

Fig. 3 FTIR spectra of the pepsin soluble collagen from unicorn fish (*N. reticulatus*) skin. UCP: Unicorn fish skin hydrolyzed with pepsin from porcine; UCB: Unicorn fish skin hydrolyzed with pepsin from bovine; CFC: Commercial fish collagen.

TABLE II
THE PEAK AREA AND DESCRIPTION OF PSC DERIVED FROM UNICORN FISH (*N. RETICULATUS*) SKIN

| Peak | Type of collagen | | | Peak assignment |
|-----------|-----------------------------|-----------------------------|-----------------------------|--|
| | UCP | UCB | CFC | |
| Amide A | 3280.14 cm ⁻¹ | 3285.73 cm ⁻¹ | 3259.64 cm ⁻¹ | Stretching of N-H with hydrogen chain |
| Amide B | 2926.03 cm ⁻¹ | 2922.31 cm ⁻¹ | 2931.62 cm ⁻¹ | Asymmetric stretching of CH ₂ |
| Amide I | 1628.89 cm ⁻¹ | 1628.89 cm ⁻¹ | 1636.34 cm ⁻¹ | Stretching of C=O / hydrogen chain with COO- |
| Amide II | 1541.29 cm ⁻¹ | 1541.29 cm ⁻¹ | 1522.66 cm ⁻¹ | N-H bond with stretching of C-N |
| Amide III | 1235.64 cm ⁻¹ | 1235.64 cm ⁻¹ | 1241.23 cm ⁻¹ | N-H bond with C-H dan C-O stretching |

UCP: Unicorn fish skin hydrolyzed with pepsin from porcine.
UCB: Unicorn fish skin hydrolyzed with pepsin from bovine.
CFC: Commercial fish collagen.

G. Thermostability Assessment

Fig. 4. presents the thermograms of extracted collagens from the unicorn fish (*N. reticulatus*) skin. The thermostability of UCP sample ($T_{max} = 43.63^{\circ}\text{C}$) was greater than that of UCB ($T_{max} = 35.25^{\circ}\text{C}$). The reason might be due to the content of hydroxyproline in the fish collagens, which play an essential role in the formation of pyrrolidine rings partially stabilized by hydrogen bonding through the hydroxyl group of hydroxyproline, as suggested by Benjakul et al. [34]. Furthermore, hydroxyproline has the capacity to enhance the stability of the triple helical structure through hydrogen bonding within the coiled-coil alpha chains, as elucidated by Bae et al. [37]. The mentioned data (Table 1) showed a high content of hydroxyproline. These findings also agreed with the experiments on fish collagens, including lizardfish (*S. tumbil*) ($T_{max} = 40.24^{\circ}\text{C}$) [8], seabass (*Lates calcarifer*) skin ($T_{max} = 39.32^{\circ}\text{C}$) [38], and loach (*Misgurnus anguillicaudatus*) skin ($T_{max} = 36.03^{\circ}\text{C}$) [39].

