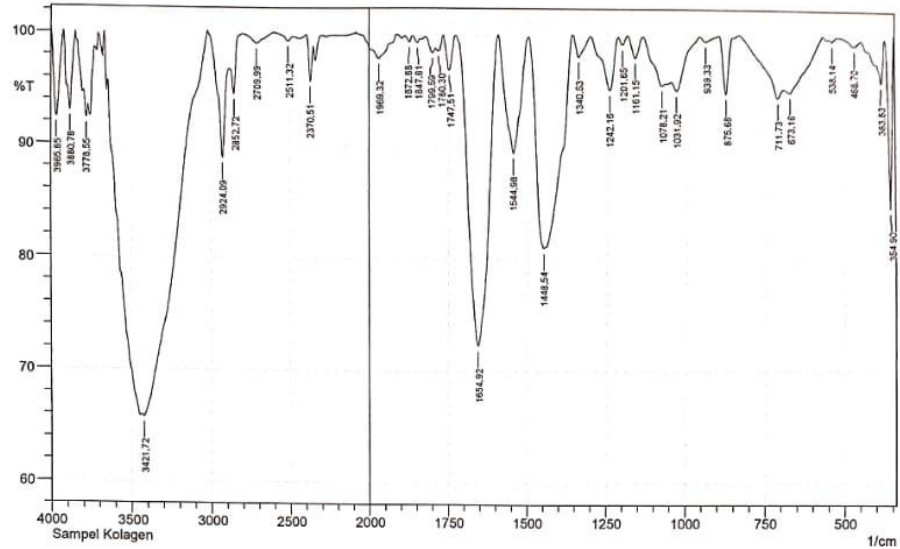
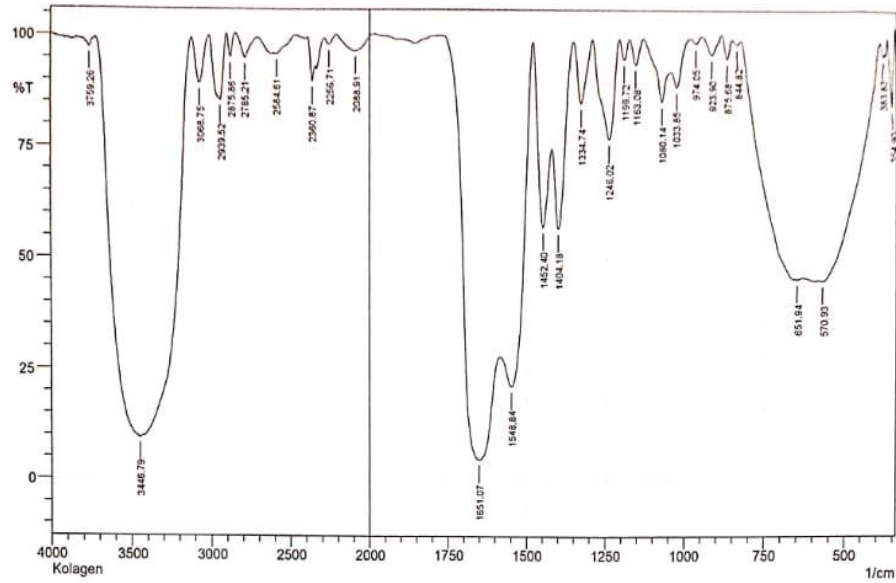| No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	354.9	84.565	15.423	372.26	341.4	0.871	0.867
2	383.83	95.892	3.015	401.19	372.26	0.356	0.19
3	468.7	99.198	0.544	516.92	447.49	0.13	0.07
4	538.14	99.563	0.268	549.71	516.92	0.03	0.009
5	673.16	94.909	0.98	690.52	569	1.626	0.359
6	711.73	94.416	1.456	833.25	692.44	1.682	0.188
7	875.68	94.738	5.164	898.83	854.47	0.454	0.436
8	939.33	99.421	0.477	958.62	898.83	0.086	0.064
9	1031.92	94.979	1.57	1049.28	958.62	1.016	0.137
10	1078.21	95.423	0.959	1138	1064.71	0.901	0.159
11	1161.15	97.904	1.958	1186.22	1138	0.226	0.197
12	1201.65	99.064	0.773	1215.15	1186.22	0.069	0.049
13	1242.16	94.986	4.811	1301.95	1215.15	0.936	0.861
14	1340.53	97.953	1.965	1357.89	1301.95	0.265	0.239
15	1448.54	80.914	18.861	1494.83	1359.62	7.396	7.288
16	1544.98	89.366	10.428	1591.27	1496.76	2.455	2.369
17	1654.92	72.237	27.643	1726.29	1593.2	9.208	9.137
18	1747.51	96.754	3.121	1766.8	1728.22	0.288	0.267
19	1780.3	98.474	0.739	1789.94	1766.8	0.114	0.044
20	1799.59	98.337	0.691	1822.73	1789.94	0.156	0.046
21	1847.81	99.212	0.43	1863.24	1840.09	0.054	0.022
22	1872.88	99.284	0.536	1888.31	1863.24	0.047	0.027
23	1969.32	97.776	1.152	1990.54	1913.39	0.471	0.214
24	2370.51	95.71	3.555	2397.52	2351.23	0.441	0.31
25	2511.32	99.334	0.447	2605.83	2476.6	0.147	0.076
26	2709.99	99.068	0.915	2798.71	2605.83	0.355	0.346
27	2852.72	94.533	3.326	2881.65	2798.71	0.822	0.212
28	2924.09	88.792	9.02	3016.67	2883.58	2.819	1.915
29	3421.72	65.696	1.35	3435.22	3018.6	38.649	2.47
30	3778.55	92.31	1.698	3793.98	3766.98	0.823	0.11
31	3880.78	92.934	3.937	3898.14	3849.92	0.923	0.397
32	3965.65	92.358	7.95	4000.36	3923.21	1.344	1.436

