Food Reviews International

ISSN: 8755-9129 (Print) 1525-6103 (Online) Journal homepage: https://www.tandfonline.com/loi/lfri20

Functional and Bioactive Properties of Gelatin Extracted from Aquatic Bioresources – A Review

R. A. S. N. Ranasinghe, W. L. I. Wijesekara, P. R. D. Perera, S. A. Senanayake, M. M. Pathmalal & R. A. U. J. Marapana

To cite this article: R. A. S. N. Ranasinghe, W. L. I. Wijesekara, P. R. D. Perera, S. A. Senanayake, M. M. Pathmalal & R. A. U. J. Marapana (2020): Functional and Bioactive Properties of Gelatin Extracted from Aquatic Bioresources – A Review, Food Reviews International, DOI: 10.1080/87559129.2020.1747486

To link to this article: https://doi.org/10.1080/87559129.2020.1747486

Published online: 14 Apr 2020.

FOOD REVIEWS INTERNATIONAL
https://doi.org/10.1080/87559129.2020.1747486

Functional and Bioactive Properties of Gelatin Extracted from Aquatic Bioresources – A Review

R. A. S. N. Ranasinghe [a], W. L. I. Wijesekara[a], P. R. D. Perera[a], S. A. Senanayake[a], M. M. Pathmalal[b], and R. A. U. J. Marapana[a]

[a]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka; [b]Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

ABSTRACT

Gelatin, which is widely used in the food industry, is a protein derived from partial denaturation of collagen. The primary sources of industrial gelatin are porcine skin or bovine hide, but their utilization is restricted due to disease outbreaks and on religious grounds. Therefore, gelatin derived from aquatic organisms is attracting widespread interest and can be a good source for harvesting of such proteins. Also, numerous studies have demonstrated bioactive (mainly antioxidant and antihypertensive) properties of enzymatic hydrolysates of aquatic gelatins. This review summarizes sources, significance, functional and bioactive properties of aquatic gelatins as revealed by recent studies.

KEYWORDS

Fish gelatin; gel strength; antioxidant properties; antihypertensive properties; surface-active properties

Introduction

Gelatin is a fibrous protein derived from partial thermal denaturation of collagen,[1–3] which is the main protein component in skin, bones and cartilages.[4] It holds specific rheological property of thermo-reversible transformation between sol and gel.[5,6] Gelatin macromolecules can possess a wide variety of conformations under specific conditions of pH, temperature and solvent, and the molecular structure of gelatin is a key factor that determines its characteristics and functionalities.[1] Most of the commercial gelatins are derived from porcine skin or bovine hide or bones,[7,8] but alternative sources such as poultry[9] and fish byproducts can also be employed for the manufacture of gelatin.[10] Johnston-Banks[11] has reported that the type (the amino acid composition), the source of raw material and the manufacturing method to be key for the overall quality of gelatin. Apart from these factors, the relative content of α-chains and β or γ components, high molecular weight aggregates, the presence of low molecular weight protein fragments and hydrophobicity have an effect on the physical properties of gelatin.[3,11,12]

Generally, gelatin constitutes of collagen fractions that exceed the molecular weight of 30 kD, and lower molecular weight fractions are not considered as gelatins since they do not possess gel forming ability.[13] Gelatin has become crucial in different applications owing to specific characteristics including its amphoteric nature, specific triple-stranded helical structure and its interaction with water, which are not observed in other synthetic hydrophilic polymers.[14] The physical properties of gelatin rely on its structure and have a greater effect

CONTACT R. A. U. J. Marapana umarapana@sci.sjp.ac.lk Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

on the quality and its potential applications.[15] Hence, it is important to investigate the structure of gelatin from different sources before their ensuing application.[15] Gelatin is composed of 20 different amino acids along its polypeptide chains.[16] The amino acid composition of gelatin is almost identical to that of the original collagen which it was derived from.[17] The major amino acids in gelatin are glycine, proline and hydroxyproline.[18] The differences in amino acid sequence of gelatins from different animal species lays the foundation for differentiation of gelatins based on marker peptide detection.[19] The structure and molecular weight distribution of gelatin extracted from different sources have been studied by different technologies including Ultraviolet Spectrum Profile analysis,[19] Fourier Transform Infrared (FTIR) Spectrum analysis,[1,19–22] Scanning Electron Microscopy (SEM),[20–24] Circular Dichroism Analysis,[12,25–27] Atomic Force Microscopy (AFM),[15] Q-TOF Mass Spectrometry[28] and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).[1,19,20,29–32]

Collagen consists of a right-handed triple helical structure composed of three parallel intertwined α-chains to form three dimensional structures.[11,33] Each α-chain is composed of a repeated sequence of (Gly-X-Y) units where X and Y usually represent proline and hydroxyproline, respectively.[34,35] The hydroxyl groups in hydroxyproline, has a major role in the stabilization of triple helix. The very short terminal regions known as telopeptides are not involved in the formation of the triple helical structure, but contains inter- and intra-molecular covalent crosslinks that are formed by lysine and hydroxylysine residues.[31] The hydrogen bonds and some of the covalent bonds are cleaved at temperatures above 40°C, and this leads to the destabilization of triple helix structure leading to the formation of soluble gelatin.[36]

The lower melting point of gelatin below the human body temperature makes it ideal for most of the food applications, owing to its melting propensity in the mouth cavity.[37] Gelatin has broad applications in food industry as an emulsifier, foaming agent, fining gent, colloid stabilizer, biodegradable film forming material, dispersing, aeration, glazing and microencapsulating agent[4] in various food products including jellies, desserts, candies, meat products, ice-creams and dairy products.[24,32] Due to its neutral flavor and color, addition of gelatin does not affect the flavor and tonal properties of the original food.[17] Gelatin is used widely not only in food industry, but also in nutraceutical and pharmaceutical industry for manufacture of capsules, tablet coatings, emulsions and cosmetics,[2,38] and photographic industries.[5,15,29] In addition, new applications of gelatin as a functional food have been investigated recently.[5] Several studies have focused on the preparation of biodegradable packaging materials using gelatin from aquatic sources including brown-stripe snapper and bigeye snapper,[39] cuttlefish,[16] jellyfish,[40] puffer fish[41] and Argentine Shortfin Squid,[38] as an alternative for synthetic packaging materials, which can cause severe environmental problems.

Sources and extraction of gelatin

Gelatin is mostly extracted from porcine and bovine skins and bones,[30,42] and poultry and fish by-products are rarely used as a gelatin source.[10] The collagen pretreatment method may influence the extraction efficiency of gelatin. Based on the pretreatment method there are two main types of gelatin; type-A (isoelectric point 8–9), which is derived by acidic hydrolysis of porcine skin (because alkali treatment may result in

saponification due to high fat content in pig skin), and type-B (isoelectric point 4–5) generated by hydrolysis of cattle bone and cattle hide with alkaline treatment.[20,43,44] Generally, acid treatment is carried out for porcine collagen and fish skins, which are less fully cross-linked collagens, while alkaline treatment is appropriate for collagen extracted from bovine hides due to its complex nature.[12]The types of gelatin, based on the pretreatment methods are demonstred in Figure 1.

According to Ahmad and Benjakul,[1] the annual world production of gelatin is nearly 326,000 tons, with highest percentage of gelatin derived from pig skin (44%), followed by bovine hides (28%), bones (27%) and other sources (1%). The worldwide consumption of gelatin is increasing annually,[1,45] and it is estimated that the global gelatin consumption in 2018 will be about 450 thousand tons.[32] The increasing global demand for gelatin requires a surge in production from alternative sources.

More than 50% accounts for heads, bones and skin of fish, which are underutilized and discarded as waste, which contributes for environmental pollution[42] and emit offensive odors.[46] Despite their high value, such items are mostly used in low value products such as animal feed and fertilizer production.[47] Therefore, optimizing the utilization of these waste materials in value addition would be practical.[46] Although, fish skin is the most widely used raw material for fish gelatin production, other sources such as bones and scales are also potential sources for gelatin extraction.[48] It is reported that collagen accounts for 70% of the dry matter of fish skin[49] and when heated above 40°C, collagen can be converted to gelatin. The amount of fish skin may vary according to the species, size of the fish and processing styles and when compared with bony fishes, cartilaginous fishes contain higher amount of collagen.[3] Also, Sinthusamran et al.[50] found that the yield, composition and the properties of seabass skin gelatin to vary with the size of the fish used for the gelatin extraction, where the highest yield was found in gelatin extracted from the skin of larger size fish compared to the smaller ones. Fish wastes including skin and bones, which are generated from canning operations, can be used for the production of gelatin, but, a major drawback is the peculiar odor of fish skins which is considered to be due to basic nitrogenous compounds and fats containing large percentages of unsaturated acids.[51] Gelatin that is extracted from fish skin usually undergo a pretreatment with acid or alkali prior to extraction.[52] Fish scales accounts for about 5% of collagenous fish waste,[53] and several studies have investigated the extraction of collagen and gelatin from scales of different fish species including, *Pagrus major* and *Oreochromis niloticas*,[54] Black drum (*Pogonia cromis*) and Sheepshead seabream (*Archosargus probatocephalus*),[55] sardine.[56] Deep-sea redfish (*Sebastes mentella*),[46] silver carp (*Hypophthalmichthys*

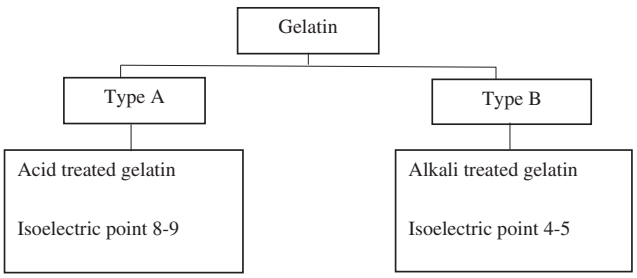

Figure 1. Types of Gelatin.

molitrix),[53] sea bream[48] and Lizard fish (*Saurida* spp).[24] Since fish scales are rich in calcium, demineralization prior to gelatin extraction is crucial to improve the yield, purity and the gel strength.[4,53] Most of these studies have pretreated the fish scales with EDTA for calcium salt removal.[46,53,54] However, Wang and Regenstein[53] compared the efficiency of calcium removal of pretreatments with different chemicals in varying concentrations. According to the study, treatment with EDTA, hydrochloric acid and citric acid was able to decalcify the scales, but the protein loss with EDTA was lower compared to HCl and citric acid since acids can hydrolyze the proteins in fish scales. Scale gelatins with highest yield and gel strength was recorded when the EDTA concentration was 0.20 mol/L.[53] Moreover, Chandra et al.[57] has extracted gelatin with a yield of 13.5% (w/w) and a satisfactory gel strength from the swim bladders of freshwater fish *Catla catla*, indicating the possibility to use the swim bladders as a source of gelatin extraction.

Although mammalian gelatins have been widely studied for their properties, only a few researches have been dedicated to processing and properties of fish skin,[37] and most of the researches on fish gelatins have been found in literature only in recent years.[37,58] Due to its distinctness compared to mammalian gelatins, fish gelatin may extend the applications of gelatin in food products.[59] Even though many fish species have been studied for extraction of gelatin from their skins and bones,[60] only 1% of the annual world gelatin production is contributed by fish gelatins.[52]

Some researchers have suggested that the extraction of gelatin from the whole body of marine species like jellyfish would be a better alternative rather than the fish skins due to their high availability of collagen.[61] Jellyfish are marine invertebrate animals belonging to the phylum Cnidaria,[62] which are known for their interferences on human activities, fisheries, aquaculture and tourism activities.[63] Therefore, some researchers have focused on extraction of collagen and gelatin from jellyfish and other marine species including *Catostylus tagi*,[64,65] *Rhopilema hispidum*,[66] *Rhopilema esculentum* Kishinouye,[35] *Lobonema smithii*,[8,61] Giant Red Sea Cucumber (*Parastichopus californicus*)[67] and marine snail (*Rapana venosa*).[68] However, there is a limited number of studies regarding the gelatin extracted from jellyfish and other species till now.[66]

Figure 2 demonstrates the gelatin extraction process from different aquatic sources.

Different studies conducted for the extraction of gelatin from aquatic sources are listed in Table 1.

The gelatin production process consists of three main steps.[85]

(1) Removal of non-collagenous material
(2) Control hydrolysis of collagen to gelatin
(3) Recovery and drying of the final product

The pre-treatment method has a major impact on the characteristics of the final product.[32] Initially, acid or alkali treatments are carried out with the purpose of swelling of the skin collagens and removal of non-collagenous protein. Hot water extraction is the most widely used method for the gelatin extraction.[86] During hot water extraction, the triple helix structure of collagen breaks down and smaller gelatin molecules are produced.[5,30] These processes are greatly affected by the pH, temperature and the time applied for pre-treatment and extraction.[85]

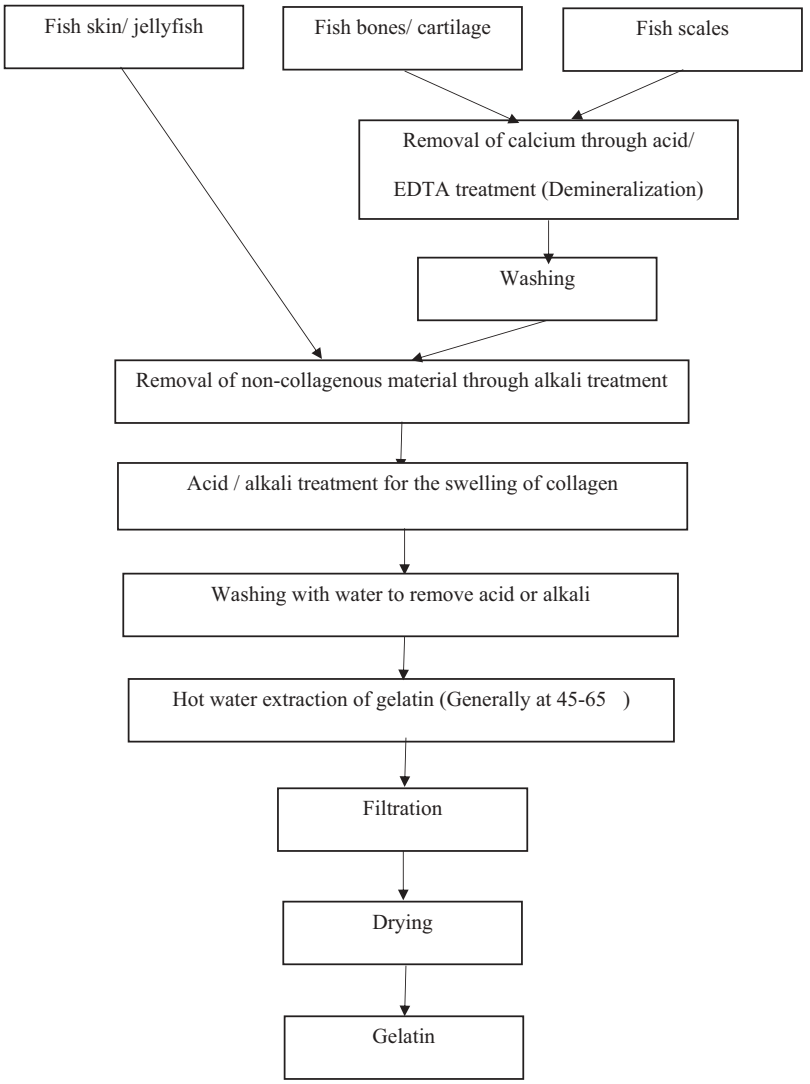

Figure 2. Gelatin extraction procedure from different aquatic sources.

The process of swelling is selected based on the raw material.[38] Since fish collagen contain low levels of non-reducible intra and inter-chain cross links, mild acid pretreatments are appropriate for the extraction of gelatin. However, it has been found that rather than using a single treatment method, combination of both acid and alkali treatments to result higher yields of gelatin with better quality.[32]

The yield of gelatin is affected by several parameters such as the extraction solvent, extraction time and temperature.[87] A study conducted by Ahmad and Benjakul[1] has revealed that the type of acid used for the pretreatment and duration of the extraction time to have an effect on the gelatin extracted from fish skin. According to the study, pretreatment with phosphoric acid resulted in a higher yield than that of acetic acid pretreatment, even at the same concentration. This may be due to the higher ionic

Table 1. Gelatin extraction from different aquatic sources.

Source	Extraction method	Reference
Thilapia skin	Pretreatment of fish skins with 0.2% Sodium hydroxide (1:14 w/v), 0.2% sulfuric acid (1:14 w/v) and 1% citric acid (1:14 w/v) followed by the extraction of gelatin with distilled water at 45-50°C overnight with subsequent filtration and lyophilization of the filtrate until a powder was obtained	[51]
Cod skin	Pretreatment of fish skins with 0.1–0.4% Sodium hydroxide, 0.1–0.4% sulfuric acid and 0.4–1.4% citric acid (1:7 w/v) followed by the extraction of gelatin with distilled water at 45°C, filtration and vacuum drying of the filtrate at 43–45°C and then air drying in a fume hood at 45°C to remove remaining water	[29,59]
Megrim (*Lepidorhombus boscii*) skin	Pretreatment of fish skins with 0.2 N Sodium hydroxide (1:6 w/v) and treatment with 0.05–0.5 M organic acids (formic, acetic, propionic, lactic, malic, tartaric and citric acid) (1:20 w/v) followed by the extraction of gelatin with distilled water at 45°C for 30 min	[69]
Harp seal (*Phoca Groendlandica*) skin	Dehairing of the seal skin, pretreatment with 0.65% sulfuric acid and extraction of gelatin with distilled water at 60°C for 7 h followed by a secondary extraction with distilled water at 75°C for 1–5 h and then concentration by vacuum evaporation and drying in a ventilated cabinet at 43°C	[70]
Megrim (*Lepidorhombus boscii*), Dover sole (*Solea vulgaris*), Cod (*Gadus morhua*) and Squid (*Dosidicus gigas*) skin	Pretreatment of fish skins with 0.05 M acetic acid followed by the extraction of gelatin with distilled water at 45°C overnight (Higher extraction temperature of 80°C was used for Squid skin) and drying until the moisture content reached less than 15%.	[12]
Nile perch (*Lates niloticus*) skin and bones	Pre-treatment of the fish skin and bones with 0.01 M sulphuric acid (1:2 w/v) or 3% hydrochloric acid followed by three sequential 5-h extraction of gelatin at 50,60 and 70°C and boiling for 5 h	[71]
Yellowfin tuna (*Thunnus albacares*) skin	Pretreatment of fish skin with 1–3% Sodium hydroxide (1:8 w/v) followed by the extraction of gelatin with distilled water (1:6 w/v) at 40–80°C for 1–9 h and then vacuum-concentration of filtered solution to 10 brix at 60°C subsequent drying in a hot-air dryer for 24 h	[10]
Fermented Skate (*Raja kenojei*) skin	Pretreatment of fermented skate skins with 0.5–2% calcium hydroxide followed by extraction of gelatin with hot distilled water and treatment with activated carbon and freeze drying	[72]
Sin croaker (*Johnius dussumieri*) and Shortfin Scad (*Decapterus macrosoma*) skin	Pretreatment of fish skins with 0.2% Sodium hydroxide (1:7 w/v), 0.2% sulfuric acid (1:7 w/v) and 1.0% citric acid (1:7 w/v) followed by the extraction of gelatin with distilled water (1:3 w/v) at 45–50°C for 12 h and vacuum evaporation of the clear filtrate and subsequent freeze-drying	[60]
Cuttlefish (*Sepia pharaonic*) dorsal and ventral skin	Pretreatment of skins with 0.05 M Sodium hydroxide (1:10 w/v), then bleaching with 2% and 5% hydrogen peroxide (1:10 w/v) for 24 and 48 h followed by the extraction of gelatin with distilled water (1:2 w/v) at 60°C for 12 h and freeze drying of the resulting filtrate.	[73]
Bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*) skin	Pretreatment of fish skins with 0.025 M Sodium hydroxide (1:10 w/v) and 0.2 M acetic acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 45°C for 12 h and freeze drying of the resulting filtrate	[20]
Brownbanded bamboo shark and blacktip shark skin	Pretreatment of fish skins with 0.1 M Sodium hydroxide (1:10 w/v) and 0.2 M acetic acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:2 w/v) at 45, 60 and 75°C for 6–12 h and filtration with subsequent freeze-drying of the filtrate	[22]
Rainbow trout (*Onchorhynchus mykiss*) skin	Pretreatment of fish skins with 0.01–0.21 N Sodium hydroxide (1:3 w/v) and 0.01–0.21 N acetic (1:3 w/v) acid followed by the extraction of gelatin with distilled water (1:3 w/v) at 45°C for 12 h and concentration of the filtrate in a rotavapor at 50°C for 30 min with subsequent freeze drying.	[74]

Unicorn leatherjacket (*Aluterus monoceros*) skin	Pretreatment of fish skin with 0.1 M Sodium hydroxide (1:10 w/v) and 0.2 M acetic acid or 0.2 M phosphoric acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 45°C and freeze drying of the resulting filtrate	[1]
Pacific cod (*Gadus macrocephalus*) skin	Pretreatment of fish skin with 1% $Ca(OH)_2$ (1:4 w/v) and extraction of gelatin with distilled water (1:4 w/v) at 60°C for 30 h followed by freeze-drying of the supernatant	[75]
Bigeye snapper (*Priacanthus tayenus*) skin	Pretreatment of fish skins with 0.025 N Sodium hydroxide (1:10 w/v) and 0.02 M acetic acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 80, 90, 100°C for 1, 2, 3 h and 110°C, 120°C, 130°C for 30 min	[30]
Cuttlefish (*Sepia officinalis*) skin	Pretreatment of the skins with 0.05 M sodium hydroxide (1:10 w/v) and 0.2 M acetic acid (1:10 w/v) followed by the treatment Smooth Hound crude acid protease (0–15 units/g) and extraction of gelatin at 50°C for 18 h and freeze drying of the supernatant resulting from centrifugation	[76]
Tilapia (*Oreochromis niloticus*) skin	Pretreatment of fish skin with 0.2 g/100 mL sodium hydroxide (1:8 w/v) and 0.2 g/100 mL sulfuric acid (1:8 w/v) and extraction of gelatin with distilled water (1:8 w/v) at 60°C for 8 h and lyophilization of the resulting filtrate	[28]
Splendid squid (*Loligo formosana*) skin	Pretreatment of skins with 0.05 M Sodium hydroxide (1:10 w/v), then bleaching with 0–8% hydrogen peroxide (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 60°C for 12 h and freeze drying of the resulting filtrate.	[77]
Skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*) and rohu (*Labeo rohita*) skin	Pretreatment of fish skins with 0.1 M Sodium hydroxide (1:10 w/v) and 0.2 M acetic acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 50°C for 16 h with subsequent fat separation, vacuum evapouration at 5°C and freeze drying	[3]
Seabass (*Lates calcarifer*) skin	Pretreatment of fish skins with 0.1 M Sodium hydroxide (1:10 w/v) and 0.05 M acetic acid (1:10 w/v) followed by the extraction of gelatin with distilled water (1:10 w/v) at 45°C and 55°C for 3,6 and 12 hours and freeze drying of the resulting filtrate	[23]
Puffer fish (*Lagocephalus gloveri*) skin	Pretreatment of fish skins with 0.1 M Sodium hydroxide (1:5 w/v) and 0.1 M acetic acid (1:5 w/v) followed by the extraction of gelatin with distilled water (1:5 w/v) at 70°C for 3 h and freeze drying of the filtrate	[41]
Chum salmon (*Oncorhynchus keta*) skin	Pretreatment of fish skin with 0.05 M NaOH (1:10 w/v), bleaching using 5 g L^{-1} hydrogen peroxide (1:10 w/v) and swelling using 0.05 M phosphoric acid (1:10 w/v) followed by extraction of gelatin with distilled water at different temperatures (40–90°C) for 10 h and subsequent freeze-drying of the filtrate.	[78]
Carp (*Cyprinus carpio*) skin	Pretreatment of fish skin with 2.6% sodium chloride, 0.1 N sodium hydroxide and ethanol or combination of 0.2% sodium hydroxide, 0.2% sulfuric acid and 1.0% citric acid followed by extraction of gelatin with distilled water at 45°C for 1–24 h and lyophilization of the resulting filtrate	[32]
Channel catfish (*Ictalurus punctatus*) skin	Pretreatment of fish skin with 0.1 M sodium hydroxide and (1:8 w/v) and 10% butyl alcohol (1:10 w/v) followed by extraction of gelatin with distilled water (1:10 w/v) at 45°C for 12 h and centrifugation with subsequent lyophilization of the supernatant.	[79]
Splendid squid (*Loligo formosana*) skins	Pretreatment of fish skin with 0.05 M sodium hydroxide (1:10 w/v) and 0.05 M phosphoric acid (1:10 w/v) followed by extraction of gelatin with distilled water (1:10 w/v) at 60°C for 12 h with subsequent filtration and dry of filtrate by freeze drying or spray drying.	[80]

(*Continued*)

Table 1. (Continued).