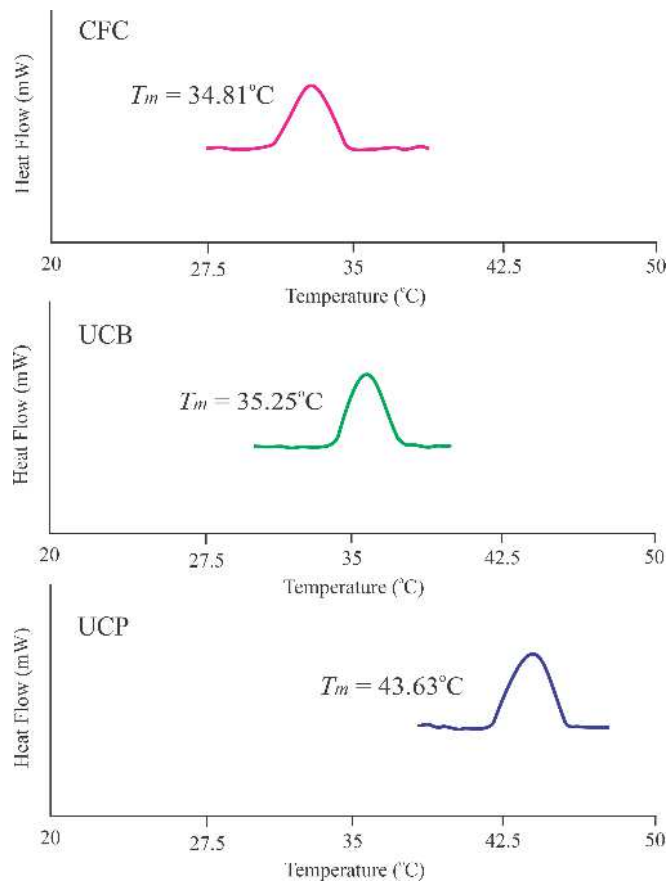


Fig. 4 Thermogram graph of the pepsin soluble collagen from unicorn fish (*N. reticulatus*) skin. UCP: Unicorn fish skin hydrolyzed with pepsin from porcine; UCB: Unicorn fish skin hydrolyzed with pepsin from bovine; CFC: Commercial fish collagen.

IV. CONCLUSION

The pepsin-soluble collagens derived from the skin of unicorn fish (*N. reticulatus*) have been isolated using two pepsin sources. UCP sample had a significantly higher yield (15.60%) than UCB (10.40%), which might be due to the swelling rate during the pre-treatment process. Both UCP and UCB were thought of as type I owing to the two alpha chains available after SDS-PAGE analysis, and the triple helical structures of those collagens were preserved, indicating no structural change during the extraction process. These analyses were proved using FTIR and UV-visible experiments. Interestingly, all collagens showed high thermal ability, with a higher observed in UCP compared to the UCB sample. This result could be influenced by the hydroxyproline content that plays a vital role in stabilizing the triple helical structure of fish collagen. Overall, the UCP sample may be electable in this study because it also has a high collagen extractability and thermostability.

ACKNOWLEDGMENT

This project gained external funding from the Ministry of Education Malaysia through the Fundamental Research Grant Scheme (FRGS) with grant number FRGS/1/2019/STG03/UMS/02/5. The authors are also grateful to Universiti Malaysia Sabah (UMS) for the support to finish this project.