Date/Time; 12/30/2020 2:54:43 PM

No. of Scans;

Resolution;

Lampiran 9. Spektrum inframerah kolagen komersial

No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	354.9	84.286	17.036	370.33	345.26	0.825	0.936
2	383.83	95.842	1.47	393.48	378.05	0.208	0.048
3	570.93	44.972	3.414	582.5	393.48	39.344	6.57
4	651.94	45.288	0.195	655.8	634.58	7.26	0.018
5	844.82	98.359	0.934	858.32	833.25	0.136	0.06
6	875.68	95.098	4.914	893.04	858.32	0.351	0.352
7	923.9	95.966	4.391	954.76	893.04	0.484	0.579
8	974.05	98.459	1.451	991.41	954.76	0.123	0.111
9	1033.85	88.572	5.741	1051.2	991.41	1.592	0.561
10	1080.14	85.389	9.076	1138	1053.13	2.932	1.302
11	1163.08	93.734	5.813	1182.36	1139.93	0.611	0.528
12	1199.72	94.826	4.695	1215.15	1182.36	0.411	0.343
13	1246.02	76.922	22.555	1298.09	1215.15	5.259	5.063
14	1334.74	84.969	14.114	1357.89	1300.02	2.154	1.94
15	1404.18	56.821	25.731	1425.4	1359.82	8.686	4.518
16	1452.4	57.167	27.861	1485.19	1427.32	9.09	5.122
17	1548.84	21.149	33.216	1585.49	1487.12	43.957	17.102
18	1651.07	4.588	48.746	1768.72	1587.42	115.744	67.428
19	2088.91	96.722	3.796	2210.42	1988.61	1.766	2.258
20	2256.71	98.14	1.747	2285.65	2212.35	0.288	0.283
21	2360.87	89.867	5.651	2389.8	2345.44	1.227	0.505
22	2584.61	95.954	0.62	2600.04	2466.96	1.239	0.191
23	2785.21	95.029	5.078	2843.07	2709.99	1.246	1.268
24	2875.86	95.216	5.232	2899.01	2843.07	0.436	0.547
25	2939.52	85.524	14.715	3008.95	2900.94	4.253	4.34
26	3068.75	89.288	10.36	3118.9	3010.88	2.624	2.471
27	3446.79	9.396	6.383	3712.97	3427.51	167.986	25.626
28	3759.26	97.237	0.173	3793.98	3757.33	0.294	0.006

