ORIGINAL ARTICLE

Extraction and Characterisation of Suckermouth Catfish Collagen

Rifqa Danisha Ramlan[1], Wan Azman Wan Ismail[2], Muhammad 'Izzuddin Zamery[2], Nor Amlizan Ramli[2], Tommy Julianto Bustami Effendi[2], Haslawati Baharuddin[3], Mizaton Hazizul Hasan[2]

[1] Faculty of Pharmacy, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, Bandar Puncak Alam, 42300 Selangor, Malaysia.
[2] Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[3] Fisheries Research Institute Glami Lemi, Titi, 71650 Jelebu, Negeri Sembilan, Malaysia

ABSTRACT

Introduction: Suckermouth catfish are invasive alien species in Malaysia with rapid population propagation, causing negative impacts on the local ecology and economy. Presently, there are no available methods to sufficiently control their populations. The aim of this study is to explore the potential of suckermouth catfish as a source of ingredients for the pharmaceutical industry, which could encourage their utilisation and indirectly control their population. **Methods:** In this study, acid-soluble collagen extraction was carried out, followed by identification tests, yield calculations, and some basic physical characteristic tests. **Results:** The extracted collagen was described as off-white and had a strong acetic acid odour. The mean yield of collagen on a wet basis was 10±2.3%, with a hydroxyproline content of 1.6%, while the viscosity and pH of a 20% collagen solution were 0.6±0.25 mPa.s and 5.51±0.1, respectively, comparable to previous studies using various species of fish. Heavy metal contents of the extracted collagen were also found to be within the acceptable limits for use in pharmaceutical and cosmetic applications. **Conclusion:** This study concludes that the skin of suckermouth catfish is a promising source of collagen for potential industrial applications. However, further studies are needed to improve the yield and purity and establish the physicochemical characteristics, safety, and applications of the extracted collagen.
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Corresponding Author:
Wan Azman Wan Ismail, MMed Sc.
Email: wanazman@uitm.edu.my
Tel: +60122677165

INTRODUCTION

Similar to many other invasive alien species (IAS) around the world, the rapid spread of suckermouth catfish in local water bodies has resulted in many adverse impacts on the ecosystem and is also affecting the economy (1,2,3). Suckermouth catfish originate from South America and are considered invasive species in many tropical, sub-tropical, and warm-watered regions around the world (4,5). They are commonly known so due to their special mouths structure which is shaped like a suction cup along with spiny body and able to live in derelict water bodies with low oxygen levels. Since the emergence of the aquarium industry in the 1970s, three members of the *Loricariidae* family, namely vermiculated sailfin catfish (*Pterygoplichthys disjunctivus*), Amazon sailfin catfish (*Pterygoplichthys pardalis*) and algae suckermouth catfish (*Hypostomus plecostomus*) have been imported into Malaysia for the fish hobbyist as ornamental freshwater fish. They are popular due to their habit of cleaning aquarium bases containing algae and other organic materials (1), which gives rise to their popular name of 'janitor fish' or *ikan bandaraya* in Malay. Later, they unintentionally or purposely escaped from the aquaria, resulting in the introduction of suckermouth catfish into local freshwater bodies such as rivers, ponds, and lakes (4,6). They have anti-predatory adaptations such as being covered in bony plates and the presence of dorsal and pectoral spines (7). This causes a lack of native predators, allowing them to successfully invade non-native environments. In suitable environmental conditions, they can grow to sizes of up to over 50 cm especially in the bottom most layers. They also have good survival abilities, including surviving in poor water conditions (3,8,9). Suckermouth catfish

are known to have the ability to disrupt the aquatic ecosystem. They feed on fish larvae and compete with native species for food such as aquatic plants, affecting the sustainability of native fish habitats. They also have a habit of burrowing where the burrows are used as nesting tunnels to lay eggs (3,9). In the long run, this natural habit of burrowing may increase the water turbidity and cause erosion of the canal banks and lake shorelines, affecting native fish survival (1,7,10). They also cause damage to fishing nets, such as gillnets, which may affect the fishermen's income (4). Due to their adverse impacts on the local economy and ecology, suckermouth catfish are classified as invasive alien species (7) and controlling their population is one of the global agenda, including in Malaysia, as outlined in the National Action Plan on Invasive Alien Species 2021-2025, a consensus developed throughout the consultation of the National Committee on Invasive Alien Species, under purview of the Ministry of Agriculture and Food Industry, Malaysia.