Source	Extraction method	Reference
Golden carp (*Probarbus Jullieni*)	Pretreatment of fish skin with 0.1 M Sodium hydroxide (1:10 w/v), 10% butyl alcohol (1:15 w/v) and 0.05 M acetic acid (1:10 w/v) or 0.05 M acetic acid (1:10 w/v) and 0.05 M sulfuric acid followed by ultrasonication (20 kHz and 750 W) and extraction of gelatin with distilled water (1:10 w/v) at 55°C for 3 and 6 h and subsequent freeze-drying of the filtrate	[81]
Tiger puffer (*Takifugu rubripes*) skin	Pretreatment of fish skin with 0.05 M sodium hydroxide and 0.05 M phosphoric acid followed by extraction of gelatin with distilled water at 45°C, 55°C and 65°C for 3, 6 and12 h and centrifugation with subsequent freeze drying of the supernatant.	[82]
Unicorn leatherjacket (*Aluterus monoceros*) and reef cod (*Epinephelus diacanthus*) skin	Pretreatment with 0.2 M NaOH (1:10 w/v) at 4°C for 2 h and 0.05 M phosphoric acid for 2 h, followed by the extraction of gelatin with potable water (1:2 w/v) at 50°C for 18 h with subsequent vacuum drying of the filtrate at different temperatures (50°C, 60°C and 70°C)	[83]
Shark (*Isurus oxyrinchus*) cartilage	Pretreatment of fish cartilage with 1–2 N Sodium hydroxide (1:8 w/v) followed by the extraction of gelatin with distilled water (1:7 w/v) at 40–80°C for 1–5 hours and vacuum concentration of the filtered solution to 10 brix at 60°C and drying for 24 h in a hot-air dryer	[5]
Tuna (*Thunnus thynnus*) head bones	Pretreatment of head bones with 0.4 M hydrochloric acid (1:5 w/v) and 0.9 g l^{-1} calcium hydroxide (1:2 w/v) followed by the extraction of gelatin with distilled water (1:4 w/v) at 75°C for 4 h and freeze drying of the extracted gelatin	[13]
Alaska Pollock (*Theragra chalcogramma*) and Yellowfin Sole (*Limanda aspera*) bones	Fish bones were pretreated with 0.6 M HCl (1:5 w/v) for 7.5 h at 20°C, followed by limited enzymatic hydrolysis with papain, with the enzyme/substrate ratio of 0.3% (w/w), and 3:1 (v/w) liquid to solid proportion at 45°C for 2 h. Then the enzyme was inactivated by heating the samples in a microwave oven for 5 min, and the gelatin was extracted with distilled water at 50°C for 5 h followed by centrifugation with subsequent freeze drying of the supernatant.	[84]
Lizardfish (*Saurida* spp) scales	Pretreatment of fish scales with 0.1–0.9% Sodium hydroxide (1:2 w/v) followed by the extraction of gelatin with distilled water at 70–90°C for 1–5 h and vacuum drying of the filtrate at 60°C	[24]
Silver carp (*Hypophthalmichthys molitrix*) scales	Pretreatment of fish scales with EDTA, 0.06–0.40 M HCl or 0.3–1.5 M citric acid followed by extraction of gelatin in distilled water (1:20 w/v) for 1 h at 60°C, then for 1.5 h at 65°C, and finally 2 h at 70°C	[53]
Swim bladders of freshwater fish *Catla catla*	Pretreatment of fish skins with 0.15% Sodium hydroxide (1:7 w/v), 0.15% sulfuric acid (1:7 w/v) and 0.5% citric acid (1:7 w/v) followed by the extraction of gelatin with distilled water at 45–50°C for 17 h with subsequent deionization and concentration of the filtrate using rotary flash evaporator followed by oven drying at 60°C for 70–72 h.	[57]
Jellyfish *Rhopilema hispidum*	Pretreatment of jellyfish with 2% Sodium hydroxide (1:5 w/v) followed by the extraction of gelatin with distilled water (1:6 w/v) at 60°C for 5 h and concentration of the filtrate at 60°C with subsequent drying at 50°C	[66]
White jellyfish (*Lobonema smithii*)	Pretreatment of dried jellyfish with sulfuric acid (pH 2, 1:15 w/v) for acid-treated (type A) and sodium hydroxide (pH 14, 1:15 w/v) for alkaline-treated (type B) followed by extraction of gelatin with distilled water at 60 and 75°C for 6–12 h and subsequent drying at 50°C for 24 h	[61]
White jellyfish (*Lobonema smithii*)	Pretreatment of jellyfish with hydrochloric acid (pH 1, 1:15 w/v) followed by extraction of gelatin with distilled water at 45°C, 60°C and 75°C for 6–12 h and subsequent drying at 50°C for 24 h	[8]
Marine Snail *Rapana venosa*	Acidic extraction was carried by the pretreatment with 0.5 M acetic acid (1:10 w/v) at room temperature for 24 h, followed by extraction of gelatin at 60°C in a shaking water for 20 h, with subsequent dialysis of the filtrate and freeze-drying. Enzymatic extraction was carried out using pepsin from porcine gastric mucosa in 0.5 M acetic acid solution at a pepsin/dry tissue ratio of 1:10 (w/w) at room temperature for 24 h. The sample was centrifuged at 8000 g for 40 min and the resulting solution was subjected to thermal treatment at 98°C for 1 min for the enzyme inactivation. Then, gelatin was extracted at 60°C for 20 h, with subsequent dialysis of the filtrate and freeze-drying.	[68]

strength of phosphoric acid compared to acetic acid, which induce more repulsive forces among the collagen molecules, facilitating the swelling process, making it easier for extraction of gelatin.[1] Tabarestani et al.[74] has also found that the gelatin yield to increase proportionally to acid concentration and pretreatment times. Citric acid is most widely used for the manufacture of food grade gelatin from fish as it does not impart obnoxious color or odor to the gelatin.[51,59]

According to Gómez-Guillén et al.,[31] utilization of high pressure for prolonged time periods during gelatin extraction; either in acid swelling or actual gelatin extraction with distilled water, result in reduced gelatin yield, but produced more high molecular weight polymers with better viscoelastic properties. The study further revealed that the use of high pressure in gelatin extraction rather than conventional methods may significantly reduce the extraction time, and improve the gelling quality.

For the preservation of the functional properties, most of the former studies have adhered to mild treatment conditions (45–60°C) when extracting gelatin from fish.[1,31,69] However, some studies have tried higher temperatures up to 100–130°C[30] when extracting fish gelatins and have found that the gelatin yield and hydroxyproline content to increase with increasing extraction time and temperature.[22,23,30,72,82] The yield of gelatin extracted from jellyfish also increased with increasing extracting temperature and time.[8,61] This may be due to the increased rate of collagen breakdown, through the effective destabilization of the bonds between the α-chain and native mother collagen, which results in an amorphous triple helix structure, that easily release more gelatinous proteins to the extracting solvent.[8,23,60] However, according to Sinthusamran et al.[23] increasing the extraction conditions beyond a certain point does not increase the yield as the crosslinked proteins in the skin network, which are stabilized by covalent bonds may not be destroyed even at higher temperatures or extended extraction periods. Moreover, it was revealed that the increased extraction temperature and prolonged extraction time, decline the lightness of tiger puffer skin gelatin, while enhancing the redness and yellowness.[82] Liu et al.[78] has discovered the yield of gelatin extracted from chum salmon skin increased with increasing temperature, but the purity was decreased at high temperatures due to the elevated contamination with non-collagenous proteins.

The variation of physicochemical properties of seabass skin gelatin and splendid squid skin gelatin with drying method have been investigated by Sae-leaw, et al.[88] and Hamzeh et al.[80] As revealed by the studies, freeze-dried gelatin had a lower solubility compared to spray-dried gelatin. This may be due to the formation of more hydrophilic and lower molecular weight peptide chains at high temperatures.[88] Also, Renuka et al.[83] have reported no significant difference in yield, nutritional value, quality, functional properties and the secondary structure of gelatin dried at different oven drying temperatures, and these properties were comparable with freeze-dried gelatin. However, a higher yield of gelatin extracted from the Marine Gastropod *Ficus variegate* have been resulted when freeze-drying method was used in the drying step, compared to drying using the hot plate.[89] As revealed by Ali et al.[81] prior ultrasonication coupled with acid pretreatment have improved the yield of golden carp skin gelatin due to the induced transition of collagen to gelatin through the destabilization of the collagen structure.

Enzymatic pretreatments using proteolytic enzymes such as papain, neutrase, bromelain, pepsin, proctase and crude proteases as an alternative to acid and alkali treatments can improve the gelatin yield and purity while reducing the processing time and waste

generation. Enzymes ease the conversion of collagen to gelatin through hydrolyzation of the crosslinks in the terminal regions. However, the continuous action of the enzymes can lead to the degradation of α and β chains lowering the gelatin quality and gel strength.[85,90] Balti et al.[76] states that use of proteases such as pepsin extracted from fish stomach can improve the extractability of gelatin. The extraction of gelatin from squid skin with smooth hound crude acid protease treatment has result in five-fold yield compared to non-enzyme treated methods. Also, the yield increased with the increasing levels of protease enzymes.[76]

The yield of gelatin extracted from cuttlefish skin[73] and splendid squid skin[77] have increased with the increasing concentration of hydrogen peroxide, which can be attributed either to the degradation of hydrogen bonds in collagen molecules by peroxide or cleavage of peptide chain of collagen by the radicals generated by H_2O_2-related reactions, thereby reducing the chain length, making the way to the easy extraction of gelatin.[77]

The effect of soybean trypsin inhibitor (SBTI) on the bigeye snapper skin gelatin has been discovered by Intarasirisawat et al.[91] and according to the study, with the presence of SBTI the degradation was inhibited, and retained α and β chains at temperatures lower than 50°C resulting a lower yield. Kaewruang et al.[92] also have found that the addition of soybean trypsin inhibitor to decrease the yield of gelatin extracted from the skin of unicorn leatherjacket at different temperatures. This can be associated with the inhibition of indigenous proteases in the fish skin matrix, thus, preventing the cleavage of collagen polypeptide chains.[92]

Traditional gelatin extraction methods include several extractions steps consuming large amounts of solvents and they are more time consuming. Multiple extraction steps can also lead to product losses, resulting lower extraction yields.[27] However, a novel cheap and environmental friendly extraction method at mild conditions, which involves water acidified with carbon dioxide for the extraction of collagen/gelatin from marine sponges has been proposed by Barros et al.[26] and Silva et al.[27]

Importance of gelatin from aquatic sources

The use of gelatin from mammalian sources have major drawbacks due to disease outbreaks and religious reasons.[10,30] This encouraged the search for alternative sources for gelatin production.[2] It is considered that gelatin extracted from aquatic sources are to be free of infections such as bovine spongiform encephalopathy (BSE) and Foot and Mouth Disease (FMD), which are associated with bovine gelatin.[8,29] They are also acceptable in Muslim communities, in which porcine based food products are prohibited,[2] and in Hindu communities which do not permit to consume cattle based products.[8,44,71]

Also, of the fish and sea food catch, only about 25–30% is eaten, and the rest is discarded or converted to fish meal leading to high economical loss and environmental issues.[93] The consumer demand for convenient foods have been increased leading to increased value addition processing of sea food before marketing.[86] A large biomass of by-products is generated during fish processing operations, and it is about 75% of the total fish weight. Out of this, 30% accounts for fish skin, bones and fins.[94] Surimi processing generates an enormous amount of fish wastes,[24] and Kittiphattanabawon et al.[22] state that 50-70% of solid fish wastes were generated during surimi processing in Thailand, in the form of heads, skin, bones and viscera. These waste materials can be a valuable source

of fish oil, proteins, enzymes, minerals, collagen and gelatin.[95] The wastes from fish processing operations, especially bones and skin by-products can be potential raw materials for gelatin extraction and the fish gelatins can be used for food and pharmaceutical applications[1] facilitating economical and eco-friendly management of industrial waste.[4] Therefore, aquatic sources are a better option for the gelatin extraction due to their high availability, reduction of pollution and minimum risk of disease transmission and free of religious restrictions.[34,37,61]

Potential applications of aquatic gelatins

Fish gelatins are available either in non-gelling form (which are used as stabilizers, film forming, clarifying and binding agents) or gelling form.[71] Due to its essential and non-essential amino acid contents, fish gelatin has been considered for dietary use.[96] Duan et al.[79] has reported that ice-cream incorporated with channel catfish skin gelatin to possess a better mouthfeel due to high emulsion stability, foaming capacity and foam stability. Also, incorporation of channel catfish skin gelatin has improved the clarification process in beer due to the flocculation effect of peptides.[79]

Porous scaffolds with higher swelling ratio, *in vitro* degradation, and the protein adsorption capacity have been prepared from gelatin extracted from the Marine Gastropod *Ficus variegate* crosslinked with chitosan, revealing their possibility to be used in biomedical applications.[89] Etxabide et al.[97] has developed lactose-crosslinked fish gelatin-based porous scaffolds as a carrier of Tetrahydrocurcumin, which have shown high water-resistant properties. Moreover, phosphate-glass fibers coated with Tilapia (*Oreochromis* spp.) scales gelatin, which were crosslinked using 0.10% and 0.15% glutaraldehyde has shown accelerated *in vitro* artificial wound healing, revealing their potential to be used in wound healing scaffolds.[98] Lower content of imino acids (proline and hydroxyproline) in fish gelatins results in a sol state without gelation at room temperature. Kwak et al.[99] have fabricated ultrafine fish gelatin nanofibrous web by electrospinning technique, using water as the solvent and, without any additional polymer or temperature control facilities. These nanofibrous scaffolds were crosslinked with glutaraldehyde for the enhancement of the water stability and mechanical properties, and have shown good cell adhesion and proliferation rates, as well as good bio-compatibility compared to mammalian gelatin, suggesting a potential environmental friendly and simple alternative to mammalian-gelatin derived electrospinning process.[99] Also, porous materials prepared by the addition of chitin and tetrahydocurcumin to fish gelatin have shown potential bioactive delivery properties.[100] Kang et al.[101] have formed fish gelatin methacryloyl nano gels as a nano carrier without aggregation and cytotoxicity to deliver small-molecule drugs, suggesting their potential application as a drug delivery system in treatments for variety of disorders. Oral administration of Chum Salmon (*Oncorhynchus keta*) skin gelatin have resulted in accelerated wound healing in hyperglycemic diabetic rats, revealing its potential as an effective treatment method for diabetic associated wound disorders.[102] Also, fish gelatin microgels have been successfully used as injectable cell carriers for enhanced skin wound healing in 8-week old nude mice.[103]

Several studies have focused on production of biodegradable packaging materials using gelatin extracted from fish skin and jellyfish.[16,38–41,104,105] This can be a sustainable solution for the serious environmental issues caused by non-biodegradable plastic

packaging materials.[40,106] Due to the higher amounts of hydrophobic amino acids, films obtained from cold water fish gelatins have exhibited lower water vapor permeability compared to the films based on warm water fish and mammalian gelatins.[107] However, gelatin-based films have drawbacks due to their hydrophilic nature, high brittleness and moisture sensitivity,[108] and low antioxidant activity limit the use of pure gelatin films.[109] Hence, recent studies have focused on improving the structure and mechanical properties of gelatin films through different approaches such as blending with biopolymers, manufacture of multilayers and chemical cross-linking which involve the formation of stable covalent bonds between the protein segments.[110] However chemical cross-linking agents are disadvantageous due to their high cost and cytotoxic properties. Therefore, a rising interest on the application of natural antioxidant agents as alternatives to synthetic cross-linking agents can be observed.[111] Fish gelatin films cross-linked using citric acid has shown enhanced mechanical and light barrier properties.[110] Benbattaieb et al.[111] has discovered the synergistic effect of the incorporation of natural antioxidant agents such as ferulic acid, quercetin and tyrosol and electron beam irradiation on the structural, mechanical, thermal and barrier properties of chitosan-fish gelatin edible films. The study revealed a notable enhancement in oxygen barrier properties and tensile strength of irradiated and antioxidant containing films. This could be resulted from the conversion of phenolic compounds to quinones under irradiation and in the presence of oxygen, which could act as a protein-crosslinker by reacting with pendent nucleophilic amino group of gelatin.[111] Santos et al.[112] have discovered that the chemical cross-linking of gelatin, especially with nonelectrolytes such as gallic acid can improve the mechanical properties of fish gelatin films. Etxabide et al.[108,113] have produced cod fish gelatin films with improved film solubility and UV barrier properties by glycation with lactose at high temperatures. Moreover, increased tensile strength, low water vapor permeability and excellent UV barrier properties were reported in fish gelatin films incorporated with chitosan nanoparticles.[114] Preparation of multi-layer films based on poly(Lactic acid) and fish gelatin has resulted in eight-fold reduction in water vapor permeability, while increasing the water resistance and tensile strength compared to the bare gelatin film.[115] Furthermore, active packaging of fish gelatins with antioxidant and antimicrobial properties have been developed by the incorporation of natural plant extracts such as *Moringa oleifera* Lam. leaf extract,[41] *Morinda citrifolia* Oil,[116] aqueous extracts from blue-berried honeysuckle and chokeberry pomace.[117]

As discovered by Abdelhedi et al.[118] smooth hound fillets coated with black-barred halfbeak (*Hemiramphus far*) skin gelatin enriched with its hydrolysate have exhibited lower water loss and color degradation, slower lipid and protein oxidation, microbial growth, and nucleotides and proteins degradation compared to uncoated gelatin. Moreover, vacuum impregnated fish gelatin and grape seed extract was capable of preserving the quality and freshness of tilapia fillets by reducing the metabolic changes.[119] As revealed by Damodaran et al.[120] peptides (1000–2000 Da) resulted from the alcalase hydrolysis of fish gelatin were capable of inhibiting the ice crystal growth in ice cream mix and 23% sucrose solution.

Functional properties of aquatic gelatins

The most important properties of gelatins for food applications are gel strength, viscosity and the melting point.[37] Yet, other parameters such as color, transparency, flavor and easy dissolution also have a greater influence on the quality of gelatin.[45]

Gelatin structure is composed of 20 amino acids and, the content of these discriminate the different types of gelatin sources. The amino acid composition of gelatin derived from different sources is listed in Table 2. Table 2 demonstrates that bovine gelatin possesses higher imino acid content compared to the gelatin extracted from aquatic sources. Generally, gelatin contains higher amount of glycine (about 30%), proline and hydroxyproline, which originates from the repeated Gly-X-Y sequence. The thermal and rheological properties such as the gel strength and gelling and melting temperatures depend on the physicochemical characteristics, which are determined by the source of gelatin[6] and the method of production.[3] The quality of gelatin depends on the molecular weight and the length of collagen chains.[38] Better functional properties including gel strength, foaming and emulsifying properties have been reported in gelatin with higher α-chain content.[12] Comparison of functional properties of aquatic gelatins with mammalian gelatin is shown in the Table 3.