Comment;

Kolagen

Date/Time; 12/30/2020 2:47:32 PM

No. of Scans;

Resolution;

Apodization;

Lampiran 10. Perhitungan Kadar Asam Amino Kolagen

$$\text{Kadar asam amino (mg/kg : mg/L)} = \frac{A/B \times C\text{ std}/1000000 \times BM \times Va \times FP}{Wx \text{ atau } Vx}$$

$$\text{Kadar Asam Amino (\%)} = \frac{\text{Kadar asam amino (mg/Kg : mg/L)}}{1000}$$

Contoh Perhitungan;

$$\text{Kadar asam amino (mg/kg : mg/L)} = \frac{32{,}72/1{,}01 \times 100/1000000 \times 75.07 \times 1000 \times 1000}{0{,}1031}$$

$$= 235528{,}06$$

$$\text{Kadar Asam Amino (\%)} = \frac{235528{,}06}{1000} = 23{,}55 \text{ \%}$$

Keterangan:
A   : Rasio sampel
B   : Rasio standar
Ax   : Luas area analit asam amino
A IS   : Luas area internal standar
C Std : Konsentrasi larutan standar asam amino (pmol/μL)
BM   : Bobot molekul asam amino (mg/mmol)
Va   : Volume akhir sampel (μL)
FP   : Faktor pengenceran
Wx   : bobot penimbangan sampel (g)
Vx   : Volume pemipetan sampel (mL)

Lampiran 11. Data Penentuan Komposisi Asam Amino Kolagen (UPLC)

Paraf	
Supervisor	
	Eka R.

Tgl Pengujian : 01.03.21
Tgl Pelaporan : 02.03.21
Metode Acuan : IK No.18-5-17/MUSMM-SIG
No. Instrumen : SIG/FWA/ALB/IN-0092

Standar Pembanding : Amino Acid Standard (SIGMA-ALDRICH, AAS18-10X1ML, Lot# SLBZ 9656)
Tgl Pembuatan Standar Induk : 01.03.21
Tgl Pembuatan Standar Injeksi : 01.03.21

No. Sampel : 101.R.1295
Matriks : Ekstrak

REKAMAN PENGUJIAN ASAM AMINO

Bobot Sampel (g)	Volume 1 (µL)	Volume Pembelian (µL)	Volume Akhir (µL)
0.1038	50000	500	1000