To control their population, some methods have been suggested, among others, through physical removal, encouraging their consumption, and through public awareness campaigns (9,11,12). Encouraging their consumption could be a good solution, but besides their unappealing appearance, the suckermouth catfish are also covered with bony plates and are considered non-edible. Furthermore, the common nature of their habitat also creates an impression that they might be contaminated with heavy metals and are toxic to eat. Although some people eat them (8,13), it is still in debate whether they are suitable for human consumption due to their high level of metal content (9,14). Previous studies also proposed physically removing the suckermouth catfish from freshwater by catching the fish and their eggs during the spawning season using hands, raps, or netting gears (9,11,15). Pesticide control was also proposed to reduce the suckermouth catfish burrowing activity. However, it would most likely affect the aquatic ecosystem negatively as it alters both the chemical and physical properties of water. Furthermore, awareness campaigns aimed at the public against releasing young and adult suckermouth catfish into the freshwater could also be done to prevent a further increase in the population of this fish (4,9,11). One of the methods to control the suckermouth catfish population might be through introducing their industrial applications, as it will require massive and continuous removal of the suckermouth catfish from their habitats. Previous studies have shown that the skin of the suckermouth catfish, specifically *P. pardalis* (16,17) and *P. disjunctivus* (18), contains a high percentage of collagen. Hence, the suckermouth catfish might offer a possible alternative to collagen or gelatin production from other mammals sourced in industries like pharmaceuticals, nutraceuticals, and cosmeceuticals.

Collagen is the major insoluble fibrous protein that makes up approximately 25% to 35% of the protein in the body and 70% of the skin's protein. It is found in large amounts in the extracellular matrix and connective tissue, especially in joints, tendons, bones, and skin (2,19,20). There are various applications of collagen extract in the fields of biomedical, health, and cosmetic. For example, collagen is used in a wound dressing to accelerate the healing process in biomedical applications and exists in several forms, such as sponges, gels, and membranes. It is also used as an implantable biomaterial in ophthalmology (21), as a health supplement to help with increasing and strengthening muscle mass (22), reducing the development and progression of arthritis (23), and improving skin health (24). The primary sources of collagen extraction are from land mammals such as bovines and porcine. However, there are some drawbacks to using mammalian collagens, such as religious restrictions, zoonotic issues, and immune responses. To overcome the issues associated with mammalian collagen, collagen is also extracted from other sources such as marine life. In the past few years, aquatic organisms have been gaining interest for their potential as an alternative source of collagen. It has overcome the limitations of mammalian collagen, yet still has comparable characteristics to porcine collagen (16,25,26). Fish-extractable collagen is mainly type I collagen. These collagens have been widely utilised as scaffolds for tissue engineering due to their excellent bioactive properties, such as cell growth potential, low antigenicity, and biocompatibility (26–29). Fish collagen also has higher bioavailability and is absorbed into the body up to 1.5 times more effectively than bovine and porcine collagen due to its low molecular weight and size (2,27). Furthermore, the amino acid composition of fish collagen is comparable to that of mammalian collagen, which is primarily composed of glycine and has a degree of proline hydroxylation of 35–48% (30). Some of the fish species used to extract collagen are red tilapia (30,31), Pacific cod, and eel (30).

There are limited studies related to the collagen extracted from the suckermouth catfish, especially those from the genus *Pterygoplichthys*. Thus, this research aims to focus on the utilisation of suckermouth catfish as a potential alternative source of halal collagen from an abundant, underutilised, and low-value fish species in Malaysia. Utilisation of this fish could also indirectly serve to control its population by increasing the demand and encouraging extensive removal of the fish from the ecosystems.

MATERIALS AND METHODS

Materials

Pterygoplichthys spp. was collected from the Langat River, Selangor using various fishing gear, mainly using cast nets. Genus identification and verification were done by the Fisheries Research Institute Glami Lemi, Malaysia. Sodium hydroxide, sodium chloride, and acetic acid were purchased from Sigma-Aldrich, and

butyl alcohol was purchased from R&M Chemicals.

Extraction Procedures

Extraction of acid-soluble collagen was done according to the method described by Nagai & Suzuki (2000) with some modifications. A total of 450 g of the skin of suckermouth catfish, genus *Pterygoplichthys*, were collected and verified by the Fisheries Research Institute. The skins were separated, labelled, and cut into small pieces of 1 Ч 1 cm2 in length and stored at -20°C until further use. All pre-treatment, washing, and extraction procedures were carried out at 25°C.