Gel strength

Gel strength can be considered as one of the most important functional properties of gelatin, which is determined by the amino acid composition and ratio of α chain and β component.[74,76] Gelatin is characterized, based on the gel strength as low (<150 g), medium (150–220 g) and high gel strength (220–300 g) gelatins.[11] Physical cross linking which form junction zones resulting in a three dimensional branched network leads to the gelation of gelatin.[7] The source of raw materials also have an impact on gelling properties of gelatins due to the varying content of proline and hydroxyproline.[39] The thermostability and the gel strength of gelatins largely depend on the amino acid composition and molecular weight distribution, which differ according to species and the processing conditions respectively.[12] Also, Sinthusamran et al.[50] has revealed that the gel strength of seabass skin gelatin increased as the size of the fish increased, which can be attributed to the increased number of cross-links with the age of the animal and higher amount of high molecular weight components. The structural stability of gelatin mainly depends on the hydroxyproline content.[5] Higher imino acid content and less amounts of high molecular weight aggregates favors a more stable gel structure.[12] The gel strength of fish gelatins are found to be less, compared to the gelatins from mammalian origin,[13,76,121] which can be attributed to their low hydroxyproline content. Arnesen and Gildberg[52] have stated that the formation of hydrogen bonds between the free hydroxyl groups of gelatin and water molecules are crucial for the gel strength and low imino acid content in fish gelatin results in less organized triple helical structure, consequently resulting in a lower gel strength.[76] However, as shown in the Table 3, the gel strenghts of gelatin extracted from Tilapia skin[51] and *Catla catla* swim bladder[57] were comparable with mammalian gelatin. The gel strength also depends on the isoelectric point and can be controlled by adjusting the pH. Usually, the gelatin polymers are positively charged and they repel each other. The gels with higher stability can be resulted in pH near its isoelectric point as the gelatin

Table 2. Comparison of amino acid composition of gelatin from different sources.

	Amino acid content (Number of residues/1000 residues)															
Amino acid	Halal bovine gelatin [76]	Sole skin gelatin [12]	Megrim skin gelatin [12]	Cod skin gelatin [12]	Hake skin gelatin [12]	Squid skin gelatin [12]	*P. tayenus* skin gelatin [20]	*P. macracanthus* skin gelatin [20]	Giant catfish skin gelatin [87]	Brown banded bamboo shark skin gelatin [22]	Blacktip shark skin gelatin [22]	Cuttlefish skin gelatin [76]	Tuna skin gelatin [93]	Halibut skin gelatin [93]	Jellyfish *Rhopilema hispidum* gelatin [66]	Carp skin gelatin [32]
Hydroxyproline	96	61	60	50	59	80	87.75	90.86	87	95	91	84	78	67	139.3	N/M
Aspartic acid	63	48	48	52	49	65	N/M	N/M	15	40	40	63	44	49	54.6	15.74
Threonine	23	20	20	25	22	24	25.03	26.08	24	22	20	23	21	22	19.9	33.82
Serine	29	44	41	64	49	37	29.80	31.85	36	41	29	49	48	65	25.3	21.55
Glutamic acid	74	72	72	78	74	90	92.94	95.34	62	76	76	92	71	71	60.9	32.35
Proline	123	113	115	106	114	95	98.54	96.56	124	113	110	96	107	94	81.5	N/M
Glycine	341	352	350	344	331	327	246.57	259.38	359	322	321	321	336	365	189.0	351.70
Alanine	115	122	123	96	119	89	49.04	108.31	106	106	120	81	119	108	68.8	147.16
Valine	21	17	18	18	19	21	17.82	19.06	22	24	25	22	28	15	19.4	32.92
Methionine	5	10	13	17	15	13	20.04	20.55	10	12	15	6	16	12	N/M	24.20
Isoleucine	11	8	8	11	9	18	10.30	7.16	13	17	18	22	7	8	10.7	16.88
Leucine	25	21	21	22	23	32	22.32	23.60	23	22	23	29	21	22	22.2	30.05
Tyrosine	1	3	3	3	4	6	6.07	6.11	3	2	2	5	3	3	4.5	2.56
Phenylalanine	12	14	14	16	15	10	20.01	20.62	13	13	13	10	13	13	19.3	20.15
Histidine	5	8	8	8	10	8	4.46	4.36	4	7	7	18	7	5	6.2	10.33
Hydroxylysine	7	5	5	6	5	15	7.37	652	5	6	5	15	6	6	N/M	N/M
Lysine	26	27	27	29	28	13	35.02	36.40	32	27	28	13	25	25	24.9	42.16
Arginine	48	55	54	56	54	57	87.36	91.76	63	51	54	51	52	50	56.2	73.57
Cysteine	0	N/M	N/M	N/M	N/M	N/M	N/M	N/M	N/M	1	1	0	N/M	–	–	N/M
Imino acid (Pro +Hyp)	219	174	175	156	173	175	186.26	187.42	211	208	201	180	185	161	220.8	144.86

N/M – Not mentioned

Table 3. Comparison of functional properties of gelatins extracted from different sources.

Gelatin source	Reference	Gel strength (g)	Viscosity (MP)	Gelling point (° C)	Melting point (° C)
Bovine hide	[10]	216	–	23.8	25.6
Porcine skin	[10]	295	–	33.8	36.5
Thilapia skin	[51]	263	51.0 (at 60°C)	–	–
Cod skin	[59]	180–110	75 (at 40°C)	–	–
Megrim skin	[69]	16.1–386.7	–	9.4–12.5	15.7–20
Yellowfin tuna skin	[10]	426	–	18.7	24.3
Skate skin	[72]	43.84–76.48	187.1–222.5	16.12	19.3
Sin croaker	[60]	124.94	–	7.1	17.7
Shortfin Scad	[60]	176.92	–	9.9	23.8
Rainbow trout (*Onchorhynchus mykiss*) skin	[74]	459	12.6–35.3	–	23
Skipjack tuna (*Katsuwonus pelamis*) skin	[3]	177 ± 3.51	43.7	18.7	24.2
Dog shark (*Scoliodon sorrakowah*) skin	[3]	206 ± 3.51	56.0	20.8	25.8
Rohu (*Labeo rohita*) skin	[3]	124 ± 3.61	25.0	13.8	18.2
Catla catla swim bladder	[57]	264.6	–	13.7	23.3
Jellyfish (*Rhopilema hispidum*)	[66]	–	–	18.0	22.3
Tiger puffer (*Takifugu rubripes*)	[82]	144.5–79.8	–	9.13–19.34	15.26–22.42

polymers are nearly neutrally charged at pH closer to their isoelectric point and can form more compact and stronger gels.[59]

Tabarestani et al.[74] has found that the gel strength to depend on pretreatment conditions and recorded a positive covariance among the gel strength, alkali and acid concentrations. The study found that gelatin produced with treatment in high acid and alkali concentrations to possess higher gel strength due to the presence of high molecular weight fragments. Gómez-Guillén and Montero[69] compared the effectiveness of several organic acids (formic, acetic, propionic, lactic, malic, tartaric and citric acid) and pretreatment with dilute sodium hydroxide for collagen extraction from skins of Megrim (*Lepidorhombus boscii*) by evaluating viscoelastic and gelling properties of the resultant gelatins. The study revealed that the functional effectiveness of the extraction depends on the swelling capacity of collagen, pH of extraction, and ionic strength, which vary with the type of acid used. The highest swelling capacity and the pH of extraction, which are essential for the highest viscoelastic and gelling properties has been observed in gelatins treated with acetic and propionic acids when pretreated with sodium hydroxide. Although a low pH can favor a higher extraction rate, it can be unfavorable for development of gel network.[69] Ahmad and Benjakul[1] has found that gelatin extracted from unicorn leatherjacket skin to possess a higher gel strength when pretreated with phosphoric acid compared to acetic acid. Improved gel strength of golden carp skin gelatin extracted with prior ultrasonication coupled with acid pretreatment has been reported by Ali et al.[81]

Although, the yield increased with the extraction time and temperature, higher extraction temperatures and times may result in low bloom strength gelatin gels due to the degradation of fish gelatins.[23,30] Several studies have reported lower gel strengths of gelatins of Nile perch,[71] Yellow fin tuna,[10,122] brownbanded bamboo shark and blacktip shark,[22] bigeye snapper[30] and blue shark (*Prionace glauca*) skins[122] and jellyfish (*Lobonema smithii*)[8] extracted at higher temperatures with prolonged extraction times. Harsh extracting conditions results in gelatins with poor gel strengths due to the

formation of low molecular weight fractions, even though the imino acid content is high.[22] Shorter chain lengths contain less inter-junction zones hindering the formation of strong gels.[91] Chancharern et al.[61] has reported gel formation of gelatin obtained from white jellyfish (*Lobonema smithii*) when pretreated with acid and extracted at 75°C for 6 and 12 h, but no gel formation was observed when the alkali pretreatment was carried out. Cho et al.[66] and Rodsuwan et al.[8] has reported lower imino acid content in jellyfish gelatin, resulting low gel strength and gelling and melting points. Therefore, jellyfish gelatins have useful applications different from mammalian gelatins.[66] Aewsiri et al.[73] has observed the gel strength of cuttlefish ventral skin gelatin to increase with increasing H_2O_2 concentration and bleaching time. However, the gel strength of splendid squid skin gelatin has decreased with increasing concentration of hydrogen peroxide as reported by Nagarjan et al.[77]

As revealed by Gudmundsson and Hafsteinsson[59] the gel forming properties of air-dried gelatins were much lower compared to that of lyophilized gelatins due to the increased protein denaturation during the air-drying process. Sae-leaw et al.[88] and Hamzeh et al.[80] also have reported the gel strength of gelatin to depend on the drying method. The studies have revealed that the gel strength of freeze-dried gelatin to be higher compared to that of spray dried gelatin, which may be attributed to the increased thermal degradation at high temperature. Besides, the gel strength decreased with the increasing inlet temperature of spray drying. However, splendid squid skin gelatin spray dried at 200°C exhibited high gel strength than that of 180°C dried gelatin, and this may be due to the aggregation of peptides induced at high drying temperatures.[80]

Benjakul et al.[20] have reported higher bloom strength of gelatin extracted from two bigeye snapper species (*Priacanthus tayenus* and *Priacanthus macracanthus*) compared to previously studied fish species, but the values were slightly lower than that of commercial bovine gelatin. However, higher gel strength of yellowfin tuna skin and shark skin gelatin compared to bovine and porcine gelatins has been recorded by Cho, et al.[10] and Kittiphattanabawon et al.[22] while Tabarestani et al.[74] have recorded a remarkable higher gel strength of 459 g for gelatin extracted from rainbow trout skin at multiple response optimized pretreatment condition. However, gelatins with physical and chemical characteristics that are compatible with other mammalian gelatins could be extracted from harp seal skin as described by Arnesen and Gildberg.[70] Also, seabass (*Lates calcarifer*) skin gelatin have exhibited a higher gel strength compared to bovine gelatin and could be a potential replacement for mammalian gelatin.[23] Although, similar Pro and Hyp contents were recorded, the gel strength of sea bream scale gelatin was higher compared to bone gelatins[48] and this can be attributed to high protein content of former than the latter since the gel strength also depends on the protein content.[37]

Addition of sucrose and sorbitol has increased the gel strength of rainbow trout (*Oncorhynchus mykiss*) skin gelatin, which could be due to the attraction of more water by the solutes, leading to formation of more junction zones through enhanced association between the gelatin chains.[123] Norziah et al.[6] has found that the addition of transglutaminase enzyme up to a certain concentration to increase the gel strength of fish gelatins, but further addition caused a decline in gel strength. Moreover, Sinthusamran and Benjakul[124] have observed the gel strength of fish gelatin gels to decrease when incorporated with β-glucan. This can be attributed to the excessive cross linking, which may hinder the intermolecular aggregation, resulting reduced gel network formation.[39] In

addition to fragmentation, large protein aggregates also possess poor gelling ability due to the lowering of binding sites between adjacent protein molecules.[77] Santos et al.[112] have reported a higher gel strength in Tilapia gelatin crosslinked with gallic acid compared to commercial bovine gelatin, but crosslinking Tilapia and carp gelatin with sodium chloride has negatively affected the gel strength.

Viscosity

Viscosity can be considered as the second most important physical property of gelatin.[74] The viscosity of gelatin depends on the concentration, pH and molecular weight, molecular size distribution, source of collage and polydispersity.[59,87] The species also have a significant effect on the viscosity, although similar extraction conditions were applied.[3] Usually, the viscosity of most gelatin lays between 20 and 70 mpoise, but higher values up to 130 mpoise has been reported.[11]

Jongjareonrak et al.[87] has reported higher viscosity value in giant catfish skin gelatin compared to calf skin gelatin, while channel catfish skin gelatin have exhibited superior viscosity properties compared to calf bone gelatin upon heating and cooling.[79] According to Yang and Wang[15] the viscosity of channel catfish gelatin increased with the concentration and Johnston-Banks[11] has reported that relationship between viscosity of gelatin and concentration to be nearly logarithmic.

The viscosity also decrease with increasing temperature.[29] Higher viscosities of gelatin solutions at low temperature have been reported due to the formation of triple helical junction zones which develop more cross-linking.[107] Higher temperatures cause the destruction of hydrogen bonds which cause a reduction of viscosity,[47] and the gelatin molecules behave as random coils in solution.[66] As reported by Cho et al.[66] viscosity of jellyfish gelatin solution have a tendency to decrease rapidly until the temperature reached 32°C and then decreased gradually between 33°C and 50°C.

Tabarestani et al.[74] have recorded the viscosity of 6.67% rainbow trout skin gelatin samples to range between 1.26 and 3.53 mPa s, depending on the pretreatment condition, and the study also have found a synergetic effect between the acid and alkali concentrations on the viscosity. At pH near the isoionic point, gelatin molecules become neutral, suppressing the repulsion forces, which results in high viscosity.[59] Gudmundsson and Hafsteinsson[59] have reported higher viscosity (75 mpoise) for 6.67% solution of gelatin extracted from cod skin, which was higher compared to mammalian gelatin measured at the same concentration. According to Santos et al.[112] crosslinking of carp and Tilapia gelatin with nonelectrolytes such as gallic acid can increase the gelatin viscosity, while phosphorylation using sodium trimetaphosphate has decreased the apparent viscosity of bighead carp scales gelatin, correspondingly to the phosphorylation time.[125]

Gelling and melting temperatures

The gelling point is the temperature at which gelling or setting begins, which involves the transition from random coils to triple helical structure of gelatin.[33] As shown in Table 3, several studies have reported lower melting and gelling temperatures in fish gelatins compared to that of conventional gelatins.[1,10,69] Cold water fish species possess collagen with lower imino acid content than do warm water fish species,[70] and the

Table 4. Surface active properties of gelatin from different sources.

Gelatin source	Reference	Emulsion Activity Index (EAI) (m^2/g)	Emulsion stability index (SI) (min)	Foam Expansion (%)	Foam stability (%)	Water binding capacity (%)	Fat binding capacity (%)
Bovine hide	[13]	19.0–39.0	30.0–41.0	110.0–138.0	70.0–102.0	280	310
Tuna *(Thunnus thynnus)* head bones	[13]	12.0–32.0	31.0–43.0	64.0–80.0	37.0–54.0	250	350
Unicorn leatherjacket (*Aluterus monoceros*) skin	[1]	13.49–39.17	8.58–39.63	110.34–152.63	98.86–147.35	–	–
Cuttlefish (Sepia officinalis) skin	[76]	17.22–41.87	28.33–51.27	103.44–134.52	70.42–105.83	–	–
Skipjack tuna (*Katsuwonus pelamis*) skin	[3]	–	–	19.2 ± 0.36	14.4 ± 0.25	214 ± 3.61	452 ± 6.51
Dog shark (*Scoliodon sorrakowah*) skin	[3]	–	–	21.5 ± 0.45	17.6 ± 0.35	256 ± 4.51	341 ± 5.51
Rohu (*Labeo roh ita*) skin	[3]	–	–	17.4 ± 0.15	10.5	163 ± 4.00	360 ± 29.19
Unicorn leatherjacket (*Aluterus monoceros*) skin	[83]	21.62–22.40	29.87–30.16	120.37–127.59	111.48–114.41	250.17–273.35	527.37–545.62
Reef cod (*Epinephelus diacanthus*) skin	[83]	24.95–26.94	26.04–27.57	110.80–113.97	102.76–103.21	270.68–288.82	550.72–563.40
Alaska Pollock (*Theragra chalcogramma*) bones	[84]	21.36–33.61	30.69–42.75	105.37–129.36	88.26–120.51	–	–
Yellowfin Sole *(Limanda aspera)* bones	[84]	23.82–39.34	28.75–40.61	114.58–135.62	97.58–130.24	–	–

gelatin prepared from such collagens form lower number of hydrogen bonds in solutions resulting in gels with lower melting points.[4,29,37,59] No significant difference of melting points between mammalian/warm water fish blends and mammalian gelatin has been observed by Gilsenan and Ross-Murphy,[7] but the melting point was lower in cold-water fish gelatin blends. Also, the gelling and melting temperatures increase with the increasing size of the animal, which can be attributed to the increased amount of cross links.[50]

Furthermore, Muyonga[71] has identified the imino acid content as a key factor of the denaturation temperature of collagen. Choi and Regenstein[37] have recorded that the gel strength and the melting point of fish gelatins to depend on the gel concentration, maturation time and temperature, pH, and the concentrations of NaCl and sucrose, similar to those of porcine gelatin. Changes in ionic strengths also have an influence on gelling and melting temperatures,[3] where the higher ionic strengths has caused a decrease in gelling and melting temperatures as a result of reduced electrostatic interactions, which prevent ionic inter-chain bridging and gelation of fish gelatin.[33] High extraction temperatures and prolonged extraction time can reduce the gelling and melting temperatures of gelatin, which can be attributed to the formation of low molecular weight fragments and changes in the structure and functional groups of gelatin at harsh extraction conditions.[82,122] Tabarestani et al.[74] have found a direct relationship between the melting point and acid and alkali concentrations of the pretreatment due to formation of high molecular weight fragments. The melting point also increases with maturation time.[3] As revealed by Ali et al.,[81] gelatin extracted from golden carp skin with prior ultrasonication coupled with acid pretreatment have exhibited high gelling and melting temperatures compared to gelatin extracted by conventional method.

The addition of transglutaminase also increased the melting temperature due to the polymerization induced by the enzyme.[6] Inverse relationship between the phosphorylation time and gelling and melting points of fish gelatin has been discovered by Huang et al.[125] Short-term phosphorylation with sodium trimetaphosphate have improved the gelling and melting point of bighead carp scales gelatin, but phosphorylation beyond 30 min have decreased the gelation rate constant.[125]

Lower melting temperatures of fish gelatins may facilitate better releases of flavor and aroma in jelly-based food products, extending the applications of gelatin in food industry.[37] Also, low temperature characteristics of fish gelatins make it suitable for diverse coating applications,[70] refrigerated and low gelling temperature required products.[3]

Emulsifying properties

Emulsifying and foaming ability of gelatin is mainly related to its surface active properties.[76] The stability of emulsions are influenced by number of factors such as, interfacial tension between the two phases, characteristics of the absorbed film in the interface, magnitude of the electrical charge on the globules, size and surface per volume ratio of the globules, weight per volume ratio of dispersed and dispersion phases and viscosity of the dispersion phase.[8] The emulsion stability also depends on the electrostatic repulsion forces between the adsorbed proteins on the interfacial protein films,[1] and the

emulsifying efficiency increases with the increasing solubility of proteins due to the ability for rapid migration to the lipid droplet surface.

Generally, Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI) of fish gelatins are lower compared to bovine and porcine gelatin[73] and this can be due to the differences of inherent properties, compositions and conformation of gelatin from the different sources.[20] As shown in Table 4, Balti et al.[76] have observed lower emulsion activity index in cuttlefish skin gelatin than halal bovine gelatin. However, high stability of fish gelatin emulsions compared to that of bovine gelatin emulsions has been reported in Tuna head bone gelatin[13] and cuttlefish skin gelatin.[76] Rodsuwan et al.[8] have also observed a higher ESI in jellyfish gelatin extracted at 75°C for 6 h, than bovine gelatin. Higher stability of high molecular weight fish gelatin emulsions compared to that of low molecular weight fish gelatin emulsions have been reported by Surh, et al.[126] The thickness of an adsorbed gelatin membrane increased with increasing molecular weight, which makes it more stable against coalescence during homogenization. The emulsion stability of oil-in-water emulsions prepared with high average molecular weight fish gelatin (~120 kDa) was higher than the emulsions prepared with low average molecular weight fish gelatin (~50 kDa).[126] Duan et al.[79] has reported high emulsion stability index in channel catfish skin gelatin compared to calf bone gelatin. This can be attributed to higher content of high molecular weight α and γ chains that can cause more hydrophobic interactions with oil.

Ahmad and Benjakul[1] have observed that EAI and ESI of gelatin extracted from unicorn leatherjacket, pretreated with phosphoric acid to be higher compared to gelatin pretreated with acetic acid. The EAI decrease with increasing extraction time due to the increased degradation of proteins which result in shorter peptide chains which are more hydrophilic and tend to localize in the aqueous phase.[1] Also, the study found that the pretreatment to have an effect on ESI, in which phosphoric acid treated gelatin showed a higher ESI than acetic acid treated gelatin, and in both cases, with the increasing concentration, the ESI also increased. Studies have reported that the EAI of cuttlefish skin gelatin,[76] Tuna head bone gelatin,[13] Alaska Pollock and Yellowfin Sole bone gelatin[84] to increase with increasing concentrations, since proteins at high concentrations may facilitate more protein adsorption at interface. In contrast, a decrease in ESI with the increasing concentration of Alaska Pollock and Yellowfin Sole bone gelatin was reported by Mi et al.[84] Aewsiri et al.[73] have reported decreased emulsion activity index with extended bleaching time with hydrogen peroxide due to increased aggregation of proteins. The extraction time and temperature also have an effect on the emulsifying properties of gelatin.[82] Furthermore, Renuka et al.[83] have reported that the emulsifying properties of oven dried unicorn leatherjacket and reef cod gelatins were not affect by the drying temperature.