Analit	C. Standar Induk (pmol/µL)	C. Standar Injeksi (pmol/µL)	BM (mg/mmol)	RT (menit)	Area Standar	Rasio Standar (terhadap AABA)	RT (menit)	Area Sampel	Rasio Sampel (terhadap AABA)	Verifikasi Rasio	C. Injeksi (pmol/µL)	Kadar Asam Amino (mg/Kg : mg/L)	Kadar Asam Amino (%)	Keterangan
AMO				2.4	78047411.67		2.41	3012254.33						
NH3				3.44	43922.94		3.36	100556.47						
L-Histidine	2500	100	155.16	4.21	52047.59	1.04	4.14	14957.16	0.39	OK	37.32	5578.88	0.56	-
L-Serine	2500	100	105.09	5.57	50067.17	1.00	5.54	123007.39	3.20	OK	321.71	32571.06	3.26	-
L-Arginine	2500	100	174.29	5.7	54644.28	1.09	5.67	204529.30	5.33	OK	490.12	82295.41	8.23	-
Glycine	2500	100	75.07	5.97	50870.90	1.01	5.92	1252330.05	32.61	OK	3223.58	233135.36	23.31	-
L-Aspartic Acid	2500	100	133.10	6.52	46558.21	0.93	6.52	107036.63	2.79	OK	301.04	38601.71	3.86	-
L-Glutamic Acid	2500	100	147.13	7.09	45284.51	0.90	7.1	177672.39	4.63	OK	513.76	72822.21	7.28	-
L-Threonine	2500	100	119.12	7.53	50294.36	1.00	7.53	94427.51	2.46	OK	245.85	28213.46	2.82	-
L-Alanine	2500	100	89.10	8.06	51902.95	1.03	8.07	369557.51	9.62	OK	932.35	80031.33	8.00	-
L-Proline	2500	100	115.13	8.76	47534.17	0.95	8.78	353618.90	9.21	OK	974.14	108046.43	10.80	-
AABA				9.63	50285.17		9.68	38401.68						
Derivated Peak				9.8	385494.81		9.85	385431.55						
L-Ornitine	1250	50	121.16	10	42520.74	0.85	10	0.00	0.00	OK	0.00	0.00	0.00	-
L-Lysine	2500	100	146.19	10.09	78730.77	1.57	10.15	116805.11	3.04	OK	194.27	27360.71	2.74	-
L-Tyrosine	2500	100	181.19	10.39	56286.32	1.12	10.44	8167.32	0.21	OK	19.00	3316.68	0.33	-
L-Methionine	2500	100	149.21	10.55	53310.96	1.06	10.6	32798.16	0.85	OK	80.56	11580.39	1.16	-
L-Valine	2500	100	117.15	10.67	51737.42	1.03	10.72	74451.92	1.94	OK	188.43	21266.98	2.13	-
L-Isoleucine	2500	100	131.17	11.51	51975.17	1.03	11.57	34297.98	0.89	OK	86.41	10919.42	1.09	-
L-Leucine	2500	100	131.17	11.62	51329.80	1.02	11.68	78454.88	2.04	OK	200.14	25291.66	2.53	-
L-Phenylalanine	2500	100	165.19	11.8	54965.45	1.09	11.85	54011.54	1.41	OK	128.67	20477.30	2.05	-
TOTAL	41250				9145935.37			6632767.78			7937.37	801508.98	80.15	