During pre-treatment stage, the skin samples were soaked and gently stirred in 0.1 M sodium hydroxide (NaOH) at a sample to solution ratio of 1:10 (w/v) continuously for 24 hours to remove non-collagenous proteins from the skins. The solution was changed every 12 hours. Then, the skins were washed several times using cold distilled water until the samples achieved pH≈7. Next, the collagenous skins were soaked in 10% butyl alcohol (1:10 w/v) for 24 hours to remove fat from the skin. Following that, the defatted skins were thoroughly washed with cold distilled water.

For the extraction of the collagen, the defatted skin samples were suspended in 0.5 M acetic acid (1:10 w/v) for 90 hours with continuous stirring. Then, the suspended samples were filtered, and the filtrate was centrifuged at 4,000 rpm for 50 minutes using a centrifuge machine (Heraeus Megafuge 200). The filtrate was then salted out by adding sodium chloride (NaCl) to a final concentration of 0.7 M. Following that, a white precipitate was formed and collected by centrifuging at 4,000 rpm for 60 minutes. The wet precipitate was weighed using an analytical balance. Then, the wet precipitates were kept at -20°C, continuing to -86 °C before being lyophilised by using a freeze dryer for 5 days (Scanvac Coolsafe). The freeze-dried samples were weighed using an analytical balance and recorded.

Yield Calculation

The final weight of the extracted collagen sample was used to calculate the collagen's percentage yield. The yield was calculated using the equation based on the wet weight basis.

$$\text{Yield (\%)} = \frac{\text{Weight of collagen samples (g)}}{\text{Weight of initial sample (g)}} \text{ X } 100$$

Identification Tests

Identification tests were based on the Gelatine Manufacturers of Europe (GME)'s standardised methods for testing of edible collagen peptides, which consists of Biuret method, gelling/non-gelling test and hydroxyproline content test (33).

Physical Characteristics

Physical characteristics methods are based on the methods described in the standardised methods for testing edible collagen peptides, including viscosity, pH, colour, and odour (33).

Heavy Metal Content Determination

Content of iron, mercury, methylmercury, arsenic and inorganic arsenic, cadmium, chromium, copper, lead, and zinc were determined using in-house methods by ALS Technichem (M) Sdn Bhd. Iron content was determined by digesting 1.5 g of the samples using 3% nitric acid at 95°C for 1.5 hours. Then, the digested samples were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent, USA). Mercury and methylmercury content were determined by digesting 1.5 g of the samples using three types of acids (37% hydrochloric, 65% nitric acid, and 98% sulphuric acid) at 95°C for half an hour. Then, the digested samples were analysed using the Flow Injection Mercury System (FIMS) (Perkin Elmer, USA). Finally, arsenic, inorganic arsenic, cadmium, chromium, copper, lead, and zinc content were determined by digesting 1.5 g of the samples using 65% nitric acid. 1 ml of hydrogen peroxide was added, and the mixture was then heated at 95°C for 1.5 hours. The digested samples were then analysed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) (Agilent, USA).

RESULT

The yield and physical characteristics of the extracted collagen are summarised in Table I.

Table I: Yield and physical characteristics of extracted suckermouth catfish collagen

Properties	Value/Description
Identification tests	
Biuret test	Positive
Gelling/non-gelling	Non-gelling
Hyp content	Positive (1.6%)
Physical characteristics	
Yield	10±2.3%
pH	5.51±0.1
Viscosity (mPa.s)	0.6±0.25
Colour	Off-white
Odour	Strong acetic acid odour

Heavy metal content of the extracted collagen is shown in Table II.

Table II: Heavy metal content of the extracted suckermouth catfish collagen and ASEAN and NPRA limits for heavy metal contents in health supplement and cosmetic products.

Heavy Metals	Content (mg/kg)	Limit for Health Supplement Products (mg/kg)[1]	Limit for Cosmetic Products (mg/kg)[2]
Mercury	<0.05	0.5	1
Iron	6.2	-	-
Arsenic	0.327	5	5
Cadmium	<0.100	0.3	5
Chromium	0.124	-	-
Copper	0.470	-	-
Lead	0.294	10	20
Zinc	3.23	-	-
Methyl Mercury	0.0012	-	-
Inorganic Arsenic	0.19	-	-

[1] Annex III: ASEAN Guidelines on Limits of Contaminants for Health Supplements.

[2] Annex I, Part 14: Heavy Metal and Microbiological test Limit for Cosmetic Product, NPRA.