Phosphorylation of bighead carp scales gelatin with sodium trimetaphosphate has improved the emulsifying properties proportionally to the phosphorylation time due to increased surface hydrophobicity and electrostatic repulsion between protein and oil droplets that prevent them from coming together.[125]

Foaming properties

Foaming ability of gelatin is of great importance in food products such as marshmallows. Transportation, penetration and reorganization of the protein molecules in air–water interface are the main factors that control the foam formation of gelatin.[3] The stability of foams depends on several parameters such as the rate of attaining equilibrium surface tension, bulk and surface viscosities, steric stabilization and electrical repulsion between the two sides of the foam lamella.[127] Better foaming ability is presented by gelatins with smaller molecular weight peptides due to their ability for rapid migration to the air-liquid interface and unfold and rearrange at the interface.[78] The foaming characteristics of gelatin is positively correlated with the hydrophobicity of the unfolded proteins.[32] Ahmad and Benjakul[1] and Mi et al.[84] have reported that the foam expansion and foam stability of fish gelatin to increase with increasing concentration. Higher concentrations of proteins produce more dense and stable foams due to the increased thickness of interfacial films.[128] Tkaczewska et al.[32] have reported the foaming ability of carp skin gelatin to vary with the pretreatment. However, the foaming properties of unicorn leatherjacket and reef cod skin gelatin were not negatively affected by different oven drying temperatures.[83]

As shown in Table 4, the foaming properties of skipjack tuna, dog shark and rohu skin gelatins[3] and tuna head bone gelatin[13] were lower compared to bovine gelatin. Also, foaming properties of cuttlefish skin gelatin[76] and jellyfish gelatin[8] has found to be poorer compared to bovine gelatin, while gelatin extracted from shark cartilages have exhibited lower foam formation ability and foam stability compared to porcine skin gelatin.[5] This can be attributed to the interferences caused by aggregation of proteins on interactions between the protein and water needed for foam formation.[129] However, gelatin extracted from the skins of unicorn leatherjacket[1,83], cuttlefish[76] and reef cod[83], as well as Alska pollock and yellowfin sole bones[84] have exhibited foaming properties that were comparable to bovine gelatin (Table 4). Duan et al.[79] have reported better foaming capacity and foam stability in channel catfish skin gelatin compared to calf bone gelatin. Higher foam capacity and foam stability of giant catfish skin gelatin compared to calf skin gelatin was reported by Jongjareonrak et al.[87] and this can be attributed to the higher amount of hydrophobic amino acids in the former compared to the latter. Bleaching of cuttlefish skin gelatin with hydrogen peroxide have improved the foaming properties through oxidation of the gelatin molecule.[73]

Water and fat binding properties

Water binding and fat binding properties are important functional properties that determine the texture of a product through the interactions between water, oil and other components.[5] The water-holding capacity of gelatin imparts its ability to reduce the drip loss of frozen fish and meat products during thawing.[32] According to Zayas,[128] the water-holding capacity of proteins depend on several factors including the amino acids composition of the protein, number of polar groups within the particle, availability of hydrophilic spots, pH of the environment, ionic strength, temperature and protein concentration. Fat-binding capacity depends on the degree of exposure of the hydrophobic residues inside gelatin and amount of hydrophobic amino acids, while the amount of

hydrophilic amino acids such as hydroxyproline and the particle size which affect the surface tension of water, determine the water-binding ability. As shown in Table 4, most of the fish gelatins have shown lower water biding capacity, but higher fat-binding capacity than bovine gelatin.[3,13,83] Cho et al.[5] has also observed a higher fat biding capacity, but lower water binding capacity in gelatin extracted from shark cartilage, compared to commercial porcine gelatin. These observations could be related to the higher amount of tyrosine, which is a hydrophobic amino acid, and low hydroxyproline content of shark cartilage gelatins. Tkaczewska et al.[32] reported a higher water binding capacity, but lower fat binding capacity of carp skin gelatin and this may be due to the presence of a lower proportion of hydrophobic as compared to hydrophilic groups on the protein surfaces in different treatment methods. According to Razali et al.[42] the low molecular weight fraction of cobia skin gelatin hydrolysates exhibited high water binding capacity, but the fat-binding capacity decreased with the lower molecular weight.

The surface active properties of gelatin derived from different sources are given in Table 4.

Bioactive properties of aquatic gelatins

Functional foods possess potential health promoting benefits and ability to reduce the risks of chronic diseases.[130] Bioactive peptides are food-derived ingredients that perform a physiological function in the body, in addition to their functional value.[131] The bioactive peptides of dietary proteins are usually inactive in its native protein sequence, but can be liberated through gastrointestinal digestion, fermentation or processing, and affect various physiological functions of the organisms.[4,132] Many studies have been focused on investigation of bioactive properties of peptides derived from enzymatic hydrolysis of collagen and gelatin,[4] and in recent years, a great interest on fish collagen and gelatin as a source of biologically active peptides with possible nutritional and pharmaceutical applications can be observed.[48] Fish gelatin hydrolysates and peptides have displayed a broad spectrum of bioactivities including antioxidant, anti-anemia, immunoregulatory, Ca binding, mineral chelating, antimicrobial and anti-hypertensive activities.[133] The biological properties of gelatin peptides may depend on the gelatin source and their molecular weight and conformational structure, which are greatly affected by processing conditions.[134,135] Moreover, the amino acid composition and sequence has a major effect on the bioactive properties of peptides.[136,137] Usually, bioactive peptides contain 3–20 amino acid residues, and the low molecular weight promotes the bioactive properties.[138] Gelatin hydrolysates are generally obtained by enzymatic hydrolysis using commercial proteases such as alcalase, collagenase, pepsin and trypsin.[134] Since it can modify the protein functionality,[93] depending on the enzymes used, considerable differences in the degree of protein breakdown have been revealed leading to different molecular weight profiles.[94] Besides, enzymatic extracts from different sources such as ginger protease[135] and smooth hound crude acid protease[76] have been used for the preparation of bioactive hydrolysates of aquatic gelatins.

Most of the studies on bioactive properties of gelatin hydrolysates have focused on their antioxidant and antihypertensive activities.[4] These properties are seemed to be associated with the unique amino acid composition, which is the repeated sequence of Gly-Pro-Hyp.

Antioxidant properties

A number of human chronic diseases are a result of oxidative damage, which is caused by the oxidative stress due to the excessive production of reactive oxygen species (ROS) through metabolism. Also, iron has the ability to generate hydroxyl radicals by the reaction with peroxides, which can initiate the lipid peroxidation within the human body,[139] and oxidation of lipids also leads the deterioration of food,[140] leading to poor quality, shortening of shelf life along with production of potentially harmful reaction products.[141] The mechanism of antioxidant activity of peptides can be as free radical scavengers, lipid peroxidation inhibitors or transition metal ion chelators.[4] Hence, scavenging free radicals for the inhibition of lipid peroxidation in human body and foodstuffs is of great importance.[135] Although, the synthetic antioxidants possess strong antioxidant activities, their utilization is restricted owing to their potential health hazards,[28,93,142] and there is a rising interest on derivation of bioactive peptides with antioxidant properties by enzymatic hydrolysis of various proteins, to replace synthetic antioxidants in food and pharmaceutical industries.[93] Gelatin peptides are rich in hydrophobic amino acids, which have high affinity to oils and have better emulsifying properties, thereby can possess antioxidant properties.[140]

A great interest on extraction of antioxidative peptides from aquatic gelatin hydrolysates has been raised as a solution for increasing waste generation from fish sources.[4] Several studies have focused on extraction of antioxidative peptides from aquatic gelatin hydrolysates including jumbo squid skin,[140] inner and outer tunics of jumbo flying squid (*Dosidicus gigas*), skins of Tuna (*Thunnus* spp.) and Halibut (*Hypoglossus* spp.),[93] blue shark (*Prionace glauca*) skin,[143] Nile Tilapia skin[139] and Jellyfish (*Rhopilema esculentum*).[144]

Generally gelatin hydrolysates exhibit higher antioxidant activities compared to non-hydrolyzed gelatin.[109,135,145] It has been recorded, that there is an approximately two-fold ferrous reducing capacity in squid, Halibut and Tuna gelatin hydrolysates, compared to the corresponding gelatins by Alemán et al.[93] and in squid skin gelatin hydrolysates by Giménez et al.[146] Both Giménez et al.[146] and Alemán et al.[134] have reported a 10-fold higher vitamin C equivalent antioxidant capacity in fish gelatin hydrolysates compared to the native form. This increase of antioxidant activities of gelatin hydrolysates may be associated with either the exposition of previously hidden amino acid residues or side chains which possess antioxidant properties or release of sequences of peptides with antioxidant activities.[146]

The antioxidant activities of Nile Tilapia skin gelatin hydrolysates catalyzed by different enzymes were observed to be different by Choonpicharn et al.[139] which can be attributed to the variations in amino acid sequences. The study found the highest ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonicacid) radical scavenging and lipid peroxidation inhibition abilities in flavourzyme and trypsin hydrolysates, while greater FRAP (Ferric Reducing Antioxidant Power) activity and ferrous ion chelating activity were found in alcalase and bromelain hydrolysates, respectively. Alemán et al.[93] have reported that the antioxidant activity of fish gelatin hydrolysates measured as FRAP and ABTS radical assay to vary with the fish species, type of enzymes used and the amino acid composition. The peptides that are rich in hydrophobic amino acids may inhibit lipid peroxidation either as proton donors to hydrophobic peroxyl radicals or as metal ion chelators.

Apart from the enzymes, the antioxidant properties of gelatin hydrolysates vary with the hydrolysis conditions such as pH, temperature and hydrolysis time duration.[147] The variation of antioxidant activity of seabass (*Lates calcarifer*) skin gelatin hydrolysates depending on the production process has been investigated by Sae-leaw et al.[148] The study revealed that the alcalase hydrolysates produced during gelatin extraction to exhibit higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, FRAP activity and metal chelating activity compared to the hydrolysates prepared after gelatin extraction. Moreover, the antioxidant activity of the hydrolysates decreased when subjected to gastric phase digestion, which can be attributed to the disruption of the structures of antioxidative peptides by pepsin or acidic pH. However, following the intestinal digestion, the antioxidant activities increased, which may be due to the further cleavage of peptides by pancreatin, leading to the release of new antioxidant peptides.[148] A similar pattern of variation of ABTS radical scavenging activity in fish gelatin gels incorporated with β-glucan has been observed by Sinthusamran and Benjakul.[124] However a gradual decrease in FRAP activity was observed. Nevertheless, the antioxidant activity of fish gelatin was enhanced proportionally to the concentration of β-glucan.[124] However, *in vitro* gastrointestinal digestion of giant catfish skin gelatin hydrolysates have led to a significant higher radical scavenging, reducing ferric ions and chelating activities.[145] Moreover, Yang et al.[149] have purified five peptides with the amino acid sequences Gly-Pro-Asp-Gly-Arg (500.43 Da), Gly-Ala-Asp-Ile-Val-Arg (544.55 Da), Gly-Ala-Pro-Gly-Pro-Glu-Met-Val (756.84 Da), Ala-Gly-Pro-Lys (374.39 Da) and Gly-Ala-Glu-Gly-Phe-Ile-Phe (739.76 Da), by stimulated *in vitro* gastrointestinal digestion of Skipjack Tuna (*Katsuwonus pelamis*) bone gelatin. Of these, Gly-Ala-Asp-Ile-Val-Arg and Gly-Ala-Glu-Gly-Phe-Ile-Phe have exhibited the strongest DPPH, hydroxyl, superoxide anion and ABTS radical scavenging activities and lipid peroxidation inhibition capacity. Also, Zhang et al.[28] have reported, progressive hydrolysis of Tilapia skin gelatin using multifect neutral and properase E to produce hydrolysates with high radical scavenging activity. The study further purified two peptides with higher hydroxyl radical scavenging activity, which possess the amino acid sequence of Glu-Gly-Leu (317.33 Da) and Tyr-Gly-Asp-Glu-Tyr (645.21 Da). Also, Alemán et al.[94] have found higher antioxidant capacities in fish gelatin hydrolysates obtained by the hydrolysis with alcalase and collagenase under high pressure. The study also suggests that the enzymatic hydrolysis in combination with pressurization as a useful method to promote rapid release of antioxidant products. A noticeable free radical scavenging ability and inhibition of lipid peroxidation of thermally hydrolyzed Tilapia skin gelatin have been investigated by Yang et al.[150] The study further found that the phosphoric acid concentration, extraction water to skin ratio and the retorting time to have a significant effect on the antioxidant activities.

The antioxidant activity of gelatin hydrolysates is determined by the molecular structure and average molecular weight of the peptide fractions, which in turn are greatly affected by the processing conditions.[4,93] Generally, low molecular weight gelatin hydrolysates possess higher antioxidant activities.[28] Moreover, it has been found a positive relationship between the degree of hydrolysis and the antioxidant activities of the gelatin peptides.[28] However, the findings of Ketnawa et al.[145] demonstrated that the radical scavenging activity of giant catfish skin gelatin hydrolysates and the degree of hydrolysis were not directly correlated, since the highest radical scavenging activity was reported in Izyme AL®, despite its low degree of hydrolysis. Nevertheless, the radical scavenging

activities of individual enzyme hydrolysates increased with the progress of hydrolysis, as the degree of hydrolysis increased. Hence, the study revealed that the specific amino acid composition and sequence, structure and hydrophobicity of the peptides in hydrolysates have a profound effect on the antioxidant activities rather than the degree of hydrolysis.[145]

The gelatin hydrolysates of Tilapia skin, with molecular weight below 2000 Da have shown high hydroxyl, superoxide, hydrogen peroxide, peroxynitrite and nitric oxide radical scavenging activity.[151] Weng et al.[143] have also isolated a low molecular weight fraction of 2000–150 Da with higher antioxidant activity similar to glutathione, and this was mainly composed of four peptides (Glu-Gly-Pro, Gly-Pro-Arg, Gly-Tyr and Gly-Phe) and four amino acids (Leu, Arg, Tyr and Phe). Of these, the dipeptide Gly-Tyr and the amino acid Tr has exhibited strong DPPH and hydroxyl radical scavenging activities. Zhuang et al.[144] have found the progressive hydrolysis of jellyfish (*Rhopilema esculentum*) gelatin with trypsin and Properase E to exhibit high antioxidant activity. The fraction of molecular weight of 2–6 kDa has shown high hydroxyl radical and hydrogen peroxide scavenging activities and metal chelating ability, while the fraction of <2 kDa had strong reducing power, inhibition of oxidation of linoleic acid in its emulsion form and superoxide anion radical scavenging activity. Mendis et al.[140] have recorded the lipid peroxidation inhibition activity of Jumbo squid skin gelatin to exceed the activity of α-tocopherol, and it was very closer to the activity of highly active synthetic antioxidant butylated hydroxytoluene (BHT). This can be related to the abundance of hydrophobic amino acids which exert high affinity oils. The study also purified two peptides, Phe-Asp-Ser-Gly-Pro-Ala-Gly-Val-Leu (880.18 Da) and Asn-Gly-Pro-Leu-Gln-Ala-Gly-Glu-Arg (1241.59 Da), with high antioxidant potential, which could enhance the viability of radical mediated oxidation-induced human lung fibroblasts. However, the hydrolysates exhibited less metal ion chelating ability. Hence, free radical scavenging can be considered as the main antioxidative mechanism in jumbo squid skin gelatin hydrolysates.[140] A superior free radical scavenging ability in alcalase-derived Nile Tilapia scale gelatin hydrolysates has been recorded by Ngo et al.[152] and the antioxidant activity showed the dependence on the type of the enzyme and the concentration of the hydrolysate. Moreover, the study also purified a peptide with the amino acid sequence of Asp-Pro-Ala-Leu-Ala-Thr-Glu-Pro-Asp-Pro-Met-Pro-Phe (1382.57 Da) which possessed the ability to retard the oxidative damage of DNA. In Ngo et al.[75] the skin gelatin hydrolysates of Pacific cod (*Gadus macrocephalus*) were obtained through the hydrolysis with alclalase, neutrase, papain, trypsin, pepsin and α-chymotrypsin. Among them, the highest antioxidant activity was revealed by the papain hydrolysate and two peptides with amino acid sequences of Thr-Cys-Ser-Pro (388 Da) and Thr-Gly-Gly-Gly-Asn-Val (485.5 Da), which exhibited high radical scavenging activity and inhibited the radical mediated oxidation in mouse macrophages (RAW 264.7 cells). A peptide with the amino acid sequence of Pro-Ala-Gly-Tyr (PAGT) with the molecular mass of 405.99 Da has been purified from the alcalase hydrolysate of Amur sturgeon skin gelatin by Nikoo et al.[153] The peptide showed the radical scavenging activity against DPPH, ABTS and hydroxyl radicals, but no metal chelating activity. It was also capable of preventing the lipid peroxidation in sea bass mince when incorporated at 25 ppm and decreased the denaturation of actin and myosin. Moreover, the PAGT peptide showed a cryoprotective effect in minced fish.[153] Qiu

et al.[154] have isolated three peptides with the amino acid sequences His-Gly-Pro-Hyp-Gly-Glu (608.57 Da), Asp-Gly-Pro-Lys-Gly-His (609.61 Da) and Met-Leu-Gly-Pro-Phe-Gly-Pro-Ser (804.92 Da), from Skipjack Tuna (*Katsuwonus pelamis*) scales gelatin hydrolysates, which have shown high DPPH, hydroxyl radical and superoxide anion radical scavenging activities. The alcalase hydrolysates (5 kDa fraction) of cobia skin gelatin has showed high ferrous ion chelating ability and superoxide anion scavenging activity than BHT, but the reducing power and DPPH radical scavenging activity were lower compared to BHT.[42] The gelatin hydrolysate extracted from salmon (*Salmo salar*) skin, bone and residual meat, generated with Corolase PP and Promod 144 MG have exhibited high oxygen radical absorbance capacity (ORAC).[133]

Other than commercial enzymes, proteases extracted from natural sources can be a promising method to prepare antioxidant peptides. The fish skin gelatin hydrolysates prepared by hydrolysis with the proteases extracted from pyloric caeca of brownstripe red snapper (L*utjanus vitta*), bigeye snapper (*Priacanthus tayenus*) and threadfin bream (*Nemipterus marginatus*) under optimum conditions were found to possess enhanced radical scavenging and reducing properties.[155] Zheng et al.[135] have reported that fish skin gelatin hydrolysates prepared using ginger protease to possess high antioxidant activities, especially regard to DPPH radical scavenging and lipid peroxidation inhibition, compared to porcine and bovine gelatin hydrolysates. Also, ginger protease fish gelatin hydrolysates were high in antioxidant activity than that of pepsin-pancreatin hydrolysates. As revealed by Aewsiri et al.[156] the antioxidative activities of cuttlefish skin gelatin modified with oxidized phenolic compounds (caffeic acid, ferulic acid and tannic acid) was higher compared to gelatin without modification and the antioxidative activity increased with concentration of the oxidized phenolic compound. Moreover, the DPPH and ABTS radical scavenging activities and reducing power of grass carp scales gelatin hydrolysates (FSGH) have been increased by Millard reaction with ribose, and ribose-fish gelatin hydrolysate Millard reaction products (ribose-FSGH MRPs) have exhibited flavor enhancement due to the formation of volatile flavor compounds.[157] Although the antioxidant capacities of ribose-FSGH MRPs were reduced when subjected to *in vitro* simulated gastrointestinal digestion, still they were higher compared to FSGH.[157]

The antioxidant activities of aquatic gelatin based edible films including Atlantic Bluefin Tuna *(Thunnus thynnus)* skin gelatin films containing brown algae (*Cystoseira barbata*) extracts,[142] fish gelatin films incorporated with pomegranate (*Punica granatum* L.) peel powder[104] and modified with Millard reactions[109] have been reported.

The antioxidant activities of different aquatic gelatin hydrolysates are depicted in Table 5.

Antihypertensive properties

Hypertension has become a common health problem in the world,[139] and affects 15–20% of adults.[141] It is considered to be a major risk factor for cardiovascular diseases[159] such as coronary heart disease, peripheral artery disease and stroke.[75] The renin-angiotensin system (RAS) plays a key role in regulating blood pressure.[132] Renin converts angiotensinogen in liver to angiotensin I, which is a decapeptide, and it is further converted to an octapeptide, angiotensin II by the Angiotensin Converting Enzyme (ACE),[160–162] which is a zinc metallocarboxydipeptidase that needs zinc and chloride for its activity.[163] ACE is

Table 5. Antioxidant activities of different aquatic gelatin hydrolysates.