UKS

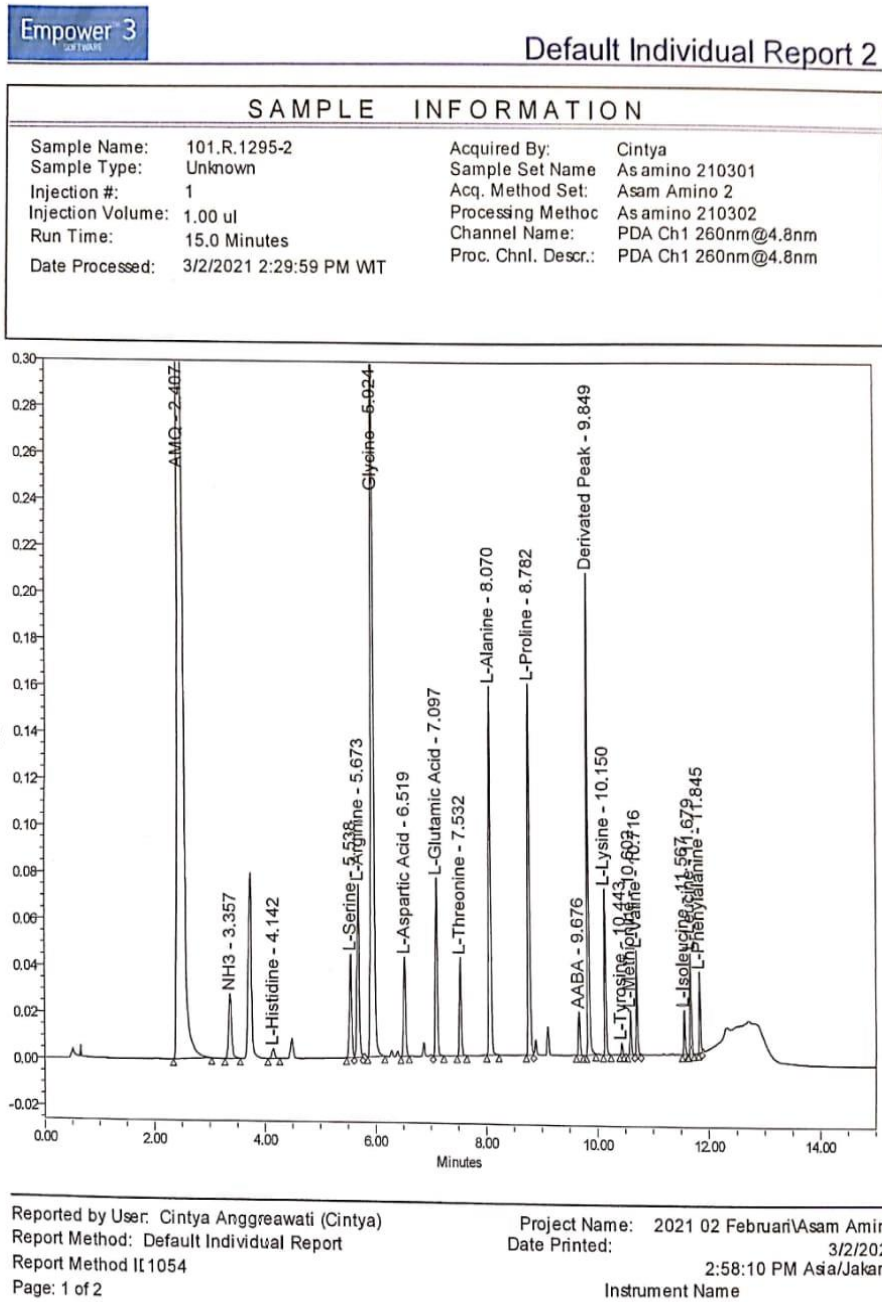| Syarat	%RSD RT ≤2	%RSD Area ≤2	Tailing Factor ≤2	Theoritical Plate ≥1000	Resolusi ≥1.5
AABA	0.13	1.03	1.03	542830.42	-
Keterangan	OK	OK	OK	OK	OK

Perhitungan:

Rasio = Area Analit / Area AABA

FP = Volume 1 / Volume Pempelian

C. Injeksi (pmol/µL) = (Rasio Sampel/Rasio Standar) x C. Standar Injeksi

Kadar Asam Amino (mg/Kg : mg/L) = ((Rasio Sampel / Rasio Standar) x (C. Standar Injeksi/1000000000) x BM x FP x Volume Akhir x 1000) / Bobot Sampel (g)

Kadar Asam Amino (%) = Kadar Asam Amino (mg/Kg : mg/L) / 10000

**Lampiran 12.** Kromatogram analisis asam amino kolagen kulit ikan tawes

Empower 3

Default Individual Report 2

S A M P L E   I N F O R M A T I O N

Sample Name:	101.R.1295-2
Sample Type:	Unknown
Injection #:	1
Injection Volume:	1.00 ul
Run Time:	15.0 Minutes
Date Processed:	3/2/2021 2:29:59 PM WIT

Acquired By:	Cintya
Sample Set Name	As amino 210301
Acq. Method Set:	Asam Amino 2
Processing Method	As amino 210302
Channel Name:	PDA Ch1 260nm@4.8nm
Proc. Chnl. Descr.:	PDA Ch1 260nm@4.8nm

Peaks labeled:
AMQ - 2.407
NH3 - 3.357
L-Histidine - 4.142
L-Serine - 5.538
L-Arginine - 5.673
Glycine - 6.024
L-Aspartic Acid - 6.519
L-Glutamic Acid - 7.097
L-Threonine - 7.532
L-Alanine - 8.070
L-Proline - 8.782
AABA - 9.676
Derivated Peak - 9.849
L-Tyrosine - 10.443
L-Lysine - 10.150
L-Methionine - 10.602
L-Valine - 10.716
L-Isoleucine - 11.567
L-Leucine - 11.679
L-Phenylalanine - 11.845