DISCUSSION

Extraction and Yield of Suckermouth Catfish Collagen

The percentage yield of acid-soluble collagen from suckermouth catfish skin in this study was found to be lower (10±2.3%) compared to a study on *P. pardalis* by Nurubhasha et al. (16) and *P. disjunctivus* by Herath et al. (18), which had a percentage yield of 19.6% and 26.2%, respectively. This might be due to the differences in the experimental procedure, such as temperature and processing time. This collagen yield however is higher than the yield obtained from the skin of *parang-parang* fish (*Cirocentrus dorab*) which is 1.915% (34) and eel (*Evenchelys macrura*), which is 4.2% (35), but lower than tilapia (*Oreochromis niloticus*) which is 27.2% (36), South Asian carp (*Catla catla*) which is 13% (37), and sole fish (*Aseraggodes umbratilis*) which is 19% (38). Generally, the collagen yield is linked to the species of fish and its age, as well as the body sections used, and environment. The experimental settings and preparative procedures also play a crucial role (16,36).

According to Herath et al. (18), suckermouth catfish account for 28% of their total body weight in meat, 23% in bone, 20% in skin, and 6% in fin. These bodily components can be used as the primary raw material for collagen extraction. The usable fraction for collagen extraction accounts for roughly 75% of total body weight. Herath et al. (18) also investigated the percentage yield of acid-soluble collagen in several body parts of this fish and found that skin has the highest percentage yield of acid-soluble collagen among others. Therefore, this study focuses more on the skin of the suckermouth catfish. An acid extraction procedure was used to extract the collagen in this study after considering the cost and time-effectiveness. In comparison to hydrochloric acid, citric acid, and lactic acid, acetic acid is the most commonly used acid for extraction (2). The concentration range for the acid extraction solution (acetic acid) is between 0.2 and 1 M, with the yield increasing steadily as the acetic acid concentration increases, with a maximum yield at 0.6 M of acetic acid. However, a study reported that the collagen yield decreased after 0.6 M (39). The skin samples were thoroughly washed with distilled water after being soaked in the NaOH and butyl alcohol solutions during the extraction process to reduce the introduced impurities from the samples as much as possible.

Identification Tests

Biuret method is used to detect the presence of peptide bonds. Development of violet colour indicates the presence of at least two peptide bonds in the compound. In this study, the sample showed a positive result for peptide bonds.

The gelling/non-gelling test was used to differentiate the collagen from gelatine, as gelatine is a degraded form of collagen and will form a gel in the solution (40). As the gelling/non-gelling test revealed a flowable without forming a gel, it is not gelatine and might be collagen.

The hydroxyproline test was done because there is a significant amount of hydroxyproline in collagen but negligible in other proteins. Thus, measuring the hydroxyproline in a sample will give an indication of both the presence and the purity of the collagen in the samples (2). The amount of hydroxyproline in the triple helix structure of collagen shows how stable it is at high temperatures (18). In this study, the hydroxyproline percentage was 1.6%, which indicates a lower denaturation temperature and stabilisation of the triple helix structure. This percentage is significantly lower compared to the studies by Herath et al. (7.57% and 9.37% according to the length classification). This might be due to the shortening duration of soaking the sample in NaOH solution (from 36 hours to 24 hours). The significance of the soaking in NaOH solution is to remove non-collagenous proteins from the skin. Thus, if the soaking duration is shortened, it may reduce the removal of non-collagenous proteins from the skins, causing the extracted collagen to become contaminated with other proteins.

Physical Characteristics

The extracted collagen was in the form of an off-white powder with a strong acetic acid odour. The physical characteristics determined in this study were pH and viscosity, which are among the tests required in the Standardised Methods for the Testing of Edible Collagen Peptides (33). For pH, the result was 5.51±0.1 (20% in water at room temperature). This is a reasonable result as the pH level is determined by the extraction method. Nurubhasha et al. (16) reported that acid-soluble collagen

had the greatest solubility in the acidic zone (pH 1-4), but it began to fall after pH 5, and the lowest was at pH 6, which might be due to the protein's isoelectric point (pI). In a study on starfish and lumpfish, Kumar Vate et al. (41) also noted this. The pI of proteins has been frequently utilised to recover myofibrillar, animal, and plant proteins after acid or alkaline protein solubilisation (42). When proteins approach pI, their entire net charge becomes zero, resulting in precipitation (16). In general, pH plays a crucial role in several formulations, such as collagen in the liquid form. This is because it can affect the solubility of the compound, which can determine the stability, biological tolerability, and activity of the formulation (43). Hence, pH 5.51±0.1 suggests good solubility, and it was below the pI.