Gelatin source	Method of Preparation	Antioxidant activities	References
Jumbo squid (*Dosidicus gigas*) skin	Hydrolysis with trypsin, α-chymotrypsin and pepsin	Inhibition of lipid peroxidation in linoleic acid, ABTS radical scavenging activity and enhancing cell viability against oxidation induced cell death	[140]
Brown stripe red snapper skin	Hydrolysis with the proteases extracted from pyloric caeca of brownstripe red snapper (*Lutjanus vitta*), bigeye snapper (*Priacanthus tayenus*) and threadfin bream (*Nemipterus marginatus*) under optimum conditions	Increase of DPPH and ABTS radical scavenging activities and FRAP activity were observed in gelatin hydrolysates. However, the degree of increase of reducing power was lower compared to the radical scavenging activities	[155]
Tilapia skin	Thermal hydrolysis at 121°C by retorting in an autoclave	DPPH radical scavenging activity and inhibition of lipid peroxidation	[150]
Nile tilapia scale	Hydrolysis of the gelatin with alcalase, pronase E, trypsin and pepsin	The alcalase hydrolysate showed the highest DPPH, hydroxyl and superoxide radical scavenging activity and DNA protection ability. Hydroxyl radical scavenging was more efficient than the other radical scavenging activities	[152]
Jellyfish (*Rhopilema esculentum*)	Progressive hydrolysis with trypsin and properase E	Hydroxyl radical and hydrogen peroxide scavenging activities and metal chelating ability, reducing power, inhibition of oxidation of linoleic acid emulsion and superoxide anion radical scavenging activity.	[144][]
Jumbo flying squid (*Dosidicus gigas*) and skins of tuna (*Thunnus* spp.) and halibut (*Hypoglossus* spp.)	Hydrolysis with alcalase, collagenase type 1A, trypsin type 1 from bovine pancreas and pepsin	Fe reducing activity and ABTS radical scavenging ability	[93]
Warm water fish skin (mainly Catfish)	Enzymatic hydrolysis with alcalase collagenase type 1A, trypsin type 1 from bovine pancreas and pepsin from porcine stomach combined with high pressure	Gelatin hydrolysates obtained by the hydrolysis with alcalase and collagenase under high pressure exhibited high ABTS radical scavenging and FRAP activities	[94]
Pacific cod (*Gadus macrocephalus*) skin	Hydrolysis with alclalases, neutrase, papain, trypsin, pepsin and α-chymotrypsin.	The papain hydrolysates exhibited the highest radical scavenging activity	[75]
Tilapia (*Oreochromis niloticus*) skin	Progressive hydrolysis with multifect neutral and properase E, at their optimum conditions	DPPH, superoxide and superior hydroxyl radical scavenging activity	[28]
Amur sturgeon skin	Hydrolysis of gelatin with alcalase	High ABTS and hydroxyl radical scavenging activity, moderate DPPH radical scavenging activity and retarded the lipid oxidation in minced fish	[153]
Nile tilapia skin	Hydrolysis with proteases, including bromelain, papain, trypsin, flavourzyme, alcalase and neutrase	Flavourzyme and trypsin hydrolysates exhibited high ABTS radical scavenging activity and inhibition of lipid peroxidation in linoleic acid system, whereas alcalase and bromelain hydrolysates found to possess greater FRAP and ferrous ion chelating activity	[139]
Thornback ray skin	Hydrolysis with Neutrase from *Bacillus amyloliquefaciens* and a Crude enzyme preparation from *Bacillus subtilis*	DPPH radical scavenging activity	[158]
Cobia skin gelatin	Hydrolysis with alcalase	High ferrous ion chelating ability and superoxide anion scavenging ability compared to BHT	[42]
Blue shark (*Prionace glauca*) skin	Hydrolysis with Protamex	DPPH and radical scavenging activity	[143]
Seabass (*Lates calcarifer*) skin	Hydrolysis with alcalase during or after the extraction of gelatin	DPPH radical scavenging activity, FRAP activity and metal ion chelating ability	[148]
Skipjack Tuna (*Katsuwonus pelamis*) Scales	Hydrolysis with alcalase	High DPPH, hydroxyl and superoxide anion scavenging activity	[154]
Skipjack Tuna (*Katsuwonus pelamis*) bone	*In vitro* Gastrointestinal digestion	DPPH, hydroxyl, superoxide anion and ABTS radical scavenging activities and lipid peroxidation inhibition capacity	[149]

typically found in vascular endothelial and neuroepithelial cells.[164] Angiotensin II is responsible for release of aldosterone increasing arterial pressure, increasing sodium and fluid retention, enhancing sympathetic adrenergic function and causing cardiac and vascular remodeling.[160] ACE also inactivates bradykinin, which is an antihypertensive vasodilator.[161,165] Hence, inhibition of ACE is believed to lower the blood pressure and has become the main therapeutic target in treatment for hypertension.[136,160,166]

Usually, synthetic ACE inhibitors such as Captopril and Enalapril are widely used to treat hypertension and other cardiovascular diseases.[134] But, they can cause numerous adverse effects.[162,167] Therefore, there is an increasing interest toward the natural ACE inhibitors.[141] Peptides derived from food proteins have exhibited significant ACE inhibitory properties,[132] and prevent hypertension by binding ACE molecule.[168] Several studies have discovered enzymatic digestion of collagen and gelatin to produce potent antihypertensive peptides.

Generally, small peptide fragments that are consisted of 2–12 amino acids possess high ACE inhibitory activity.[169] It is suggested by many studies that tripeptide residues to play an important role in competitive binding at the active site of ACE.[139,169] ACE inhibitory activity is mainly related to the C-terminal tripeptide sequence.[141] In most of the antihypertensive peptides, hydrophobic or positively charged amino acids are found in the C terminal.[139] Also, branched alpha-chain aliphatic amino acids in N-terminal increase the ACE binding activity of peptides.[170] According to Alemán et al.,[141] replacement of Hyp residues with Leu residues in positions marked as X in the peptide chain Gly-Pro-X-Gly-X-X-Gly-Phe-X-Gly-Pro-X-Gly-X-Ser could enhance the ACE inhibitory activity.

Aquatic gelatin hydrolysates produced using commercial proteases such as squid gelatin hydrolysates produced using alcalase,[134] alcalase hydrolysates of skate skin gelatin,[138] seabream scale gelatin hydrolysates produced using Esparase,[48] alcalase and trypsin hydrolysates of Nile Tilapia skin gelatin[139] have exhibited ACE inhibitory activities. Hydrolysis with different proteases have been capable of increasing the ACE inhibitory activity of gelatin compared to the parent form, but they were significantly different depending on the enzyme used.[134] A high ACE inhibitory activity has been presented by alcalase hydrolysates of squid gelatin with an IC_{50} value of 0.34 ± 0.02 mg/mL, followed by neutrase hydrolysates (IC_{50} = 0.63 ± 0.01 mg/mL), which contained peptide fractions mostly between 500 and 1400 Da.[134] A peptide purified from gastrointestinal enzyme hydrolysate of Pacific cod skin gelatin, with the amino acid sequence of Leu-Leu-Met-Leu-Asp-Asn-Asp-Leu-Pro-Pro (1301 Da) have expressed potent non-competitive ACE inhibition. This peptide has exhibited 70% hydrophobicity.[136] Several studies have discovered the ACE inhibitory activity to vary with the molecular weight distribution.[137,163,167] Zhao et al.[163] have found that ACE inhibitory activity of sea cucumber gelatin hydrolysates prepared by sequential hydrolysis with bromelain and alcalase with peptides of molecular weight less than 1 kDa to be two-fold higher compared to <10 kDa peptide fraction. The oral administration of the former (120 mg/kg dose for 1 month) has significantly reduced systolic and diastolic blood pressure of renovascular hypertensive rats (RHR) with no significant difference compared to RHR treated with captopril.[163] According to Lin et al.[167] the ACE inhibition of squid gelatin hydrolysates prepared using pepsin was the highest in peptide fraction with the molecular weight lower than 2 kDa. Also, the oral administration of 200 mg/kg dose of the peptide fraction for 30 days have significantly

decreased the systolic and diastolic blood pressure of renovascular hypertensive rats. Byun and Kim[137] have reported a marked increase of ACE inhibitory activity of Alaska Pollak skin gelatin hydrolysates with decreasing molecular weight. A high ACE inhibitory activity has been reported in peptide fractions below 3 kDa, prepared by hydrolysis of sea bream scale gelatin with Esparase, with an IC_{50} value of 59.9 ± 3.9 μg/mL[48] and peptic hydrolysates of Pacific cod skin gelatin of molecular weight below 1 kDa.[168] Around 89.02–92.55% ACE inhibitory activity have been recorded in bromelain, papain, trypsin, flavourzyme, alcalase and neutrase hydrolysates of Nile Tilapia skin gelatin and these values were not significantly different.[139] Also, Salmon (*Salmo salar*) gelatin hydrolysates prepared using Corolase PP have exhibited high ACE inhibitory activity with IC_{50} value of 0.13 ± 0.05 $mgmL^{-1}$, which remained even after the simulated gastrointestinal digestion. Spontaneously hypertensive rats (SHR) which were administrated with a dose of 50 mg/kg of above hydrolysate had lowered heart rate along with systolic, diastolic and mean arterial blood pressure.[133]

Some of the ACE inhibitory peptides derived from aquatic sources are depicted in Table 6.

Other bioactive properties

Alemán et al.[134] have revealed cytotoxic effects of Esparase hydrolysates of squid gelatin on human breast carcinoma (MCF-7) and glioma (U87) cell lines with IC_{50} values of 0.13 ± 0.01 and 0.10 ± 0.01 mg/mL, respectively. Alcalase hydrolysates also showed a high cytotoxic activity on both cancer cell lines. Esparase hydrolysates followed by alcalase hydrolysates also have presented high antiproliferative activity on both cell lines. However, squid gelatin has not exhibited any cytotoxic or antiproliferative effects against both cancer cell lines. The study further revealed that the cell viability to vary depending on the enzymes used to prepare the hydrolysates.

Moreover, peptides with MMP-1 inhibitory activity have been identified in alkaline protease and trypsin hydrolysates of cod skin gelatin[171] and gastrointestinal hydrolysates

Table 6. Comparison of ACE inhibitory activities of different aquatic gelatins.

Gelatin Source	Method of peptide preparation	Peptide sequence	IC_{50} Value (μM)	References
Alaska pollack (*Theragra chalcogramma*) skin	Serial digestion with Alcalase, pronase E, and collagenase using a three-step recycling membrane reactor	Gly-Pro-Leu	2.65	[137]
Alaska pollack (*Theragra chalcogramma*) skin	Synthesis using solid phase method with a 430A Peptide Synthesizer	Leu-Gly-Pro	0.72	[170]
Squid (inner and outer tunics)	Hydrolysis with alcalase	Gly-Pro-Leu-Gly-Leu-Leu-Gly-Phe-Leu-Gly-Pro-Leu-Gly-Leu-Ser	90.03	[134]
Pacific cod (*Gadus macrocephalus*) skin	Hydrolysis with papain	Thr-Cys-Ser-Pro	-	[75]
Pacific cod skin	Gastrointestinal hydrolysis with pepsin, trypsin and α-chymotrypsin	Leu-Leu-Met-Leu-Asp-Asn-Asp-Leu-Pro-Pro	35.7	[136]
Skate (*Okamejei kenejei*) skin	Hydrolysis with alcalase followed by protease	MVGASPGVL LGPLGHQ	3.09 4.22	[138]
Pacific cod skin	Hydrolysis with pepsin	Gly-Ala-Ser-Ser-Gly-Mmet-Pro-Gly Leu-Ala-Tyr-Ala	6.9 14.5	[168]

of Tilapia skin gelatin.[172] MMP-1 is an interstitial collagenase, belonging to the zinc-dependent endopeptidases which destroys the collagen fibers and damage extracellular matrix in connective tissues of the skin.[171,172] UV radiation leads to the generation of more reactive oxygen species (ROS), which in turn increase the MMP-1 expressing in cells through signaling pathways, inducing photoaging.[173] The balance between ROS and MMP-1 has a major impact on photoaging. The peptides with the amino acid sequences GYTGL, LGATGL and VLGL, which were prepared by the stimulated gastrointestinal digestion of Tilapia skin gelatin hydrolysates has exhibited antiphotoaging effects on mouse embryonic fibroblasts through docking the active sites of MMP-1 and inhibiting ROS production induced by UVB.[172] Moreover, Lu et al.[171] have purified two MMP-1 inhibitory peptides with the amino acid sequences of GEIGPSGGRGGKPGKDGDAGPK and GFSGLDGAKGD from cod skin gelatin hydrolysates, which have exhibited antiphotoaging effects on UVB irradiated mouse skin fibroblast *in vitro*.

Type 2 diabetes has become one of the fastest growing health concerns worldwide, and it is estimated that nearly 366 million people will be diagnosed with this condition by 2030. Glucagon like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that stimulate the release of insulin from the pancreas in a glucose-dependent manner.[174] Dipeptidyl peptidase IV (DPP-IV) is an aminopeptidase, which acts on GLP-1 and GLP and rapidly cleaves the N-terminal dipeptides of the hormones, causing the loss of their insulinotropic activity.[175] Hence, inhibition of DPP-IV has been identified as a strategy for Type 2 diabetes treatments. Recently, there is an emerging interest on the identification of natural DPP-IV inhibitors without side effects rather than synthetic therapeutic agents.[174,175] Sila et al.[176] have identified DPP-IV inhibition activity in Barbel (*Barbus callensis*) skin gelatin hydrolysates prepared with several commercial proteases, revealing potential antidiabetic applications in functional foods. Also, higher DPP-IV inhibitory activity in warm water fish (tilapia) skin gelatin hydrolysates compared to that of cold water fish (halibut) skin gelatin hydrolysates have been observed by Wang et al.[175] The study further revealed that the glucose tolerance in streptozotocin-induced diabetic rats were more effectively improved by the daily administration of tilapia skin gelatin hydrolysates, compared to the rats administrated with halibut skin gelatin hydrolysates, which can be due to the inhibition of DPP-IV activity and enhanced secretion of GLP-1 and insulin.[175] Neves et al.[133] have also revealed DPP-IV inhibitory activity in Corolase PP hydrolysates of Salmon skin gelatin.

Osteoporosis is one of the common bone disorders, which is caused by unbalanced bone remodeling processes. Post-menopausal women and elderly people are at high risk of osteoporosis. Post-menopausal osteoporosis is characterized by low bone mass, micro-architectural deterioration and decreased bone strength, that can increase the risk of bone fracture.[177] Huang et al.[177] have discovered that treatment with cod bone gelatin at 3 and 6 g/kg doses effectively prevented the mechanical property loss and improved the bone density and trabecular architecture of female ovariectomized rats. Moreover, fish gelatin hydrolysates were capable of preventing the decrease in trabecular bone mineral density of magnesium deficient rats, without affecting intrinsic biomechanical properties.[178] These findings reveal the potential applications of fish gelatin in human skeletal disorders such as postmenopausal osteoporosis.

Drawbacks of aquatic gelatins

For the sustainable production of gelatin from aquatic sources, there should be sufficient amounts of by-products with economical potential along with compatible rheological properties to conventional mammalian gelatins. However, small fish which are captured in large quantities are consumed in whole body and they cannot be considered as a resource for gelatin production,[10] limiting the available aquatic resources for gelatin production at a commercial scale. Also, compared to the gelatin extracted from traditional sources, aquatic gelatins exhibit larger variations in physical and chemical properties, which necessitates a greater need for careful optimization of extraction processes in order to achieve products with desirable properties.[90]

The production and utilization of fish gelatins in industry is still rare due its darker color and unpleasant odor.[5,45,60] Prolonged storage of raw materials before processing can lead to lipid oxidation and formation of volatile aldehydes and alcohols, which can intensify the mal-odor, Therefore, delays in processing should be avoided in order to prevent the formation of undesirable volatile compounds.[90,179] Cho et al.[72] has suggested that activated carbon treatment (250–350 mesh, 3%) followed by freeze-drying may eliminate the odor emanated from aquatic gelatins. As revealed by Sae-leaw et al.[88] and Hamzeh et al.[80] the odor of spray dried gelatin extracted from seabass skin and splendid squid skin were lower compared to the freeze-dried gelatin and the stench decreased with increasing inlet temperature of the spray drier, which can be possibly due to the elimination of low molecular weight volatiles and lipid oxidation products such as thiobarbituric acid reactive substances and peroxides contributing to the unpleasant odor at high temperatures.[80,88] However, Pan et al.[180] have reported strong odor in gelatin extracted from tiger puffer skin at higher temperatures compared to low temperature extractions and this can be triggered by increased oxidation of residual lipids in fish skin at higher temperatures, which lead to the formation of more volatile compounds which enhance the bad odor. Treatment with 0.5% powdered activated carbon can effectively reduce the odor in tiger puffer skin gelatin, without impacting the physicochemical and functional properties. However, deodorization with 3% β-cyclodextrin and fermentation with 1% yeast have significantly deteriorated the physical and functional properties of gelatin.[180] Sae-leaw et al.[88] have also identified that pretreatment with citric acid followed by defatting to be capable of lowering the mal-odor of seabass skin gelatin. Also, Montero and Gomez-Guillen[45] has found that the treatment of megrim skin collage with acetic acid to result in gelatin with a high degree of transparency and easy dissolution. Nagarajan et al.[77] have used H_2O_2 as a bleaching agent to overcome the color problems of gelatin imparted by the pigments in splendid squid skin.

Moreover, due to the lesser stability and gel strength of aquatic gelatin gels and poor rheological properties, which can be attributed to their lower proline and hydroxyproline content, they have limited industrial applications.[74,125] However, modification of fish gelatin can improve marketable properties, and chemical cross-linking using variety of cross linkers including glutaraldehyde, genipin and carbodiimide have proven to be effective means of improving the mechanical properties of gelatin-based hydrogels.[181] However, toxicity of some of the chemical cross-linking agents makes them unacceptable in food and pharmaceutical applications.[182]

Radiation can increase the thermal stability of gelatin through the induction of molecular aggregation, which leads to the formation of covalent bonding.[182] Also, the rheological

properties of fish gelatin can be improved through protein-polysaccharides interactions.[182] As discovered by Sow et al.[183] interaction of fish gelatin with κ-carrageenan in 96:4 ratio was capable of improving the rheological properties of fish gelatin. Also, addition of κ-carrageenan has increased the gel strength, gelling and melting temperatures of fish gelatin, through the interaction between negatively charged sulfate ester groups of carrageenan and the positively charged chains of fish gelatin.[184] Although combination with sodium alginate increased the gel strength and hardness of Tilapia fish gelatin, the texture profiles were not comparable to porcine gelatin.[185] Moreover, as revealed by Phawaphuthanon et al.[186] the addition of alginate have suppressed the foam formation of fish gelatin, since alginate is a highly viscous polysaccharide with poor surface activity, of which the addition could result in increased air-liquid surface tension and bulk viscosity, hindering incorporation of air during foam formation. Despite that, the foam stability was improved by the addition of alginate under acidic conditions, which could be attributed to higher molar mass and hygroscopic nature of alginate, leading to higher water-holding ability, as well as the formation of fish gelatin-alginate network in the lamella and plateau border regions of the foams, thereby preventing the coalescence of air bubbles and liquid drainage.[186] However, Tilapia skin gelatin modified with gellan and calcium chloride have exhibited comparable texture properties to commercial bovine gelatin,[187] while mixed gel of fish gelatin: low acyl gellan at 99:1 (w/w) ratio has induced rheological properties that were similar to porcine gelatin, suggesting that fish gelatin was an efficient replacement for porcine gelatin.[188] Huang et al.[189] have revealed the improved emulsifying properties of grass carps scales gelatin glycosylated with gum Arabic (GA) and octenyl succinate anhydride gum Arabic (OSA-GA), and its potential for application in coffee as a new coffee whitener instead of milk. Immersion of Tilapia skin gelatin hydrogels in appropriate concentrations of $(NH_4)_2SO_4$ solutions has strengthened the gel structure, which can be attributed to the stronger hydrophobic interactions and electrostatic interactions of gelatin chains induced by the Hofmeister effect.[190]

The intrinsic differences in collagen molecules can be attributed to a large diversity of fish species and the lower amount of intra and interchain crosslinks, which relegates them to a higher susceptibility to collagen degradation stresses. Finally, there is the need for more intensive research on the utilization of fish for gelatin production.[191]

Conclusions

Although gelatin is a widely used ingredient in food industry, there is cautious concern about gelatin originating from porcine and bovine sources due to disease outbreaks such as foot and mouth disease and religious adherences such as those practiced in halal and kosher markets. Therefore, gelatin extracted from aquatic sources would be a better alternative to mammalian gelatin due to their higher availability, reduced pollution, low risk of disease transmission and their compliance to religious restrictions. Furthermore, enzymatic hydrolysates of aquatic gelatins have exhibited bioactive properties, especially antioxidant and antihypertensive properties. However, their utilization is limited in the food industry due to their poor rheological and functional properties as well as darker color and unpleasant odor. Several studies have demonstrated that crosslinking, phosphorylation, bleaching and treatment with deodorizing agents to improve such properties of aquatic gelatins. Still, further research is required for the improvement of overall quality of gelatins extracted from aquatic sources.

Acknowledgments

This review article is based on the work supported by University Research Grants (Grant No. ASP/01/RE/SCI/2019/13), University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by University Research Grants (Grant No. ASP/01/RE/SCI/2019/13), University of Sri Jayewardenepura, Gangodawila, Nugegoda,Sri Lanka.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

R. A. S. N. Ranasinghe http://orcid.org/0000-0002-7518-3142

References

[1] Ahmad, M.; Benjakul, S. Characteristics of Gelatin from the Skin of Unicorn Leatherjacket (*Aluterus Monoceros*) as Influenced by Acid Pretreatment and Extraction Time. *Food Hydrocoll.* **2011**, *25*(3), 381–388. DOI: 10.1016/j.foodhyd.2010.07.004.

[2] Hossain, M. A.; Ganguly, A.; Rahman, S. A.; Hossain, A.; Hasan, M.; Rahman, M. S. Amino Acid Profile of the Gelatin Extracted from the Scales of Catla, Rohu, Grass Carp and Their Mixed Type. *Bangladesh J. Zool.* **2017**, *44*(2), 185–195. DOI: 10.3329/bjz.v44i2.32758.

[3] Shyni, K.; Hema, G. S.; Ninan, G.; Mathew, S.; Joshy, C. G.; Lakshmanan, P. T. Food Hydrocolloids Isolation and Characterization of Gelatin from the Skins of Skipjack Tuna (*Katsuwonus Pelamis)*, Dog Shark (*Scoliodon Sorrakowah)*, and Rohu (*Labeo Rohita*). *Food Hydrocoll.* **2014**, *39*, 68–76. DOI: 10.1016/j.foodhyd.2013.12.008.