Reported by User: Cintya Anggreawati (Cintya)
Report Method: Default Individual Report
Report Method II 1054
Page: 1 of 2

Project Name: 2021 02 Februari\Asam Amino
Date Printed: 3/2/2021
2:58:10 PM Asia/Jakarta
Instrument Name

63

	Peak Name	RT	Area	% Area	Height	Amount
1	AMQ	2.407	3010328.82	45.45	520905	
2	NH3	3.357	99664.96	1.50	27348	
3	L-Histidine	4.142	14499.25	0.22	4116	27.629
4	L-Serine	5.538	122644.86	1.85	43719	244.961
5	L-Arginine	5.673	204333.74	3.08	73382	373.934
6	Glycine	5.924	1251604.19	18.90	376493	2460.354
7	L-Aspartic Acid	6.519	106497.68	1.61	41895	228.741
8	L-Glutamic Acid	7.097	177154.31	2.67	75825	391.203
9	L-Threonine	7.532	94108.13	1.42	41446	187.115
10	L-Alanine	8.070	369017.82	5.57	157703	710.977
11	L-Proline	8.782	353213.11	5.33	158653	743.072
12	AABA	9.676	38247.46	0.58	18348	76.061
13	Derivated Peak	9.849	384912.96	5.81	206443	
14	L-Cystine	10.000				
15	L-Lysine	10.150	116507.78	1.76	70105	147.983
16	L-Tyrosine	10.443	8060.89	0.12	4868	14.321
17	L-Methionine	10.602	32662.58	0.49	18523	61.268
18	L-Valine	10.716	74387.06	1.12	43997	143.778
19	L-Isoleucine	11.567	34066.81	0.51	18582	65.544
20	L-Leucine	11.679	78104.26	1.18	42603	152.162
21	L-Phenylalanine	11.845	53591.76	0.81	34134	97.501
Sum			6623608.44			