At room temperature, the viscosity of 20% collagen in an acetic acid solution was 0.6±0.25 mPa.s indicating low viscosity where the interaction between water and collagen molecules is not very strong. Thus, it is suggested that this collagen extracted from suckermouth catfish is preferable for certain types of applications, such as liquid skincare formulations, as it can improve the processability of the collagen solution, making it easier to handle and process. Viscosity expresses the magnitude of friction in a fluid. Collagen viscosity is very important to know related to its use. For example, the viscosity is important to ensure the uniformity of the collagen potency through all stages of production. Viscosity also plays an active role in the determination of the denaturation temperature. A lower collagen viscosity is associated with a lower protein denaturation temperature. A lower denaturation temperature implies that the protein is less able to maintain its distinctive triple helical structure at elevated temperatures. This structural integrity is essential for collagen to fulfill its functional roles effectively (44). This information is particularly important in the food, medicine, pharmaceuticals, and cosmetics industries. A study by Nurubhasha et al. (16) stated that by measuring the viscosity, they found that the extracted collagen was denatured at 25°C, which was lower than bovine collagen (37°C). In short, viscosity plays a very important role in collagen application and storage.

Heavy Metal Content

It is a common conception that suckermouth catfish are contaminated with heavy metals and other contaminants due to their presence in normally polluted rivers and lakes (45). According to a study in Indonesia, Plecostomus species in the Ciliwung River in Jakarta contain up to 57 metals in their flesh, including three types of heavy metals: lead (Pb), mercury (Hg), and cadmium (Cd), with concentrations exceeding the maximum recommended levels for human consumption (46). On the other hand, a 2020 study by Amir et al. found that *Pterygoplichthys pardalis* caught from three different locations in South Sulawesi province was free from Pb but contained an amount of arsenic (As) and Hg that did not exceed the limit for metal contaminant content. However, heavy metal levels in the fish species are highly dependent on the pollution level of the river and might differ between locations (46,47). It is also interesting to investigate if the metals will be carried forward into the final products after processing.

As can be seen from the Table II, the level of mercury, arsenic, lead, and cadmium are well below the limits permitted for health supplement and cosmetic products, based on the guidelines by Association of Southeast Asian Nations (ASEAN) and the National Pharmaceutical Regulatory Agency (NPRA) of Malaysia. Furthermore, when used in such products, the heavy metal content will be further diluted and should be safe for the consumers.

CONCLUSION

In this study, it was found that the skin of suckermouth catfish produced a yield of collagen on a wet basis of 10±2.3%, with a hydroxyproline content of 1.6% while the viscosity and pH of a 20% collagen solution were 0.6±0.25 mPa.s and 5.51±0.1, comparable to other studies using various fish species. Heavy metal content lower than the limit set for health supplements and cosmetic products also suggests that it could be a promising source of collagen for potential industrial applications as it has some distinct advantages over mammalian-based collagen. Industrial utilisation could offer an effective and sustainable way of controlling the populations of suckermouth catfish, as it will create a large and continuous demand for the raw material. However, further studies should be conducted to optimise the yield and fully characterise the extracted collagen and determine the safety factors, such as toxicity studies, of suckermouth catfish collagen, and compare its characteristics with commercial mammalian and marine collagen.

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REFERENCES

1. *Hypostomus plecostomus [I*nternet]. Malaysia: Malaysia Biodiversity Information System (MyBIS);