[4] Gomez-Guillen, M. C.; Gimenez, B.; Lopez-Caballero, M. E.; Montero, M. P. Functional and Bioactive Properties of Collagen and Gelatin from Alternative Sources: A Review. *Food Hydrocoll.* **2011**, *25*(8), 1813–1827. DOI: 10.1016/j.foodhyd.2011.02.007.

[5] Cho, S. M.; Kwak, K. S.; Park, D. C.; Gu, Y. S.; Ji, C. I.; Jang, D. H.; Lee, Y. B.; Kim, S. B. Processing Optimization and Functional Properties of Gelatin from Shark (*Isurus Oxyrinchus*) Cartilage. *Food Hydrocoll.* **2004**, *18*(4), 573–579. DOI: 10.1016/j.foodhyd.2003.10.001.

[6] Norziah, M. H.; Al-Hassan, A.; Khairulnizam, A. B.; Mordi, M. N.; Norita, M. Characterization of Fish Gelatin from Surimi Processing Wastes: Thermal Analysis and Effect of Transglutaminase on Gel Properties. *Food Hydrocoll.* **2009**, *23*(6), 1610–1616. DOI: 10.1016/j.foodhyd.2008.12.004.

[7] Gilsenan, P. M.; Ross-Murphy, S. B. Rheological Characterisation of Gelatins from Mammalian and Marine Sources. *Food Hydrocoll.* **2000**, *14*(3), 191–195. DOI: 10.1016/S0268-005X(99)00050-8.

[8] Rodsuwan, U.; Thumthanaruk, B.; Kerdchoechuen, O.; Laohakunjit, N. Functional Properties of Type A Gelatin from Jellyfish *(Lobonema Smithii). Int. Food Res J.* **2016**, *23* (2), 507–514.

[9] Park, J.; Choe, J.; Kim, H.; Hwang, K.; Song, D.; Yeo, E.; Kim, H.; Choi, Y.; Lee, S.; Kim, C. Effects of Various Extraction Methods on Quality Characteristics of Duck Feet Gelatin. *Korean J. Food Sci. An.* **2013**, *33*(2), 162–169. DOI: 10.5851/kosfa.2013.33.2.162.
[10] Cho, S. M.; Gu, Y. S.; Kim, S. B. Extracting Optimization and Physical Properties of Yellowfin Tuna (*Thunnus Albacares*) Skin Gelatin Compared to Mammalian Gelatins. *Food Hydrocoll.* **2005**, *19*(2), 221–229. DOI: 10.1016/j.foodhyd.2004.05.005.
[11] Johnston-Banks, F. A. Gelatine. In *Food Gels*; Harris, P., Eds. Springer: Netherlands: Dordrecht, 1990; pp 233–289. DOI:10.1007/978-94-009-0755-3_7.
[12] Gómez-Guillén, M.; Turnay, J.; Fernández-Díaz, M.; Ulmo, N.; Lizarbe, M.; Structural, M. P. Physical Properties of Gelatin Extracted from Different Marine Species: A Comparative Study. *Food Hydrocoll.* **2002**, *16*(1), 25–34. DOI: 10.1016/S0268-005X(01)00035-2.
[13] Haddar, A.; Bougatef, A.; Balti, R.; Souissi, N.; Koched, W.; Nasri, M. Physicochemical and Functional Properties of Gelatin from Tuna (*Thunnus Thynnus*) Head Bones. *J. Food Nutr. Res.* **2011**, *50*(3), 150–159.
[14] Kozlov, P. V.; Burdygina, G. I.; Structure, T. Properties of Solid Gelatin and the Principles of Their Modification. *Polymer.* **1983**, *24*, 651–666. DOI: 10.1016/0032-3861(83)90001-0.
[15] Yang, H.; Wang, Y. Effects of Concentration on Nanostructural Images and Physical Properties of Gelatin from Channel Catfish Skins. *Food Hydrocoll.* **2009**, *23*(3), 577–584. DOI: 10.1016/j.foodhyd.2008.04.016.
[16] Hoque, M. S.; Benjakul, S.; Prodpran, T. Effects of Partial Hydrolysis and Plasticizer Content on the Properties of Film from Cuttlefish (*Sepia Pharaonis*) Skin Gelatin. *Food Hydrocoll.* **2011**, *25*(1), 82–90. DOI: 10.1016/j.foodhyd.2010.05.008.
[17] Hanani, Z. A. N. Gelatin. *Encycl. Food Heal.* **2015**, 191–195. DOI: 10.1016/B978-0-12-384947-2.00347-0.
[18] Singh, S.; Rama Rao, K. V.; Venugopal, K.; Manikandan, R. Alteration in Dissolution Characteristics of Gelatin-Containing Formulations A Review of the Problem, Test Methods, and Solutions. *Pharm. Technol.* **2002**, 36–58. www.pharmtech.com accessed October 22, 2018)..
[19] Hermanto, S.; Sumarlin, L. O.; Fatimah, W. Differentiation of Bovine and Porcine Gelatin Based on Spectroscopic and Electrophoretic Analysis. *J. Food Pharm. Sci.* **2013**, *1*(3), 68–73.
[20] Benjakul, S.; Oungbho, K.; Visessanguan, W.; Thiansilakul, Y.; Roytrakul, S. Characteristics of Gelatin from the Skins of Bigeye Snapper, *Priacanthus Tayenus* and *Priacanthus Macracanthus*. *Food Chem.* **2009**, *116*(2), 445–451. DOI: 10.1016/j.foodchem.2009.02.063.
[21] Kittiphattanabawon, P.; Benjakul, S.; Visessanguan, W.; Nagai, T.; Tanaka, M. Characterisation of Acid-Soluble Collagen from Skin and Bone of Bigeye Snapper (*Priacanthus Tayenus*). *Food Chem.* **2005**, *89*(3), 363–372. DOI: 10.1016/j.foodchem.2004.02.042.
[22] Kittiphattanabawon, P.; Benjakul, S.; Visessanguan, W.; Shahidi, F. Comparative Study on Characteristics of Gelatin from the Skins of Brownbanded Bamboo Shark and Blacktip Shark as Affected by Extraction Conditions. *Food Hydrocoll.* **2010**, *24*(2–3), 164–171. DOI: 10.1016/j.foodhyd.2009.09.001.
[23] Sinthusamran, S.; Benjakul, S.; Kishimura, H. Characteristics and Gel Properties of Gelatin from Skin of Seabass (*Lates Calcarifer*) as Influenced by Extraction Conditions. *Food Chem.* **2014**, *152*, 276–284. DOI: 10.1016/j.foodchem.2013.11.109.
[24] Wangtueai, S.; Noomhorm, A. Processing Optimization and Characterization of Gelatin from Lizardfish (*Saurida* Spp.) Scales. *LWT - Food Sci. Technol.* **2009**, *42*(4), 825–834. DOI: 10.1016/j.lwt.2008.11.014.
[25] Uriarte-Montoya, M. H.; Santacruz-Ortega, H.; Cinco-Moroyoqui, F. J.; Rouzaud-Sández, O.; Plascencia-Jatomea, M.; Ezquerra-Brauer, J. M. Giant Squid Skin Gelatin: Chemical Composition and Biophysical Characterization. *Food Res. Int.* **2011**, *44*(10), 3243–3249. DOI: 10.1016/j.foodres.2011.08.018.

[26] Barros, A. A.; Aroso, I. M.; Silva, T. H.; Mano, J. F.; Duarte, A. R. C.; Reis, R. L. Water and Carbon Dioxide: Green Solvents for the Extraction of Collagen/Gelatin from Marine Sponges. *ACS Sustain. Chem. Eng.* **2015**, *3*(2), 254–260. DOI: 10.1021/sc500621z.
[27] Silva, J. C.; Barros, A. A.; Aroso, I. M.; Fassini, D.; Silva, T. H.; Reis, R. L.; Duarte, A. R. C. Extraction of Collagen/Gelatin from the Marine Demosponge *Chondrosia Reniformis* (Nardo, 1847) Using Water Acidified with Carbon Dioxide - Process Optimization. *Ind. Eng. Chem. Res.* **2016**, *55*(25), 6922–6930. DOI: 10.1021/acs.iecr.6b00523.
[28] Zhang, Y.; Duan, X.; Zhuang, Y. Purification and Characterization of Novel Antioxidant Peptides from Enzymatic Hydrolysates of Tilapia (*Oreochromis Niloticus*) Skin Gelatin. *Peptides*. **2012**, *38*(1), 13–21. DOI: 10.1016/j.peptides.2012.08.014.
[29] Gudmundsson, M. Rheological Properties of Fish Gelatin. *J. Food Sci.* **2002**, *67*(6), 2172–2176. DOI: 10.1111/j.1365-2621.2002.tb09522.x.
[30] Sukkwai, S.; Kijroongrojana, K.; Benjakul, S. Extraction of Gelatin from Bigeye Snapper (*Priacanthus Tayenus*) Skin for Gelatin Hydrolysate Production. *Int. Food Res. J.* **2011**, *18*(3), 1129–1134.
[31] Gómez-Guillén, M. C.; Giménez, B.; Montero, P. Extraction of Gelatin from Fish Skins by High Pressure Treatment. *Food Hydrocoll.* **2005**, *19*(5), 923–928. DOI: 10.1016/j.foodhyd.2004.12.011.
[32] Tkaczewska, J.; Morawska, M.; Kulawik, P.; Zając, M. Characterization of Carp (*Cyprinus Carpio*) Skin Gelatin Extracted Using Different Pretreatments Method. *Food Hydrocoll.* **2018**, *81*, 169–179. DOI: 10.1016/j.foodhyd.2018.02.048.
[33] Haug, I. J.; Draget, K. I.; Physical, S. O. Rheological Properties of Fish Gelatin Compared to Mammalian Gelatin. *Food Hydrocoll.* **2004**, *18*(2), 203–213. DOI: 10.1016/S0268-005X(03)00065-1.
[34] Senaratne, L. S.; Park, P.-J.; Kim, S.-K. Isolation and Characterization of Collagen from Brown Backed Toadfish *(Lagocephalus Gloveri*) Skin. *Bioresour. Technol.* **2006**, *97*(2), 191–197. DOI: 10.1016/j.biortech.2005.02.024.
[35] Cheng, X.; Shao, Z.; Li, C.; Yu, L.; Raja, M. A.; Liu, C. Isolation, Characterization and Evaluation of Collagen from Jellyfish *Rhopilema Esculentum* Kishinouye for Use in Hemostatic Applications. *PLoS One*. **2017**, *12*(1), 1–21. DOI: 10.1371/journal.pone.0169731.
[36] Djabourov, M.; Lechaire, J. P.; Gaill, F. Structure and Rheology of Gelatin and Collagen Gels. *Biorheology*. **1993**; *30*, 191–205. DOI: 10.3233/BIR-1993-303-405.
[37] Choi, S.-S.; Regenstein, J. M. Physicochemical and Sensory Characteristics of Fish Gelatin. *J. Food Sci.* **2000**, *65*(2), 194–199. DOI: 10.1111/j.1365-2621.2000.tb15978.x.
[38] Waheed, H.; Development of Edible Films from Gelatin Extracted from Argentine Shortfin Squid (*Illex Argentinus*) with the Use of an Enzyme (Pepsin) Aided Process. M. Sc. Thesis, Department of Food Science and Agricultural Chemistry, Macdonald Campus, McGill University, Montreal, Quebec, **2016**.
[39] Jongjareonrak, A.; Benjakul, S.; Visessanguan, W.; Prodpran, T.; Tanaka, M. Characterization of Edible Films from Skin Gelatin of Brownstripe Red Snapper and Bigeye Snapper. *Food Hydrocoll.* **2006**, *20*, 492–501. DOI: 10.1016/j.foodhyd.2005.04.007.
[40] Lee, J. H.; Lee, J. H.; Yang, H. J.; Won, M.; Song, K. B. Characterisation of Jellyfish Protein Films with Added Transglutaminase and Wasabi Extract. *Int. J. Food Sci. Technol.* **2015**, *50*(7), 1683–1689. DOI: 10.1111/ijfs.12826.
[41] Lee, K. Y.; Yang, H. J.; Song, K. B. Application of a Puffer Fish Skin Gelatin Film Containing *Moringa Oleifera* Lam. Leaf Extract to the Packaging of Gouda Cheese. *J. Food Sci. Technol.* **2016**, *53*(11), 3876–3883. DOI: 10.1007/s13197-016-2367-9.
[42] Razali, A. N.; Amin, A. M.; Sarbon, N. M.; Activity, A. Functional Properties of Fractionated Cobia Skin Gelatin Hydrolysate at Different Molecular Weight. *Int. Food Res. J.* **2015**, *22*(2), 651–660.
[43] Chang, O. K.; Ha, G. E.; Jeong, S. G.; Seol, K. H.; Oh, M. H.; Kim, D. W.; Jang, A.; Kim, S. H.; Park, B. Y.; Ham, J. S. Antioxidant Activity of Porcine Skin Gelatin

Hydrolyzed by Pepsin and Pancreatin. *Korean J. Food Sci. Anim. Resour.* **2013**, *33*(4), 493–500. DOI: 10.5851/kosfa.2013.33.4.493.

[44] Nhari, R. M. H. R.; Ismail, A.; Che Man, Y. B. Analytical Methods for Gelatin Differentiation from Bovine and Porcine Origins and Food Products. *J. Food Sci.* **2012**, *77*(1), 42–46. DOI: 10.1111/j.1750-3841.2011.02514.x.

[45] Montero, P.; Gomez-Guillen, M. C. Extracting Conditions for Megrim (*Lepidorhombus Boscii*) Skin Collagen Affect Functional Properties of the Resulting Gelatin. *J. Food Sci.* **2000**, *65*(3), 434–438. DOI: 10.1111/j.1365-2621.2000.tb16022.x.

[46] Wang, L.; An, X.; Yang, F.; Xin, Z.; Zhao, L.; Isolation, H. Q. And Characterisation of Collagens from the Skin, Scale and Bone of Deep-Sea Redfish (*Sebastes Mentella*). *Food Chem.* **2008**, *108*(2), 616–623. DOI: 10.1016/j.foodchem.2007.11.017.

[47] Nagai, T.; Suzuki, N. Isolation of Collagen from Fish Waste Material - Skin, Bone and Fins. *Food Chem.* **2000**, *68*(3), 277–281. DOI: 10.1016/S0308-8146(99)00188-0.

[48] Akagündüz, Y.; Mosquera, M.; Giménez, B.; Alemán, A.; Montero, P.; Gómez-Guillén, M. C. Sea Bream Bones and Scales as a Source of Gelatin and ACE Inhibitory Peptides. *LWT - Food Sci. Technol.* **2014**, *55*(2), 579–585. DOI: 10.1016/j.lwt.2013.10.026.

[49] Sun, L.; Chang, W.; Ma, Q.; Zhuang, Y. Purification of Antioxidant Peptides by High Resolution Mass Spectrometry from Simulated Gastrointestinal Digestion Hydrolysates of Alaska Pollock (*Theragra Chalcogramma*) Skin Collagen. *Mar. Drugs*. **2016**, *14*(10), 1–14. DOI: 10.3390/md14100186.

[50] Sinthusamran, S.; Benjakul, S.; Molecular Characteristics, K. H. Properties of Gelatin from Skin of Seabass with Different Sizes. *Int. J. Biol. Macromol.* **2015**, *73*(1), 146–153. DOI: 10.1016/j.ijbiomac.2014.11.024.

[51] Grossman, S.; Bergman, M. Process for the Production of Gelatin from Fish Skins. U.S. Pat. 5,093, **1992**, 474.

[52] Arnesen, J. A.; Gildberg, A. Extraction of Muscle Proteins and Gelatine from Cod Head. *Process Biochem.* **2006**, *41*(3), 697–700. DOI: 10.1016/J.PROCBIO.2005.09.001.

[53] Wang, Y.; Regenstein, J. M. Effect of EDTA, HCl, and Citric Acid on Ca Salt Removal from Asian (Silver) Carp Scales Prior to Gelatin Extraction. *J. Food Sci.* **2009**, *74*(6), 426–431. DOI: 10.1111/j.1750-3841.2009.01202.x.

[54] Ikoma, T.; Kobayashi, H.; Tanaka, J.; Walsh, D.; Mann, S. Physical Properties of Type I Collagen Extracted from Fish Scales of *Pagrus Major* and *Oreochromis Niloticas*. *Int. J. Biol. Macromol.* **2003**, *32*(3–5), 199–204. DOI: 10.1016/S0141-8130(03)00054-0.

[55] Ogawa, M.; Portier, R. J.; Moody, M. W.; Bell, J.; Schexnayder, M. A.; Losso, J. N. Biochemical Properties of Bone and Scale Collagens Isolated from the Subtropical Fish Black Drum (*Pogonia Cromis*) and Sheepshead Seabream (*Archosargus Probatocephalus*). *Food Chem.* **2004**, *88*(4), 495–501. DOI: 10.1016/j.foodchem.2004.02.006.

[56] Harada, O.; Kuwata, M.; Yamamoto, T. Extraction of Gelatin from Sardine Scales by Pressurized Hot Water. *Nippon Shokuhin Kagaku Kogaku Kaishi*. **2007**, *54*, 261–265. DOI: 10.3136/nskkk.54.261.

[57] Chandra, M. V.; Shamasundar, B. A. Rheological Properties of Gelatin Prepared from the Swim Bladders of Freshwater Fish *Catla Catla*. *Food Hydrocoll.* **2015**, *48*, 47–54. DOI: 10.1016/j.foodhyd.2015.01.022.

[58] Karim, A. A.; Fish Gelatin:, B. R.; Properties, C. Prospects as an Alternative to Mammalian Gelatins. *Food Hydrocoll.* **2009**, *23*(3), 563–576. DOI: 10.1016/J.FOODHYD.2008.07.002.

[59] Gudmundsson, M.; Hafsteinsson, H. Gelatin from Cod Skins as Affected by Chemical Treatments. *J. Food Sci.* **1997**, *62*(1), 37–39. DOI: 10.1111/j.1365-2621.1997.tb04363.x.

[60] Cheow, C. S.; Norizah, M. S.; Kyaw, Z. Y.; Howell, N. K. Preparation and Characterisation of Gelatins from the Skins of Sin Croaker (*Johnius Dussumieri*) and Shortfin Scad (*Decapterus Macrosoma*). *Food Chem.* **2007**, *101*(1), 386–391. DOI: 10.1016/j.foodchem.2006.01.046.

[61] Chancharern, P.; Laohakunjit, N.; Kerdchoechuen, O.; Thumthanaruk, B. Extraction of Type A and Type B Gelatin from Jellyfish *(Lobonema Smithii)*. *Int. Food Res J.* **2016**, *23*, 419–424. http://www.ifrj.upm.edu.my/23(01) 2016/(63). pdf (accessed September 21, 2018).

[62] Brotz, L. Jellyfish Fisheries of the World. Ph.D Thesis, The University of British Columbia (Vancouver), **2016**.
[63] Leone, A.; Lecci, R. M.; Durante, M.; Piraino, S. Extract from the Zooxanthellate Jellyfish *Cotylorhiza Tuberculata* Modulates Gap Junction Intercellular Communication in Human Cell Cultures. *Mar. Drugs.* **2013**, *11*(5), 1728–1762. DOI: 10.3390/md11051728.
[64] Calejo, M. T.; Morais, Z. B.; Fernandes, A. I. Isolation and Biochemical Characterisation of a Novel Collagen from *Catostylus Tagi*. *J. Biomater. Sci. Polym. Educ.* **2009**, *20*(14), 2073–2087. DOI: 10.1163/156856208X399125.
[65] Calejo, M. T.; Almeida, A. J.; Fernandes, A. I. Exploring a New Jellyfish Collagen in the Production of Microparticles for Protein Delivery. *J. Microencapsul.* **2012**, *29*(6), 520–531. DOI: 10.3109/02652048.2012.665089.
[66] Cho, S.; Ahn, J. R.; Koo, J. S.; Kim, S. B. Physicochemical Properties of Gelatin from Jellyfish *Rhopilema Hispidum*. *Fish. Aquat. Sci.* **2014**, *17*(3), 299–304. DOI: 10.5657/FAS.2014.0299.
[67] Liu, Z.; Oliveira, A. C. M.; Su, Y.-C. Purification and Characterization of Pepsin-Solubilized Collagen from Skin and Connective Tissue of Giant Red Sea Cucumber (*Parastichopus Californicus*). *J. Agric. Food Chem.* **2010**, *58*(2), 1270–1274. DOI: 10.1021/jf9032415.
[68] Gaspar-Pintiliescu, A.; Stefan, L. M.; Anton, E. D.; Berger, D.; Matei, C.; Negreanu-Pirjol, T.; Moldovan, L. Physicochemical and Biological Properties of Gelatin Extracted from Marine Snail *Rapana Venosa*. *Mar. Drugs.* **2019**, *17*(10), 1–14. DOI: 10.3390/md17100589.
[69] Gómez-Guillén, M. C.; Montero, P. Extraction of Gelatin from Megrim (*Lepidorhombus Boscii*) Skins with Several Organic Acids. *Food Chem. Toxicol.* **2001**, *66*(2), 213–216. DOI: 10.1111/j.1365-2621.2001.tb11319.x.
[70] Arnesen, J. A.; Gildberg, A. Preparation and Characterisation of Gelatine from the Skin of Harp Seal (*Phoca Groendlandica*). *Bioresour. Technol.* **2002**, *82*(2), 191–194. DOI: 10.1016/S0960-8524(01)00164-X.
[71] Muyonga, J. H.; Nile Perch Collagen and Gelatin Extraction and Physico-Chemical Characterisation. Ph.D Dissertation, Faculty of Natural and Agricultural Sciences, University of Pretoria, **2003**.
[72] Cho, S.-H.; Jahncke, M. L.; Chin, K.-B.; Eun, J.-B. The Effect of Processing Conditions on the Properties of Gelatin from Skate (*Raja Kenojei*) Skins. *Food Hydrocoll.* **2006**, *20*(6), 810–816. DOI: 10.1016/j.foodhyd.2005.08.002.
[73] Aewsiri, T.; Benjakul, S.; Visessanguan, W. Functional Properties of Gelatin from Cuttlefish (*Sepia Pharaonis*) Skin as Affected by Bleaching Using Hydrogen Peroxide. *Food Chem.* **2009**, *115*(1), 243–249. DOI: 10.1016/j.foodchem.2008.12.012.
[74] Tabarestani, H. S.; Maghsoudlou, Y.; Motamedzadegan, A.; Sadeghi Mahoonak, A. R. Optimization of Physico-Chemical Properties of Gelatin Extracted from Fish Skin of Rainbow Trout (*Onchorhynchus mykiss*). *Bioresour. Technol.* **2010**, *101*(15), 6207–6214. DOI: 10.1016/j.biortech.2010.02.071.
[75] Ngo, D. H.; Ryu, B.; Vo, T. S.; Himaya, S. W. A.; Wijesekara, I.; Kim, S. K. Free Radical Scavenging and Angiotensin-I Converting Enzyme Inhibitory Peptides from Pacific Cod (*Gadus Macrocephalus*) Skin Gelatin. *Int. J. Biol. Macromol.* **2011**, *49*(5), 1110–1116. DOI: 10.1016/j.ijbiomac.2011.09.009.
[76] Balti, R.; Jridi, M.; Sila, A.; Souissi, N.; Nedjar-Arroume, N.; Guillochon, D.; Nasri, M. Extraction and Functional Properties of Gelatin from the Skin of Cuttlefish (*Sepia officinalis*) Using Smooth Hound Crude Acid Protease-Aided Process. *Food hydrocoll.* **2011**, *25*(5), 943–950. DOI: 10.1016/j.foodhyd.2010.09.005.
[77] Nagarajan, M.; Benjakul, S.; Prodpran, T.; Songtipya, P. Effects of Bleaching on Characteristics and Gelling Property of Gelatin from Splendid Squid (*Loligo Formosana*) Skin. *Food Hydrocoll.* **2013**, *32*(2), 447–452. DOI: 10.1016/j.foodhyd.2013.02.003.
[78] Liu, Y.; Xia, L.; Jia, H.; Li, Q.; Jin, W.; Dong, X.; Physiochemical, P. J. And Functional Properties of Chum Salmon (*Oncorhynchus Keta*) Skin Gelatin Extracted at Different Temperatures. *J. Sci. Food Agric.* **2017**, *97*(15), 5406–5413. DOI: 10.1002/jsfa.8431.