REFERENCES

- [1] J. E. Randall. "Naso reticulatus, a new unicornfish (Perciformes: Acanthuridae) from Taiwan and Indonesia, with a key to the species of Naso," *Zoological Studies*, vol. 40, no. 2, 2001.
- [2] R. Froese and D. Pauly, "FishBase. World Wide Web electronic publication," Jun. 2023. accessed on October 23, 2023. <https://www.fishbase.org/>
- [3] N. S. Fatiroi, A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical and microstructural characteristics of collagen biopolymer from unicornfish (*Naso reticulatus* Randall, 2001) bone prepared with various acid types," *Polymers (Basel)*, vol. 15, no. 4, Feb. 2023, doi:10.3390/polym15041054.
- [4] A. Sorushanova *et al.*, "The Collagen suprafamily: From biosynthesis to advanced biomaterial development," *Advanced Materials*, vol. 31, no. 1, Wiley-VCH Verlag, Jan. 04, 2019. doi:10.1002/adma.201801651.
- [5] Y. S. Lim, Y. J. Ok, S. Y. Hwang, J. Y. Kwak, and S. Yoon, "Marine collagen as a promising biomaterial for biomedical applications," *Marine Drugs*, vol. 17, no. 8, 2019, doi: 10.3390/md17080467.
- [6] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical analysis of collagens from the bone of lizardfish (*Saurida tumbil* Bloch, 1795) extracted with different acids," *PeerJ*, Mar. 2022, doi:10.7717/peerj.13103.
- [7] X. Zhang, S. Xu, L. Shen, and G. Li, "Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification," *Journal of Leather Science and Engineering*, vol. 2, no. 1, 2020, doi:10.1186/s42825-020-00033-0.
- [8] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Microstructural and physicochemical analysis of collagens from the skin of lizardfish (*Saurida tumbil* Bloch, 1795) extracted with different organic acids," *Molecules*, vol. 27, no. 8, Apr. 2022, doi:10.3390/molecules27082452.
- [9] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Physicochemical and microstructural analyses of pepsin-soluble collagens derived from lizardfish (*Saurida tumbil* Bloch, 1795) skin, bone and scales," *Gels*, vol. 8, no. 8, Aug. 2022, doi:10.3390/gels8080471.
- [10] A. A. Prihanto *et al.*, "Characteristics of collagen from parrotfish (*Chlorurus sordidus*), tiger grouper (*Epinephelus fuscoguttatus*) and pink ear emperor (*Lethrinus lentjan*): Effect of acetic acid concentration and extraction time," *Online Journal of Biological Sciences*, vol. 22, no. 1, pp. 26–35, 2022, doi:10.3844/ojbsci.2022.26.35.
- [11] N. N. Matarsim, A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Type I collagen from the skin of barracuda (*Sphyrna* sp.) prepared with different organic acids: Biochemical, microstructural and functional properties," *Journal of Functional Biomaterial*, vol. 14, no. 2, Feb. 2023, doi:10.3390/jfb14020087.
- [12] A. A. Prihanto *et al.*, "Characterization of collagen from java barb (*Barbonymus gonionotus*) skin and its effect on selected properties of yogurt," *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, vol. 6, no. 1, pp. 17–30, Jun. 2023, doi:10.20956/canrea.v6i1.716.
- [13] R. Ahmed, M. Haq, and B. S. Chun, "Characterization of marine derived collagen extracted from the by-products of bigeye tuna (*Thunnus obesus*)," *International Journal of Biological Macromolecules*, vol. 135, pp. 668–676, Aug. 2019, doi:10.1016/j.ijbiomac.2019.05.213.
- [14] W. Liu, Y. Zhang, N. Cui, and T. Wang, "Extraction and characterization of pepsin-solubilized collagen from snakehead (*Channa argus*) skin: Effects of hydrogen peroxide pretreatments and pepsin hydrolysis strategies," *Process Biochemistry*, vol. 76, pp. 194–202, Jan. 2019, doi:10.1016/j.procbio.2018.10.017.
- [15] Huda, N. Seow, E. K. Normawati, N. Aisyah, N. M. Fazilah, and A. and Easa, "Effect of duck feet collagen addition on physicochemical properties of surimi," *International Food Research Journal*, vol. 20, no. 2, pp. 537–544, Oct. 2013.
- [16] S. N. H. Oslan, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Characterization of acid- and pepsin-soluble collagen extracted from the skin of purple-spotted bigeye snapper," *Gels*, vol. 8, no. 10, Oct. 2022, doi:10.3390/gels8100665.
- [17] M. Bergman and R. Loxley, "Two improved and simplified methods for the spectrophotometric determination of hydroxyproline," *Analytical Chemistry*, vol. 35, pp. 1961–1965, doi:10.1021/ac60205a0531963.
- [18] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical and microstructural properties of lizardfish (*Saurida tumbil*) scale collagen extracted with various organic acids," *Gels*, vol. 8, no. 5, May 2022, doi:10.3390/gels8050266.
- [19] U. K. Laemmli, "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature*, pp. 680–685, 1970.
- [20] A. A. Jaziri, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Extraction and characterization of type I collagen from parrotfish (*Scarus sordidus* Forsskål, 1775) scale solubilized with the aid of acetic acid and pepsin," *International Journal of Biomaterial*, vol. 2023, pp. 1–10, Apr. 2023, doi:10.1155/2023/7312447.