2018 [cited 2022 Mar 18]. Available from: https://www.mybis.gov.my/sp/23846
2. Jafari H, Lista A, Siekapen MM, Ghaffari-Bohlouli P, Nie L, Alimoradi H, et al. Fish collagen: Extraction, characterization, and applications for biomaterials engineering. Polymers (Basel) 2020 Sep [cited 2022 Mar 18];12(10):1–37. Available from: https://pubmed.ncbi.nlm.nih.gov/32998331/
3. Maddern M. *Hypostomus plecostomus* (suckermouth catfish) [Internet]. Perth: CABI Invasive Species Compendium; 2015 [cited 2022 Mar 18]. Available from: https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.114927
4. Landau E. "Ikan bandaraya" "cleans out" local ecosystem. New Straits Times [Internet]. 2019 Feb 7 [cited 2022 Jun 18]. Available from: https://www.nst.com.my/2019/02/457980/ikan-bandaraya
5. Dignall J. Loricariidae The catfish family [Internet]. [Place unknown]: PlanetCatfish; 2018 [cited 2022 Mar 18]. Available from: https://www.planetcatfish.com/common/family.php?family_id=4
6. Saba AO, Ismail A, Zulkifli SZ, Halim MRA, Wahid NAA, Amal MNA. Species composition and invasion risks of alien ornamental freshwater fishes from pet stores in Klang Valley, Malaysia. Sci Rep [Internet]. 2020 Oct [cited 2022 Mar 19];10(1):1–13. Available from: https://www.mdpi.com/2076-2615/11/11/3152
7. Faridah-Hanum I, Latiff A. Invasive Alien Species in Malaysia. Malaysia: John Wiley & Sons Ltd; 2021. p. 151–167.
8. Mitchell M. Armored Catfish [Internet]. [Place unknown]: Eat The Invaders; 2019. [cited 2022 Mar 19]. Available from: http://eattheinvaders.org/armored-catfish/
9. Hossain MY, Vadas RL, Ruiz-Carus R, Galib SM. Amazon sailfin catfish *Pterygoplichthys pardalis* (*Loricariidae*) in Bangladesh: A critical review of its invasive threat to native and endemic aquatic species. Fishes 2018 [Internet]. 2018 Feb [cited 2022 Mar 19];3(1). Available from: https://www.mdpi.com/2410-3888/3/1/14
10. Saba AO, Rasli NF, Ismail A, Zulkifli SZ, Ghani IFA, Muhammad-Rasul AH, et al. A report on introduced Amazon sailfin catfish, Pterygoplichthys pardalis in Gombak basin, Selangor, with notes on two body patterns of the species. Pertanika J Trop Agric Sci [Internet]. 2020 Nov [cited 2022 Mar 19];43(4):693–703. Available from: http://www.pertanika.upm.edu.my/pjtas/JTAS-2095-2020
11. Baharuddin H, Salmi S, Dharim RA. Length-Weight Relationship and Condition Factor of The Suckermouth Armoured Catfish Genus in Perai River and Skudai River and The Effect of Water. Kuala Lumpur; 2019.
12. Orfinger AB, Goodding DD. The global invasion of the suckermouth armored catfish genus Pterygoplichthys (Siluriformes: Loricariidae): Annotated list of species, distributional summary, and assessment of impacts. Zool Stud [Internet]. 2018 Feb [cited 2022 Mar 20];57. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6517723/
13. Ikan Bandaraya Bakar Cicah Air Asam Menu Istimewa Di Vietnam [Internet]. Malaysia: Era; 2020 Nov [cited 2022 Mar 18]. Available from: https://era.syok.my/interaktif/trending/ikan-bandaraya-bakar-cicah-air-asam
14. Ernawati Y. The analysis of the concentration of heavy metals cadmium, mercury and lead in the flesh of suckermouth catfish (*Pterygoplichthys pardalis*) in Ciliwung River, Indonesia. Aquac Aquar Conserv Legis [Internet]. 2014 Jan [cited 2022 Mar 20];7(1):33–42. Available from: https://www.researchgate.net/publication/287523903
15. Hill JE, Sowards J. Successful eradication of the non-native loricariid catfish *Pterygoplichthys disjunctivus* from the Rainbow River, Florida. Manag Biol Invasion [Internet]. 2015 Sept [cited 2022 Mar 20];6(3):311–7. Available from: https://www.researchgate.net/publication/282480854
16. Nurubhasha R, Kumar NSS, Thirumalasetti SK, Simhachalam G, Dirisala VR. Extraction and characterization of collagen from the skin of *Pterygoplichthys pardalis* and its potential application in food industries. Food Sci Biotechnol [Internet]. 2019 Dec [cited 2022 Mar 21];28(6):1811. Available from: https://pubmed.ncbi.nlm.nih.gov/31807354/
17. Ebenstein D, Calderon C, Troncoso OP, Torres FG. Characterization of dermal plates from armored catfish *Pterygoplichthys pardalis* reveals sandwich-like nanocomposite structure. J Mech Behav Biomed Mater [Internet]. 2015 May [cited 2022 Mar 21];45:175–82. Available from: https://pubmed.ncbi.nlm.nih.gov/25732181/
18. Herath HMGK, Kalutharage NK, Cumaranatunga PRT. Solutions to an alien species invasion from aquarium aquaculture: Isolation and characterization of acid soluble collagen from sailfin catfish, *Pterygoplichthys disjuctivu*s (Weber, 1991) in Sri Lanka. Sri Lanka J. Aquat. Sci [Internet]. 2020 Mar [cited 2022 Mar 21];25(1):19. Available from: https://sljas.sljol.info/articles/10.4038/sljas.v25i1.7573
19. Sionkowska A, Adamiak K, Musial K, Gadomska M. Collagen based materials in cosmetic applications: A review. Materials (Basel) [Internet]. 2020 Sep [cited 2022 Mar 22];13(19):1–15. Available from: https://pubmed.ncbi.nlm.nih.gov/32977407/
20. Gelse K, Pıschl E, Aigner T. Collagens—structure, function, and biosynthesis. Adv Drug Deliv Rev [Internet]. 2003 Nov [cited 2022 Mar 22];55(12):1531–46. Available from: https://pubmed.ncbi.nlm.nih.gov/14623400/
21. Sangeetha K, Jisha Kumari A V., Radha E, Sudha PN. Pharmaceutical Applications of Collagen. Natural Polymers for Pharmaceutical Applications.