[79] Duan, R.; Zhang, J.; Liu, L.; Cui, W.; Regenstein, J. M. The Functional Properties and Application of Gelatin Derived from the Skin of Channel Catfish (*Ictalurus punctatus*). *Food Chem.* **2018**, *239*, 464–469. DOI: 10.1016/j.foodchem.2017.06.145.

[80] Hamzeh, A.; Benjakul, S.; Sae-leaw, T.; Sinthusamran, S. Effect of Drying Methods on Gelatin from Splendid Squid (*Loligo formosana*) Skins. *Food Biosci.* **2018**, *26*, 96–103. DOI: 10.1016/j.fbio.2018.10.001.

[81] Ali, A. M. M.; Kishimura, H.; Benjakul, S. Physicochemical and Molecular Properties of Gelatin from Skin of Golden Carp (*Probarbus Jullieni*) as Influenced by Acid Pretreatment and Prior-Ultrasonication. *Food Hydrocoll.* **2018**, *82*, 164–172. DOI: 10.1016/j.foodhyd.2018.03.052.

[82] Pan, J.; Li, Q.; Jia, H.; Xia, L.; Jin, W.; Shang, M.; Xu, C.; Dong, X. Physiochemical and Functional Properties of Tiger Puffer (*Takifugu Rubripes*) Skin Gelatin as Affected by Extraction Conditions. *Int. J. Biol. Macromol.* **2017**, *109*, 1045–1053. DOI: 10.1016/j.ijbiomac.2017.11.080.

[83] Renuka, V.; Rao Ravishankar, C. N.; Zynudheen, A. A.; Bindu, J.; Joseph, T. C. Characterization of Gelatin Obtained from Unicorn Leatherjacket (*Aluterus Monoceros*) and Reef Cod (*Epinephelus Diacanthus*) Skins. *LWT - Food Sci. Technol.* **2019**, *116*(June), 108586. DOI: 10.1016/j.lwt.2019.108586.

[84] Mi, H.; Wang, C.; Chen, J.; Xu, Y.; Li, X.; Li, J.; Sun, X.; Mao, L.; Ma, Y.; Lao, M. Characteristic and Functional Properties of Gelatin from the Bones of Alaska Pollock (*Theragra Chalcogramma*) and Yellowfin Sole (*Limanda Aspera*) with Papain-Aided Process. *J. Aquat. Food Prod. Technol.* **2019**, *28*(3), 287–297. DOI: 10.1080/10498850.2019.1577933.

[85] Ahmad, T.; Ismail, A.; Ahmad, S. A.; Khalil, K. A.; Kumar, Y.; Adeyemi, K. D.; Sazili, A. Q. Recent Advances on the Role of Process Variables Affecting Gelatin Yield and Characteristics with Special Reference to Enzymatic Extraction: A Review. *Food hydrocoll.* **2017**, *63*, 85–96. DOI: 10.1016/j.foodhyd.2016.08.007.

[86] Shavandi, A.; Hou, Y.; Carne, A.; McConnell, M.; Bekhit, A. E. din A. Marine Waste Utilization as a Source of Functional and Health Compounds. In *Advances in Food and Nutrition Research*, 1st ed.; Toldra, F., Eds.; Academic Press, London, UK, 2019; Vol. 87, pp 187-.254. DOI: 10.1016/bs.afnr.2018.08.001

[87] Jongjareonrak, A.; Rawdkuen, S.; Chaijan, M.; Benjakul, S.; Osako, K.; Tanaka, M. Chemical Compositions and Characterisation of Skin Gelatin from Farmed Giant Catfish (*Pangasianodon Gigas*). *LWT - Food Sci. Technol.* **2010**, *43*(1), 161–165. DOI: 10.1016/j.lwt.2009.06.012.

[88] Sae-leaw, T.; Benjakul, S.; O'Brien, N. M. Effect of Pretreatments and Drying Methods on the Properties and Fishy Odor/Flavor of Gelatin from Seabass (*Lates Calcarifer*) Skin. *Dry. Technol.* **2016**, *34*(1), 53–65. DOI: 10.1080/07373937.2014.1003071.

[89] Nazeer, R. A.; Sri Suganya, U. Porous Scaffolds of Gelatin from the Marine Gastropod *Ficus Variegate* with Commercial Cross Linkers for Biomedical Applications. *Food Sci. Biotechnol.* **2014**, *23*(2), 327–335. DOI: 10.1007/s10068-014-0046-z.

[90] Milovanovic, I.; Hayes, M. Marine Gelatine from Rest Raw Materials. *Appl. Sci.* **2018**, *8*(12), 1–20. DOI: 10.3390/app8122407.

[91] Intarasirisawat, R.; Benjakul, S.; Visessanguan, W.; Prodpran, T.; Tanaka, M.; Howell, N. K. Autolysis Study of Bigeye Snapper (*Priacanthus Macracanthus*) Skin and Its Effect on Gelatin. *Food Hydrocoll.* **2007**, *21*(4), 537–544. DOI: 10.1016/j.foodhyd.2006.05.012.

[92] Kaewruang, P.; Benjakul, S.; Molecular, P. T. Functional Properties of Gelatin from the Skin of Unicorn Leatherjacket as Affected by Extracting Temperatures. *Food Chem.* **2013**, *138*(2–3), 1431–1437. DOI: 10.1016/j.foodchem.2012.09.114.

[93] Alemán, A.; Giménez, B.; Montero, P.; Gómez-Guillén, M. C. Antioxidant Activity of Several Marine Skin Gelatins. *LWT - Food Sci. Technol.* **2011**, *44*(2), 407–413. DOI: 10.1016/j.lwt.2010.09.003.

[94] Alemán, A.; Giménez, B.; Gómez-Guillén, M. C.; Montero, P. Enzymatic Hydrolysis of Fish Gelatin under High Pressure Treatment. *Int. J. Food Sci. Technol.* **2011**, *46*(6), 1129–1136. DOI: 10.1111/j.1365-2621.2011.02590.x.

[95] Šližyte, R.; Mozuraityte, R.; Martínez-Alvarez, O.; Falch, E.; Fouchereau-Peron, M.; Rustad, T. Functional, Bioactive and Antioxidative Properties of Hydrolysates Obtained from Cod (*Gadus Morhua*) Backbones. *Process Biochem.* **2009**, *44*(6), 668–677. DOI: 10.1016/j.procbio.2009.02.010.

[96] Lv, L.; Fish Gelatin, H. Q. The Novel Potential Applications. *J. Funct. Foods.* **2019**, *63* (January), 1–14. DOI: 10.1016/j.jff.2019.103581.

[97] Etxabide, A.; Ribeiro, R. D. C.; Guerrero, P.; Ferreira, A. M.; Stafford, G. P.; Dalgarno, K.; de la Caba, K.; Gentile, P. Lactose-Crosslinked Fish Gelatin-Based Porous Scaffolds Embedded with Tetrahydrocurcumin for Cartilage Regeneration. *Int. J. Biol. Macromol.* **2018**, *117* (2017), 199–208. DOI: 10.1016/j.ijbiomac.2018.05.154.

[98] Sghayyar, H. N. M.; Lim, S. S.; Ahmed, I.; Lai, J. Y.; Cheong, X. Y.; Chong, Z. W.; Lim, A. F. X.; Loh, H. S. Fish Biowaste Gelatin Coated Phosphate-Glass Fibres for Wound-Healing Application. *Eur. Polym. J.* **2020**, *122*(November 2019), 109386. DOI: 10.1016/j.eurpolymj.2019.109386.

[99] Kwak, H. W.; Shin, M.; Lee, J. Y.; Yun, H.; Song, D. W.; Yang, Y.; Shin, B. S.; Park, Y. H.; Lee, K. H. Fabrication of an Ultrafine Fish Gelatin Nanofibrous Web from an Aqueous Solution by Electrospinning. *Int. J. Biol. Macromol.* **2017**, *102*, 1092–1103. DOI: 10.1016/j.ijbiomac.2017.04.087.

[100] Uranga, J.; Etxabide, A.; Cabezudo, S.; de la Caba, K.; Guerrero, P. Valorization of Marine-Derived Biowaste to Develop Chitin/Fish Gelatin Products as Bioactive Carriers and Moisture Scavengers. *Sci. Total Environ.* **2019**, 135747. DOI: 10.1016/j.scitotenv.2019.135747.

[101] Kang, M. G.; Lee, M. Y.; Cha, J. M.; Lee, J. K.; Lee, S. C.; Kim, J.; Hwang, Y. S.; Bae, H. Nanogels Derived from Fish Gelatin: Application to Drug Delivery System. *Mar. Drugs.* **2019**, *17*(4), 1–11. DOI: 10.3390/md17040246.

[102] Zhang, Z.; Zhao, M.; Wang, J.; Ding, Y.; Dai, X.; Li, Y. Oral Administration of Skin Gelatin Isolated from Chum Salmon (*Oncorhynchus Keta*) Enhances Wound Healing in Diabetic Rats. *Mar. Drugs.* **2011**, *9*(5), 696–711. DOI: 10.3390/md9050696.

[103] Zeng, Y.; Zhu, L.; Han, Q.; Liu, W.; Mao, X.; Li, Y.; Yu, N.; Feng, S.; Fu, Q.; Wang, X.;, et al. Preformed Gelatin Microcryogels as Injectable Cell Carriers for Enhanced Skin Wound Healing. *Acta Biomater.* **2015**, *25*, 291–303. DOI: 10.1016/j.actbio.2015.07.042.

[104] Hanani, Z. A. N.; Yee, F. C.; Nor-Khaizura, M. A. R. Effect of Pomegranate (*Punica Granatum* L.) Peel Powder on the Antioxidant and Antimicrobial Properties of Fish Gelatin Films as Active Packaging. *Food Hydrocoll.* **2019**, *89*, 253–259. DOI: 10.1016/j.foodhyd.2018.10.007.

[105] Thumthanaruk, B.; Rodsuwan, U.; Chancharern, P.; Kerdchoechuen, O.; Laohakunjit, N.; Chism, G. W. Physico-Chemical Properties of Extruded Copolymer Film. *J. Food Process. Preserv.* **2016**, *41*(2), 1–8. DOI: 10.1111/jfpp.12808.

[106] Leceta, I.; Urdanpilleta, M.; Zugasti, I.; Guerrero, P.; de la Caba, K. Assessment of Gallic Acid-Modified Fish Gelatin Formulations to Optimize the Mechanical Performance of Films. *Int. J. Biol. Macromol.* **2018**, *120*, 2131–2136. DOI: 10.1016/j.ijbiomac.2018.09.081.

[107] Avena-Bustillos, R. J.; Olsen, C. W.; Olson, D. A.; Chiou, B.; Yee, E.; Bechtel, P. J.; McHugh, T. H. Water Vapor Permeability of Mammalian and Fish Gelatin Films. *J. Food Sci.* **2006**, *71*(4), 202–207. DOI: 10.1111/j.1750-3841.2006.00016.x.

[108] Etxabide, A.; Uranga, J.; Guerrero, P.; de la Caba, K. Improvement of Barrier Properties of Fish Gelatin Films Promoted by Gelatin Glycation with Lactose at High Temperatures. *LWT - Food Sci. Technol.* **2015**, *63*(1), 315–321. DOI: 10.1016/j.lwt.2015.03.079.

[109] Kchaou, H.; Benbettaïeb, N.; Jridi, M.; Abdelhedi, O.; Karbowiak, T.; Brachais, C.-H.; Léonard, M.-L.; Debeaufort, F.; Nasri, M. Enhancement of Structural, Functional and Antioxidant Properties of Fish Gelatin Films Using Maillard Reactions. *Food Hydrocoll.* **2018**, *83*, 326–339. DOI: 10.1016/J.FOODHYD.2018.05.011.

[110] Uranga, J.; Leceta, I.; Etxabide, A.; Guerrero, P.; De La Caba, K. Cross-Linking of Fish Gelatins to Develop Sustainable Films with Enhanced Properties. *Eur. Polym. J.* **2016**, *78*, 82–90. DOI: 10.1016/j.eurpolymj.2016.03.017.
[111] Benbettaïeb, N.; Karbowiak, T.; Brachais, C. H.; Coupling Tyrosol, D. F. Quercetin or Ferulic Acid and Electron Beam Irradiation to Cross-Link Chitosan-Gelatin Films: A Structure-Function Approach. *Eur. Polym. J.* **2015**, *67*, 113–127. DOI: 10.1016/j.eurpolymj.2015.03.060.
[112] Santos, J. P.; Esquerdo, V. M.; Moura, C. M.; Pinto, L. A. A. Crosslinking Agents Effect on Gelatins from Carp and Tilapia Skins and in Their Biopolymeric Films. *Colloids Surf. A.* **2018**, *539*, 184–191. DOI: 10.1016/j.colsurfa.2017.12.018.
[113] Etxabide, A.; Urdanpilleta, M.; Guerrero, P.; De La Caba, K. Effects of Cross-Linking in Nanostructure and Physicochemical Properties of Fish Gelatins for Bio-Applications. *React. Funct. Polym.* **2015**, *94*, 55–62. DOI: 10.1016/j.reactfunctpolym.2015.07.006.
[114] Hosseini, S. F.; Rezaei, M.; Zandi, M.; Farahmandghavi, F. Fabrication of Bio-Nanocomposite Films Based on Fish Gelatin Reinforced with Chitosan Nanoparticles. *Food Hydrocoll.* **2015**, *44*, 172–182. DOI: 10.1016/j.foodhyd.2014.09.004.
[115] Hosseini, S. F.; Javidi, Z.; Rezaei, M. Efficient Gas Barrier Properties of Multi-Layer Films Based on Poly(Lactic Acid) and Fish Gelatin. *Int. J. Biol. Macromol.* **2016**, *92*, 1205–1214. DOI: 10.1016/j.ijbiomac.2016.08.034.
[116] Maryam Adilah, Z. A.; Nur Hanani, Z. A. Active Packaging of Fish Gelatin Films with *Morinda Citrifolia* Oil. *Food Biosci.* **2016**, *16*, 66–71. DOI: 10.1016/j.fbio.2016.10.002.
[117] Staroszczyk, H.; Kusznierewicz, B.; Malinowska-Pańczyk, E.; Sinkiewicz, I.; Gottfried, K.; Kołodziejska, I. Fish Gelatin Films Containing Aqueous Extracts from Phenolic-Rich Fruit Pomace. *LWT - Food Sci. Technol.* **2020**, *117,108613*, 1–9. DOI: 10.1016/j.lwt.2019.108613.
[118] Abdelhedi, O.; Jridi, M.; Nasri, R.; Mora, L.; Toldrá, F.; Nasri, M. Rheological and Structural Properties of *Hemiramphus Far* Skin Gelatin: Potential Use as an Active Fish Coating Agent. *Food Hydrocoll.* **2019**, *87*, 331–341. DOI: 10.1016/j.foodhyd.2018.08.005.
[119] Zhao, X.; Wu, J.; Chen, L.; Yang, H. Effect of Vacuum Impregnated Fish Gelatin and Grape Seed Extract on Metabolite Profiles of Tilapia (*Oreochromis niloticus*)Fillets during Storage. *Food Chem.* **2019**, *293*, 418–428. DOI: 10.1016/j.foodchem.2019.05.001.
[120] Damodaran, S.; Wang, S. Y. Ice Crystal Growth Inhibition by Peptides from Fish Gelatin Hydrolysate. *Food Hydrocoll.* **2017**, *70*, 46–56. DOI: 10.1016/j.foodhyd.2017.03.029.
[121] Vázquez, J. A.; Fernández-Compás, A.; Blanco, M.; Rodríguez-Amado, I.; Moreno, H.; Borderías, J.; Pérez-Martín, R. I. Development of Bioprocesses for the Integral Valorisation of Fish Discards. *Biochem. Eng. J.* **2019**, *144*, 198–208. DOI: 10.1016/j.bej.2019.02.004.
[122] Sousa, S. C.; Vázquez, J. A.; Pérez-Martín, R. I.; Carvalho, A. P.; Gomes, A. M. Valorization of By-Products from Commercial Fish Species: Extraction and Chemical Properties of Skin Gelatins. *Molecules*. **2017**, *22*(9), 1–12. DOI: 10.3390/molecules22091545.
[123] Altan Kamer, D. D.; Palabiyik, I.; Işık, N. O.; Akyuz, F.; Demirci, A. S.; Gumus, T. Effect of Confectionery Solutes on the Rheological Properties of Fish (*Oncorhynchus Mykiss*) Gelatin. *LWT - Food Sci. Technol.* **2019**, *101*, 499–505. DOI: 10.1016/j.lwt.2018.11.046.
[124] Sinthusamran, S.; Physical, B. S. Rheological and Antioxidant Properties of Gelatin Gel as Affected by the Incorporation of β-Glucan. *Food Hydrocoll.* **2018**, *79*, 409–415. DOI: 10.1016/j.foodhyd.2018.01.018.
[125] Huang, T.; Tu, Z.; Shangguan, X.; Wang, H.; Sha, X.; Bansal, N. Rheological Behavior, Emulsifying Properties and Structural Characterization of Phosphorylated Fish Gelatin. *Food Chem.* **2018**, *246*, 428–436. DOI: 10.1016/j.foodchem.2017.12.023.
[126] Surh, J.; Decker, E. A.; McClements, D. J. Properties and Stability of Oil-in-Water Emulsions Stabilized by Fish Gelatin. *Food Hydrocoll.* **2006**, *20*(5), 596–606. DOI: 10.1016/j.foodhyd.2005.06.002.
[127] Liu, J.; Xu, G.; Yuan, S.; Jiang, P. The Effect of Macromolecules on Foam Stability in Sodium Dodecyl Sulfate/Cetylpyridinium Bromide Mixtures. *J Dispers. Sci. Technol.* **2003**, *24*(6), 779–787. DOI: 10.1081/DIS-120025546.