Lampiran 13. Kromatogram standar asam amino
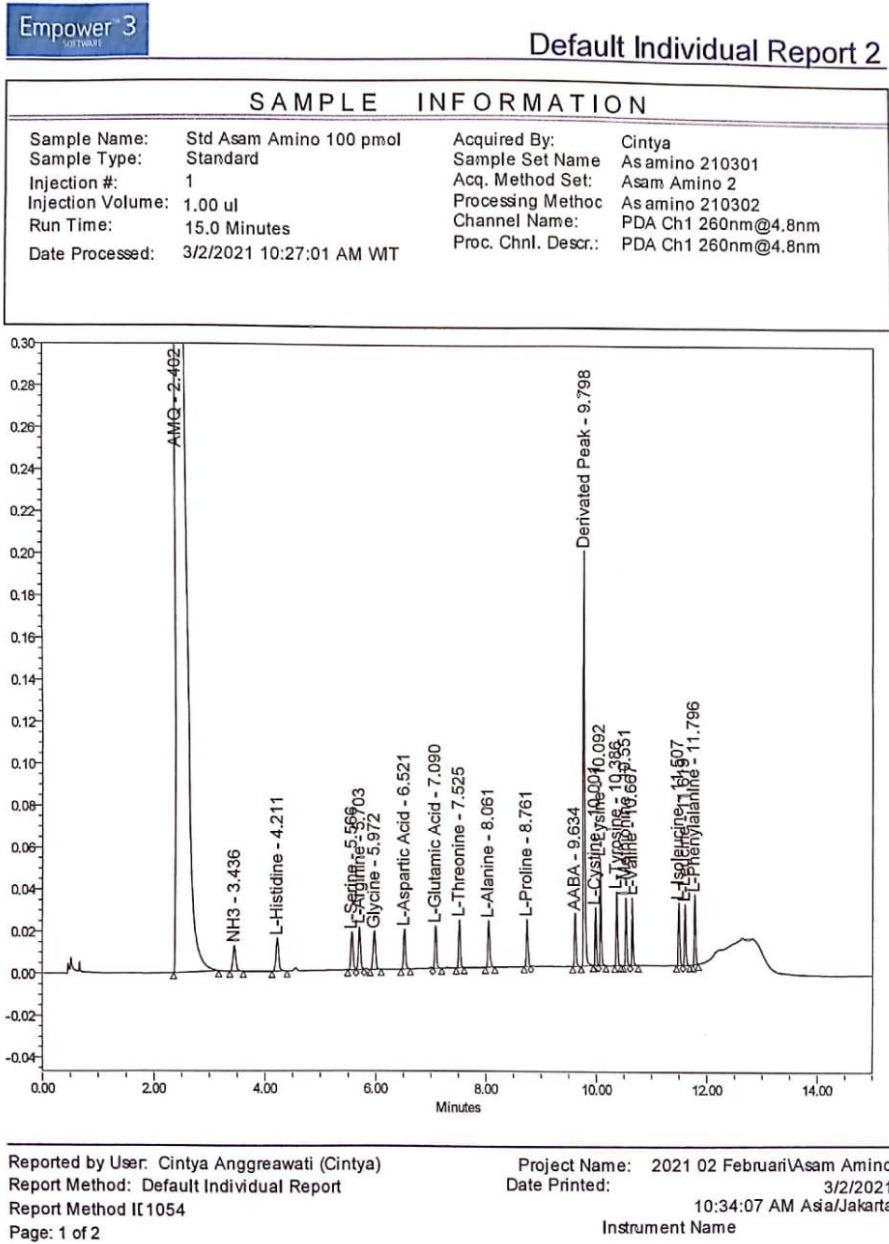
	Peak Name	RT	Area	% Area	Height	Amount
1	AMQ	2.402	7804741.67	85.34	930023	
2	NH3	3.436	43922.94	0.48	12195	
3	L-Histidine	4.211	52477.59	0.57	15659	100.000
4	L-Serine	5.566	50067.17	0.55	17958	100.000
5	L-Arginine	5.703	54644.28	0.60	20294	100.000
6	Glycine	5.972	50870.90	0.56	18113	100.000
7	L-Aspartic Acid	6.521	46558.21	0.51	18928	100.000
8	L-Glutamic Acid	7.090	45284.51	0.50	20146	100.000
9	L-Threonine	7.525	50294.36	0.55	22853	100.000
10	L-Alanine	8.061	51902.95	0.57	22288	100.000
11	L-Proline	8.761	47534.17	0.52	22453	100.000
12	AABA	9.634	50285.17	0.55	25539	100.000
13	Derivated Peak	9.798	356494.81	3.90	199692	
14	L-Cystine	10.001	42520.74	0.46	27554	50.000
15	L-Lysine	10.092	78730.77	0.86	51149	100.000
16	L-Tyrosine	10.386	56286.32	0.62	35496	100.000
17	L-Methionine	10.551	53310.96	0.58	32349	100.000
18	L-Valine	10.667	51737.42	0.57	32229	100.000
19	L-Isoleucine	11.507	51975.17	0.57	29972	100.000
20	L-Leucine	11.619	51329.80	0.56	29153	100.000
21	L-Phenylalanine	11.796	54965.46	0.60	33322	100.000
Sum			9145935.37			

Reported by User: Cintya Anggreawati (Cintya)
Report Method: Default Individual Report
Report Method IE 1054
Page: 2 of 2

Project Name: 2021 02 Februari\Asam Amino
Date Printed: 3/2/2021
10:34:07 AM Asia/Jakarta
Instrument Name