Apple Academic Press. 2020 Mar; p. 61–92.
22. Oertzen-Hagemann V, Kirmse M, Eggers B, Pfeiffer K, Marcus K, de Marées M, et al. Effects of 12 Weeks of Hypertrophy Resistance Exercise Training Combined with Collagen Peptide Supplementation on the Skeletal Muscle Proteome in Recreationally Active Men. Nutrients [Internet] 2019 May [cited 2022 Mar 24];11(5). Available from: https://pubmed.ncbi.nlm.nih.gov/31091754/
23. Dar QA, Schott EM, Catheline SE, Maynard RD, Liu Z, Kamal F, et al. Daily oral consumption of hydrolyzed type 1 collagen is chondroprotective and anti-inflammatory in murine posttraumatic osteoarthritis. PLoS One [Internet]. 2017 Apr [cited 2022 Mar 24];12(4). Available from: https://pubmed.ncbi.nlm.nih.gov/28384173/
24. Vollmer DL, West VA, Lephart ED. Enhancing Skin Health: By Oral Administration of Natural Compounds and Minerals with Implications to the Dermal Microbiome. Int J of Mol Sci [Internet]. 2018 Oct [cited 2022 Mar 24];19(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6213755/
25. Coppola D, Oliviero M, Vitale GA, Lauritano C, D'Ambra I, Iannace S, et al. Marine Collagen from Alternative and Sustainable Sources: Extraction, Processing and Applications. Mar Drugs [Internet]. 2020 Apr [cited 2022 Mar 26];18(4). Available from: https://pubmed.ncbi.nlm.nih.gov/32326635/
26. Venkatesan J, Anil S, Kim SK, Shim MS. Marine Fish Proteins and Peptides for Cosmeceuticals: A Review. Mar drugs [Internet]. 2017 May [cited 2022 Mar 26];15(5). Available from: https://pubmed.ncbi.nlm.nih.gov/28524092/
27. Subhan F, Hussain Z, Tauseef I, Shehzad A, Wahid F. A review on recent advances and applications of fish collagen. Crit Rev Food Sci Nutr [Internet]. 2020 [cited 2022 Mar 28];61(6):1027–37. Available from: https://europepmc.org/article/med/32345036
28. Subhan F, Ikram M, Shehzad A, Ghafoor A. Marine Collagen: An Emerging Player in Biomedical applications. J Food Sci Technol [Internet]. 2015 Aug [cited on 2022 Mar 29];52(8):4703–7. Available from: https://pubmed.ncbi.nlm.nih.gov/26243892/
29. Jeong HS, Venkatesan J, Kim SK. Isolation and characterization of collagen from marine fish (*Thunnus obesus*). Biotechnol Bioprocess Eng [Internet]. 2014 Jan [cited 2022 Mar 29];18(6):1185–91. Available from: https://link.springer.com/article/10.1007/s12257-013-0316-2
30. Raman M, Gopakumar K. Fish Collagen and its Applications in Food and Pharmaceutical Industry: A Review. Biology [Internet] 2018 [cited on 2022 Mar 31];12:752–67. Available from: https://www.semanticscholar.org/paper/Fish-Collagen-and-its-Applications-in-Food
31. Li J, Wang M, Qiao Y, Tian Y, Liu J, Qin S, et al. Extraction and characterization of type I collagen from skin of tilapia (Oreochromis niloticus) and its potential application in biomedical scaffold material for tissue engineering. Process Biochem [Internet] 2018 Nov [cited on 2022 Mar 31];74:156–63. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1359511318301144
32. Nagai T, Suzuki N. Isolation of collagen from fish waste material - Skin, bone and fins. Food Chem [Internet] 2000 Feb [cited on 2022 Feb 14];68(3):277–81. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0308814699001880
33. Gelatine Manufacturers of Europe. Standardised Methods For The Testing of Edible Collagen Peptides; 2020.
34. Wijaya H, Putriani S, Safithri M, Tarman K. Isolation and Allergenicity of Protein Collagen from Parang-Parang Fish Skin (*Cirocentrus dorab*). IOP Conf Ser Earth Environ Sci [Internet]. 2020 Jan [cited 2022 Jun 25];411(1). Available from: https://iopscience.iop.org/article/10.1088/1755-1315/411/1/012054/pdf
35. Govindharaj M, Roopavath UK, Rath SN. Valorization of discarded Marine Eel fish skin for collagen extraction as a 3D printable blue biomaterial for tissue engineering. J Clean Prod [Internet]. 2019 Sep [cited 2022 Jun 25];230:412–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0959652619316026
36. Chen J, Li L, Yi R, Xu N, Gao R, Hong B. Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (*Oreochromis niloticus*). LWT-Food Sci Technol [Internet]. 2016 Mar [cited 2022 Jun 25];66:453–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0023643815302887
37. Mahboob S. Isolation and characterization of collagen from fish waste material- skin, scales and fins of *Catla catla* and *Cirrhinus mrigala.* J Food Sci and Tech [Internet]. 2015 Jul [cited 2022 Jun 25];52(7):4296–305. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4486571/
38. Cheng X, Shao Z, Li C, Yu L, Raja MA, Liu C. Isolation, Characterization and Evaluation of Collagen from Jellyfish *Rhopilema esculentum Kishinouye* for Use in Hemostatic Applications. PLoS One [Internet]. 2017 Jan [cited 2022 Jun 25];12(1):e0169731. Available from: https://pubmed.ncbi.nlm.nih.gov/28103327/
39. Arumugam GKS, Sharma D, Balakrishnan RM, Ettiyappan JBP. Extraction, optimization and characterization of collagen from sole fish skin. Sustain Chem Pharm [Internet]. 2018 Sep [cited 2022 Jun 28];9:19–26. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2352554118300226
40. Lang A. Should You Choose Collagen or Gelatin? [Internet]. United States: Healthline. 2020 [cited