- [21] K. Matmaroh, S. Benjakul, T. Prodpran, A. B. Encarnacion, and H. Kishimura, "Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*)," *Food Chemistry*, vol. 129, no. 3, pp. 1179–1186, Dec. 2011, doi:10.1016/j.foodchem.2011.05.099.
- [22] F. Y. Cheng, F. W. Hsu, H. S. Chang, L. C. Lin, and R. Sakata, "Effect of different acids on the extraction of pepsin-solubilized collagen containing melanin from silky fowl feet," *Food Chemistry*, vol. 113, no. 2, pp. 563–567, Mar. 2009, doi:10.1016/j.foodchem.2008.08.043.
- [23] M. M. Schmidt, R. C. P. Dornelles, R. O. Mello, E. H. Kubota, M. A. Mazutti, A. P. Kempka, and I. M. Demiate, "Collagen extraction process," *International Food Research Journal*, vol. 23, no. 3, pp. 913–922, 2016.
- [24] W. K. Song, D. Liu, L. L. Sun, B. F. Li, and H. Hou, "Physicochemical and biocompatibility properties of type I collagen from the skin of Nile tilapia (*Oreochromis niloticus*) for biomedical applications," *Marine Drugs*, vol. 17, no. 3, Feb. 2019, doi:10.3390/md17030137.
- [25] J. Cao, Q. Duan, X. Liu, X. Shen, and C. Li, "Extraction and physicochemical characterization of pepsin soluble collagens from golden pompano (*Trachinotus blochii*) skin and bone," *Journal of Aquatic Food Product Technology*, vol. 28, no. 8, pp. 837–847, Sep. 2019, doi:10.1080/10498850.2019.1652216.
- [26] P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, T. Nagai, and M. Tanaka, "Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*)," *Food Chemistry*, vol. 89, no. 3, pp. 363–372, Feb. 2005, doi:10.1016/j.foodchem.2004.02.042.
- [27] L. Y. Li, Y. Q. Zhao, Y. He, C. F. Chi, and B. Wang, "Physicochemical and antioxidant properties of acid- and pepsin-soluble collagens from the scales of miuuy croaker (*Miichthys miuuy*)," *Marine Drugs*, vol. 16, no. 10, Oct. 2018, doi:10.3390/md16100394.
- [28] J. Bakar, U. Hartina, M. Razali, D. Hashim, and A. Q. Sazili, "Properties of collagen from barramundi (*Lates calcarifer*) skin," *International Food Research Journal*, vol. 20, no. 2, pp. 835–842, 2013.
- [29] M. Sadowska, I. Kolodziejska, and C. Niecikowska, "Isolation of collagen from the skins of Baltic cod (*Gadus morhua*)," *Food Chemistry*, vol. 81, pp. 257–262, doi: 10.1016/S0308-8146(02)00420-X
- [30] J. Chen *et al.*, "Physicochemical and functional properties of type I collagens in red stingray (*Dasyatis akajei*) Skin," *Marine Drugs*, vol. 17, no. 10, Sep. 2019, doi:10.3390/md17100558.
- [31] S. Iswariya, P. Velswamy, and T. S. Uma, "Isolation and characterization of biocompatible collagen from the skin of puffer fish (*Lagocephalus inermis*)," *Journal of Polymers and the Environment*, vol. 26, no. 5, pp. 2086–2095, May 2018, doi:10.1007/s10924-017-1107-1.
- [32] S. Chen *et al.*, "Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenops ocellatus*)," *Food Hydrocolloids*, vol. 52, pp. 468–477, Jan. 2016, doi:10.1016/j.foodhyd.2015.07.027.
- [33] S. Z. Ramle, S. N. H. Oslan, R. Shapawi, R. A. M. Mokhtar, W. N. M. Noordin, and N. Huda, "Biochemical characteristics of acid-soluble collagen from food processing by-products of needlefish skin (*Tylosurus acus melanotus*)," *Applied Sciences (Switzerland)*, vol. 12, no. 24, Dec. 2022, doi:10.3390/app122412695.
- [34] S. Benjakul *et al.*, "Extraction and characterisation of pepsin-solubilized collagens from the skin of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*)," *Journal of the Science of Food and Agriculture*, vol. 90, no. 1, pp. 132–138, Jan. 2010, doi:10.1002/jsfa.3795.
- [35] B. B. Doyle, "Infrared spectroscopy of collagen and collagen-like polypeptides," *Biopolymers*, vol. 14, no. 5, pp. 937–57, 1975. doi:10.1002/bip.1975.360140505.
- [36] M. Atef, S. M. Ojagh, A. M. Latifi, M. Esmacili, and C. C. Udenigwe, "Biochemical and structural characterization of sturgeon fish skin

- collagen (*Huso huso*)," *Journal of Food Biochemistry*, vol. 44, no. 8, Aug. 2020, doi:10.1111/jfbc.13256.
- [37] I. Bae, K. Osatomi, A. Yoshida, K. Osako, A. Yamaguchi, and K. Hara, "Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes," *Food Chemistry*, vol. 108, no. 1, pp. 49–54, May 2008, doi:10.1016/j.foodchem.2007.10.039.
- [38] S. Chuaychan, S. Benjakul, and H. Kishimura, "Characteristics of acid- and pepsin-soluble collagens from scale of seabass (*Lateolabrax japonicus*)," *LWT*, vol. 63, no. 1, pp. 71–76, Sep. 2015, doi:10.1016/j.lwt.2015.03.002.
- [39] J. Wang, X. Pei, H. Liu, and D. Zhou, "Extraction and characterization of acid-soluble and pepsin-soluble collagen from skin of loach (*Misgurnus anguillicaudatus*)," *International Journal of Biological Macromolecules*, vol. 106, pp. 544–550, Jan. 2018, doi:10.1016/j.ijbiomac.2017.08.046.