[128] Zayas, J. F. *Functionality of Proteins in Food*; Springer-Verlag Berlin: Germany, 1997; https://books.google.lk/books/about/Functionality_of_Proteins_in_Food.html?id=2
[129] Kinsella, J. E. Functional Properties of Proteins: Possible Relationships between Structure and Function in Foams. *Food Chem.* **1981**, *7*(4), 273–288. DOI: 10.1016/0308-8146(81)90033-9.
[130] Mirmiran, P.; Bahadoran, Z.; Azizi, F. Functional Foods-Based Diet as A Novel Dietary Approach for Management of Type 2 Diabetes and Its Complications: A Review. *World J. Diabetes.* **2014**, *5*(3), 267. DOI: 10.4239/wjd.v5.i3.267.
[131] Hosseini, S. F.; Ramezanzade, L.; Nikkhah, M. Nano-Liposomal Entrapment of Bioactive Peptidic Fraction from Fish Gelatin Hydrolysate. *Int. J. Biol. Macromol.* **2017**, *105*, 1455–1463. DOI: 10.1016/j.ijbiomac.2017.05.141.
[132] Li, G. H.; Le, G. W.; Shi, Y. H.; Shrestha, S. Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from Food Proteins and Their Physiological and Pharmacological Effects. *Nutr. Res.* **2004**, *24*(7), 469–486. DOI: 10.1016/j.nutres.2003.10.014.
[133] Neves, A. C.; Harnedy, P. A.; O'Keeffe, M. B.; Alashi, M. A.; Aluko, R. E.; FitzGerald, R. J. Peptide Identification in a Salmon Gelatin Hydrolysate with Antihypertensive, Dipeptidyl Peptidase IV Inhibitory and Antioxidant Activities. *Food Res. Int.* **2017**, *100*, 112–120. DOI: 10.1016/j.foodres.2017.06.065.
[134] Alemán, A.; Pérez-Santín, E.; Bordenave-Juchereau, S.; Arnaudin, I.; Gómez-Guillén, M. C.; Montero, P. Squid Gelatin Hydrolysates with Antihypertensive, Anticancer and Antioxidant Activity. *Food Res. Int.* **2011**, *44*(4), 1044–1051. DOI: 10.1016/j.foodres.2011.03.010.
[135] Zheng, L.; Yu, H.; Wei, H.; Xing, Q.; Zou, Y.; Zhou, Y.; Peng, J. Antioxidative Peptides of Hydrolysate Prepared from Fish Skin Gelatin Using Ginger Protease Activate Antioxidant Response Element-Mediated Gene Transcription in IPEC-J2 Cells. *J. Funct. Foods.* **2018**, *51*, 104–112. DOI: 10.1016/j.jff.2018.08.033.
[136] Himaya, S. W. A.; Ngo, D. H.; Ryu, B.; Kim, S. K. An Active Peptide Purified from Gastrointestinal Enzyme Hydrolysate of Pacific Cod Skin Gelatin Attenuates Angiotensin-1 Converting Enzyme (ACE) Activity and Cellular Oxidative Stress. *Food Chem.* **2012**, *132*(4), 1872–1882. DOI: 10.1016/j.foodchem.2011.12.020.
[137] Byun, H. G.; Kim, S. K. Purification and Characterization of Angiotensin I Converting Enzyme (ACE) Inhibitory Peptides from Alaska Pollack (*Theragra Chalcogramma*) Skin. *Process Biochem.* **2001**, *3'6*(12), 1155–1162. DOI: 10.1016/S0032-9592(00)00297-1.
[138] Ngo, D. H.; Ryu, B. M.; Kim, S. K. Active Peptides from Skate (*Okamejei Kenojei*) Skin Gelatin Diminish Angiotensin-I Converting Enzyme Activity and Intracellular Free Radical-Mediated Oxidation. *Food Chem.* **2014**, *143*, 246–255. DOI: 10.1016/j.foodchem.2013.07.067.
[139] Choonpicharn, S.; Jaturasitha, S.; Rakariyatham, N.; Suree, N.; Antioxidant, N. H. Antihypertensive Activity of Gelatin Hydrolysate from Nile Tilapia Skin. *J. Food Sci. Technol.* **2015**, *52*(5), 3134–3139. DOI: 10.1007/s13197-014-1581-6.
[140] Mendis, E.; Rajapakse, N.; Byun, H. G.; Kim, S. K. Investigation of Jumbo Squid (*Dosidicus Gigas*) Skin Gelatin Peptides for Their in Vitro Antioxidant Effects. *Life Sci.* **2005**, *77*(17), 2166–2178. DOI: 10.1016/j.lfs.2005.03.016.
[141] Alemán, A.; Giménez, B.; Pérez-Santin, E.; Gómez-Guillén, M. C.; Montero, P. Contribution of Leu and Hyp Residues to Antioxidant and ACE-Inhibitory Activities of Peptide Sequences Isolated from Squid Gelatin Hydrolysate. *Food Chem.* **2011**, *125*(2), 334–341. DOI: 10.1016/j.foodchem.2010.08.058.
[142] Haddar, A.; Sellimi, S.; Ghannouchi, R.; Alvarez, O. M.; Nasri, M.; Functional, B. A. Antioxidant and Film-Forming Properties of Tuna-Skin Gelatin with a Brown Algae Extract. *Int. J. Biol. Macromol.* **2012**, *51*(4), 477–483. DOI: 10.1016/j.ijbiomac.2012.06.016.
[143] Weng, W.; Tang, L.; Wang, B.; Chen, J.; Su, W.; Osako, K.; Tanaka, M. Antioxidant Properties of Fractions Isolated from Blue Shark (*Prionace Glauca*) Skin Gelatin Hydrolysates. *J. Funct. Foods.* **2014**, *11*(C), 342–351. DOI: 10.1016/j.jff.2014.10.021.

[144] Zhuang, Y. L.; Sun, L. P.; Zhao, X.; Hou, H.; Li, B. F. Investigation of Gelatin Polypeptides of Jellyfish (*Rhopilema Esculentum*) for Their Antioxidant Activity in Vitro. *Food Technol. Biotechnol.* **2010**, *48*(2), 222–228.

[145] Ketnawa, S.; Martínez-Alvarez, O.; Benjakul, S.; Rawdkuen, S. Gelatin Hydrolysates from Farmed Giant Catfish Skin Using Alkaline Proteases and Its Antioxidative Function of Simulated Gastro-Intestinal Digestion. *Food Chem.* **2016**, *192*, 34–42. DOI: 10.1016/j.foodchem.2015.06.087.

[146] Giménez, B.; Gómez-Estaca, J.; Alemán, A.; Gómez-Guillén, M. C.; Montero, M. P. Improvement of the Antioxidant Properties of Squid Skin Gelatin Films by the Addition of Hydrolysates from Squid Gelatin. *Food Hydrocoll.* **2009**, *23*(5), 1322–1327. DOI: 10.1016/j.foodhyd.2008.09.010.

[147] Tkaczewska, J.; Borawska-Dziadkiewicz, J.; Kulawik, P.; Duda, I.; Morawska, M.; Mickowska, B. The Effects of Hydrolysis Condition on the Antioxidant Activity of Protein Hydrolysate from *Cyprinus Carpio* Skin Gelatin. *LWT - Food Sci. Technol.* **2020**, *117, 108616*, 1–9. DOI: 10.1016/j.lwt.2019.108616.

[148] Sae-leaw, T.; O'Callaghan, Y. C.; Benjakul, S.; O'Brien, N. M. Antioxidant Activities and Selected Characteristics of Gelatin Hydrolysates from Seabass (*Lates Calcarifer*) Skin as Affected by Production Processes. *J. Food Sci. Technol.* **2016**, *53*(1), 197–208. DOI: 10.1007/s13197-015-1989-7.

[149] Yang, X. R.; Zhao, Y. Q.; Qiu, Y. T.; Chi, C. F.; Preparation, W. B. Characterization of Gelatin and Antioxidant Peptides from Gelatin Hydrolysate of Skipjack Tuna (Katsuwonus Pelamis) Bone Stimulated by in Vitro Gastrointestinal Digestion. *Mar. Drugs.* **2019**, *17*(2), 1–18. DOI: 10.3390/md17020078.

[150] Yang, J. I.; Liang, W. S.; Chow, C. J.; Siebert, K. J. Process for the Production of Tilapia Retorted Skin Gelatin Hydrolysates with Optimized Antioxidative Properties. *Process Biochem.* **2009**, *44*(10), 1152–1157. DOI: 10.1016/j.procbio.2009.06.013.

[151] Zhuang, Y.; Sun, L. Preparation of Reactive Oxygen Scavenging Peptides from Tilapia (*Oreochromis Niloticus*) Skin Gelatin: Optimization Using Response Surface Methodology. *J. Food Sci.* **2011**, *76*(3), 483–489. DOI: 10.1111/j.1750-3841.2011.02108.x.

[152] Ngo, D. H.; Qian, Z. J.; Ryu, B.; Park, J. W.; Kim, S. K. In Vitro Antioxidant Activity of a Peptide Isolated from Nile Tilapia (*Oreochromis Niloticus*) Scale Gelatin in Free Radical-Mediated Oxidative Systems. *J. Funct. Foods.* **2010**, *2*(2), 107–117. DOI: 10.1016/j.jff.2010.02.001.

[153] Nikoo, M.; Benjakul, S.; Ehsani, A.; Li, J.; Wu, F.; Yang, N.; Xu, B.; Jin, Z.; Xu, X. Antioxidant and Cryoprotective Effects of a Tetrapeptide Isolated from Amur Sturgeon Skin Gelatin. *J. Funct. Foods.* **2014**, *7*(1), 609–620. DOI: 10.1016/j.jff.2013.12.024.

[154] Qiu, Y. T.; Wang, Y. M.; Yang, X. R.; Zhao, Y. Q.; Chi, C. F.; Wang, B. Gelatin and Antioxidant Peptides from Gelatin Hydrolysate of Skipjack Tuna (*Katsuwonus Pelamis*) Scales: Preparation, Identification and Activity Evaluation. *Mar. Drugs.* **2019**, *17*(10), 1–17. DOI: 10.3390/md17100565.

[155] Khantaphant, S.; Benjakul, S. Comparative Study on the Proteases from Fish Pyloric Caeca and the Use for Production of Gelatin Hydrolysate with Antioxidative Activity. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* **2008**, *151*(4), 410–419. DOI: 10.1016/j.cbpb.2008.08.011.

[156] Aewsiri, T.; Benjakul, S.; Visessanguan, W.; Wierenga, P. A.; Gruppen, H. Antioxidative Activity and Emulsifying Properties of Cuttlefish Skin Gelatin Modifyed by Oxidised Phenolic Compounds. *Food Chem.* **2009**, *117*, 160–168. DOI: 10.1016/j.ifset.2010.04.001.

[157] Chen, K.; Yang, X.; Huang, Z.; Jia, S.; Zhang, Y.; Shi, J.; Hong, H.; Feng, L.; Luo, Y. Modification of Gelatin Hydrolysates from Grass Carp (*Ctenopharyngodon Idellus*) Scales by Maillard Reaction: Antioxidant Activity and Volatile Compounds. *Food Chem.* **2019**, *295*, 569–578. DOI: 10.1016/j.foodchem.2019.05.156.

[158] Lassoued, I.; Mora, L.; Barkia, A.; Aristoy, M. C.; Nasri, M.; Toldrá, F. Bioactive Peptides Identified in Thornback Ray Skin's Gelatin Hydrolysates by Proteases from Bacillus Subtilis

and Bacillus Amyloliquefaciens. *J. Proteom.* **2015**, *128*, 8–17. DOI: 10.1016/j.jprot.2015.06.016.

[159] Herregods, G.; Van Camp, J.; Morel, N.; Ghesquière, B.; Gevaert, K.; Vercruysse, L.; Dierckx, S.; Quanten, E.; Smagghe, G. Angiotensin I-Converting Enzyme Inhibitory Activity of Gelatin Hydrolysates and Identification of Bioactive Peptides. *J. Agric. Food Chem.* **2011**, *59*(2), 552–558. DOI: 10.1021/jf1037823.

[160] Hong, F.; Ming, L.; Yi, S.; Zhanxia, L.; Yongquan, W.; Chi, L. The Antihypertensive Effect of Peptides: A Novel Alternative to Drugs? *Peptides.* **2008**, *29*(6), 1062–1071. DOI: 10.1016/j.peptides.2008.02.005.

[161] Murray, B.; FitzGerald, R. Angiotensin Converting Enzyme Inhibitory Peptides Derived from Food Proteins: Biochemistry, Bioactivity and Production. *Curr. Pharm. Des.* **2007**, *13*(8), 773–791. DOI: 10.2174/138161207780363068.

[162] Pangestuti, R.; Kim, S. K. Bioactive Peptide of Marine Origin for the Prevention and Treatment of Non-Communicable Diseases. *Mar. Drugs.* **2017**, *15*(3), 1–23. DOI: 10.3390/md15030067.

[163] Zhao, Y.; Li, B.; Liu, Z.; Dong, S.; Zhao, X.; Zeng, M. Antihypertensive Effect and Purification of an ACE Inhibitory Peptide from Sea Cucumber Gelatin Hydrolysate. *Process Biochem.* **2007**, *42*(12), 1586–1591. DOI: 10.1016/j.procbio.2007.08.011.

[164] Chen, J.; Wang, Y.; Ye, R.; Wua, Y.; Xia, W. Comparison of Analytical Methods to Assay Inhibitors of Angiotensin I-Converting Enzyme. *Food Chem.* **2013**, *141*(4), 3329–3334. DOI: 10.1016/j.foodchem.2013.06.048.

[165] Wijesekara, I.; Qian, Z. J.; Ryu, B.; Ngo, D. H.; Kim, S. K. Purification and Identification of Antihypertensive Peptides from Seaweed Pipefish (*Syngnathus Schlegeli*) Muscle Protein Hydrolysate. *Food Res. Int.* **2011**, *44*(3), 703–707. DOI: 10.1016/j.foodres.2010.12.022.

[166] Zhuang, Y.; Sun, L.; Li, B. Production of the Angiotensin-I-Converting Enzyme (Ace)-inhibitory Peptide from Hydrolysates of Jellyfish (*Rhopilema Esculentum*) Collagen. *Food Bioprocess Technol.* **2012**, *5*(5), 1622–1629. DOI: 10.1007/s11947-010-0439-9.

[167] Lin, L.; Lv, S.; Angiotensin-I-Converting Enzyme, L. B. (Ace)-inhibitory and Antihypertensive Properties of Squid Skin Gelatin Hydrolysates. *Food Chem.* **2012**, *131*(1), 225–230. DOI: 10.1016/j.foodchem.2011.08.064.

[168] Ngo, D. H.; Vo, T. S.; Ryu, B. M.; Kim, S. K. Angiotensin- I- Converting Enzyme (ACE) Inhibitory Peptides from Pacific Cod Skin Gelatin Using Ultrafiltration Membranes. *Process Biochem.* **2016**, *51*(10), 1622–1628. DOI: 10.1016/j.procbio.2016.07.006.

[169] Tavano, O. L. Protein Hydrolysis Using Proteases: An Important Tool for Food Biotechnology. *J. Mol. Catal. B Enzym.* **2013**, *90*, 1–11. DOI: 10.1016/j.molcatb.2013.01.011.

[170] Byun, H.-G.; Kim, S.-K. Structure and Activity of Angiotensin I Converting Enzyme Inhibitory Peptides Derived from Alaskan Pollack Skin. *BMB Rep.* **2002**, *35*(2), 239–243. DOI: 10.5483/BMBRep.2002.35.2.239.

[171] Lu, J.; Hou, H.; Fan, Y.; Yang, T.; Li, B. Identification of MMP-1 Inhibitory Peptides from Cod Skin Gelatin Hydrolysates and the Inhibition Mechanism by MAPK Signaling Pathway. *J. Funct. Foods.* **2017**, *33*, 251–260. DOI: 10.1016/j.jff.2017.03.049.

[172] Liping, S.; Qiuming, L.; Jian, F.; Xiao, L.; Yongliang, Z. Purification and Characterization of Peptides Inhibiting MMP-1 Activity with C Terminate of Gly-Leu from Simulated Gastrointestinal Digestion Hydrolysates of Tilapia (*Oreochromis Niloticus*) Skin Gelatin. *J. Agric. Food Chem.* **2018**, *66*(3), 593–601. DOI: 10.1021/acs.jafc.7b04196.

[173] Leirós, G. J.; Kusinsky, A. G.; Balañá, M. E.; Hagelin, K. Triolein Reduces MMP-1 Upregulation in Dermal Fibroblasts Generated by ROS Production in UVB-Irradiated Keratinocytes. *J. Dermatol. Sci.* **2017**, *85*(2), 124–130. DOI: 10.1016/j.jdermsci.2016.11.010.

[174] Power, O.; Nongonierma, A. B.; Jakeman, P.; Fitzgerald, R. J. Food Protein Hydrolysates as a Source of Dipeptidyl Peptidase IV Inhibitory Peptides for the Management of Type 2 Diabetes. *Proc. Nutr. Soc.* **2014**, *73*(1), 34–46. DOI: 10.1017/S0029665113003601.

[175] Wang, T. Y.; Hsieh, C. H.; Hung, C. C.; Jao, C. L.; Chen, M. C.; Hsu, K. C. Fish Skin Gelatin Hydrolysates as Dipeptidyl Peptidase IV Inhibitors and Glucagon-like Peptide-1 Stimulators

Improve Glycaemic Control in Diabetic Rats: A Comparison between Warm- and Cold-Water Fish. *J. Funct. Foods*. **2015**, *19*, 330–340. DOI: 10.1016/j.jff.2015.09.037.

[176] Sila, A.; Martinez-Alvarez, O.; Haddar, A.; Gómez-Guillén, M. C.; Nasri, M.; Montero, M. P.; Bougatef, A. Recovery, Viscoelastic and Functional Properties of Barbel Skin Gelatine: Investigation of Anti-DPP-IV and Anti-Prolyl Endopeptidase Activities of Generated Gelatine Polypeptides. *Food Chem.* **2015**, *168*, 478–486. DOI: 10.1016/j.foodchem.2014.07.086.

[177] Huang, R.; Rong, Q.; Han, X.; Li, Y. The Effects of Cod Bone Gelatin on Trabecular Microstructure and Mechanical Properties of Cancellous Bone. *Acta Mech. Solida Sin.* **2015**, *28*(1), 1–10. DOI: 10.1016/S0894-9166(15)60010-6.

[178] Noma, T.; Takasugi, S.; Shioyama, M.; Yamaji, T.; Itou, H.; Suzuki, Y.; Sakuraba, K.; Sawaki, K. Effects of Dietary Gelatin Hydrolysates on Bone Mineral Density in Magnesium-Deficient Rats. *BMC Musculoskelet. Disord.* **2017**, *18*(1), 1–6. DOI: 10.1186/s12891-017-1745-4.

[179] Sae-leaw, T.; Benjakul, S. Physico-Chemical Properties and Fishy Odour of Gelatin from Seabass (*Lates Calcarifer*) Skin Stored in Ice. *Food Biosci.* **2015**, *10*, 59–68. DOI: 10.1016/j.fbio.2015.02.002.

[180] Pan, J.; Jia, H.; Shang, M.; Li, Q.; Xu, C.; Wang, Y.; Wu, H.; Dong, X. Effects of Deodorization by Powdered Activated Carbon, β-Cyclodextrin and Yeast on Odor and Functional Properties of Tiger Puffer (*Takifugu Rubripes*) Skin Gelatin. *Int. J. Biol. Macromol.* **2018**, *118*(2017), 116–123. DOI: 10.1016/j.ijbiomac.2018.06.023.

[181] Zhao, W.; Xu, R.; Zhang, L.; Zhang, Y.; Wang, Y. Progress in Organic Coatings Preparation and Drag Reduction Performance of Biomimetic Coatings Derived from Gelatin-3, 4-Dihydroxyhydrocinnamic Acid Gels. *Prog. Org. Coatings*. **2019**, May, 105442. DOI: 10.1016/j.porgcoat.2019.105442.

[182] Lin, L.; Regenstein, J. M.; Lv, S.; Lu, J.; Jiang, S. An Overview of Gelatin Derived from Aquatic Animals: Properties and Modification. *Trends Food Sci. Technol.* **2017**, *68*, 102–112. DOI: 10.1016/j.tifs.2017.08.012.

[183] Sow, L. C.; Nicole Chong, J. M.; Liao, Q. X.; Yang, H. Effects of κ-Carrageenan on the Structure and Rheological Properties of Fish Gelatin. *J. Food Eng.* **2018**, *239*, 92–103. DOI: 10.1016/j.jfoodeng.2018.05.035.

[184] Sinthusamran, S.; Benjakul, S.; Swedlund, P. J.; Hemar, Y. Physical and Rheological Properties of Fish Gelatin Gel as Influenced by κ-Carrageenan. *Food Biosci.* **2017**, *20*, 88–95. DOI: 10.1016/j.fbio.2017.09.001.

[185] Sow, L. C.; Toh, N. Z. Y.; Wong, C. W.; Yang, H. Combination of Sodium Alginate with Tilapia Fish Gelatin for Improved Texture Properties and Nanostructure Modification. *Food Hydrocoll.* **2019**, *94*, 459–467. DOI: 10.1016/j.foodhyd.2019.03.041.

[186] Phawaphuthanon, N.; Yu, D.; Ngamnikom, P.; Shin, I. S.; Chung, D. Effect of Fish Gelatine-Sodium Alginate Interactions on Foam Formation and Stability. *Food Hydrocoll.* **2019**, *88*, 119–126. DOI: 10.1016/j.foodhyd.2018.09.041.

[187] Sow, L. C.; Peh, Y. R.; Pekerti, B. N.; Fu, C.; Bansal, N.; Yang, H. Nanostructural Analysis and Textural Modification of Tilapia Fish Gelatin Affected by Gellan and Calcium Chloride Addition. *LWT - Food Sci. Technol.* **2017**, *85*, 137–145. DOI: 10.1016/j.lwt.2017.07.014.

[188] Sow, L. C.; Tan, S. J.; Yang, H. Rheological Properties and Structure Modification in Liquid and Gel of Tilapia Skin Gelatin by the Addition of Low Acyl Gellan. *Food Hydrocoll.* **2019**, *90*, 9–18. DOI: 10.1016/j.foodhyd.2018.12.006.

[189] Huang, T.; Tu, Z.; Zou, Z.; Shangguan, X.; Wang, H.; Bansal, N. Glycosylated Fish Gelatin Emulsion: Rheological, Tribological Properties and Its Application as Model Coffee Creamers. *Food Hydrocoll.* **2020**, *102*, 105552. DOI: 10.1016/j.foodhyd.2019.105552.

[190] Chen, H.; Shi, P.; Fan, F.; Chen, H.; Wu, C.; Xu, X.; Wang, Z.; Du, M. Hofmeister Effect-Assisted One Step Fabrication of Fish Gelatin Hydrogels. *LWT - Food Sci. Technol.* **2020**, *121*, 108973. DOI: 10.1016/j.lwt.2019.108973.

[191] Montero, P.; Alvarez, C.; Martí, M. A.; Borderías, A. J. Plaice Skin Collagen Extraction and Functional Properties. *J. Food Sci.* **1995**, *60*(1), 1–3. DOI: 10.1111/j.1365-2621.1995.tb05593.x.