2022 Jul 2]. Available from: https://www.healthline.com/nutrition/collagen-vs-gelatin#bottom-line
41. Kumar Vate N, Pawel Strachowski P, Undeland I, Abdollahi M. Structural and functional properties of collagen isolated from lumpfish and starfish using isoelectric precipitation vs salting out. Food Chem. X [Internet]. 2023 [cited Nov 9];18,100646. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC10053375/
42. Abdollahi M, Rezaei M, Jafarpour A, Undeland I. Sequential extraction of gel-forming proteins, collagen and collagen hydrolysate from gutted silver carp (*Hypophthalmichthys molitrix*), a biorefinery approach. Food Chemistry [Internet]. 2018 [cited Nov 9];242:568-578. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0308814617315005
43. Vázquez-Blanco S, González-Freire L, Dávila-Pousa MC, Crespo-Diz C. pH determination as a quality standard for the elaboration of oral liquid compounding formula. Farm Hosp [Internet]. 2018 Nov [cited Jul 5];42(6):221–7. Available from: https://pubmed.ncbi.nlm.nih.gov/30381041/
44. Zhang X, Xu S, Shen L. et al. Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. J Leather Sci Eng [Internet] 2020 [cited Nov 9];2,19. Available from: https://jlse.springeropen.com/articles/10.1186/s42825-020-00033-0
45. Amir N, Syahrul, Djamaluddin N. Suckermouth Catfish (Pterygoplichthys pardalis) In Wajo Regency, South Sulawesi Province: The Heavy Metal Content of Lead (Pb), Mercury (Hg) and Arsenic (As). Agrikan [Internet]. 2020 Dec [cited 2022 Jul 10];13(2):173–4. Available from: https://www.researchgate.net/publication/347576130
46. Elfidasari D, Ismi LN, Sugoro I. Heavy metal concentration in water, sediment, and Pterygoplichthys pardalis in the Ciliwung River, Indonesia. Aquac Aquar Conserv Legis [Internet]. 2020 Jul [cited 2022 Jul 11];13(3):1764–78. Available from: https://www.researchgate.net/publication/343390717
47. Castaceda-Chávez MDR, Lango-Reynoso F, Navarrete-Rodríguez G, Wakida-Kusunoki AT. Concentration of metals in native and invasive species of fish in the fluvial-lagoon-deltaic system of the Palizada river, Campeche. Fishes [Internet]. 2021 Dec [cited 2022 Jul 11];6(4). Available from: https://www.mdpi.com/2410-3888/6/4/72