Lampiran 13. Tahap Optimasi Ekstraksi Kolagen Kulit Ikan Tawes

1. Deproteinasi dengan larutan NaOH
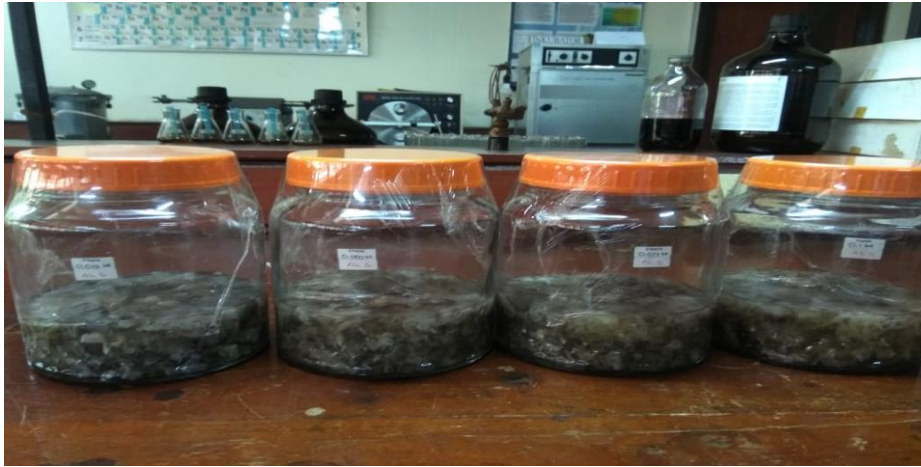
2. Perendaman kulit dengan butil alkohol

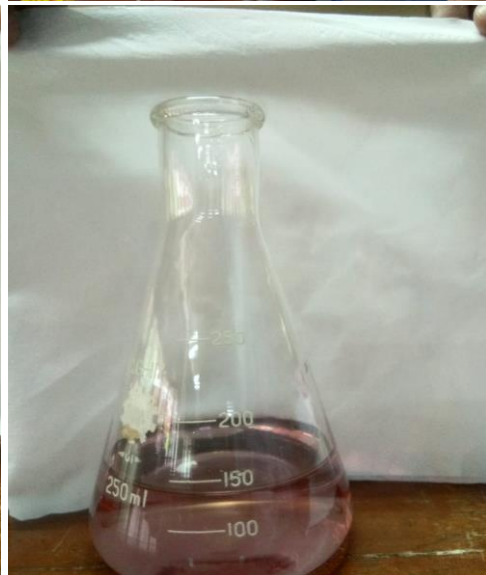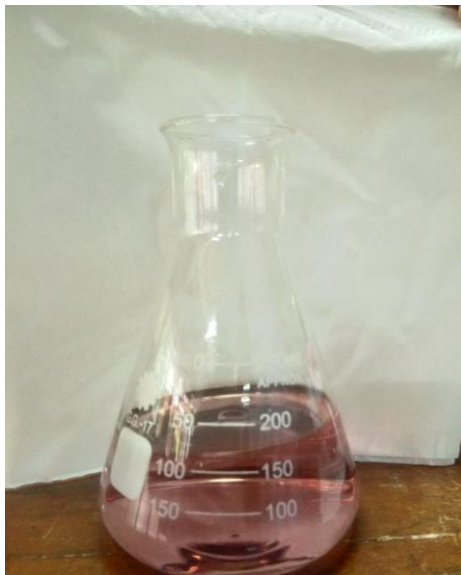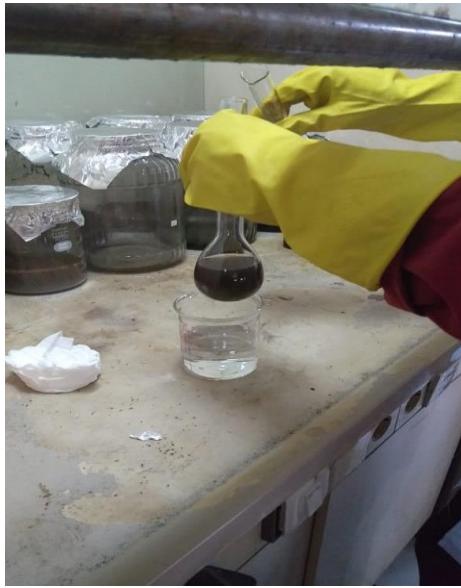3. Ekstraksi dengan larutan CH$_3$COOH

4. Penyaringan kulit

5. Salting out dengan NaCl

6. Dialisis

7. Uji proksimat

8. Sentrifugasi

9. FTIR

10. UPLC