

1 *Review*

2 **Marine gelatine from rest raw materials**

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10 **Abstract:** In recent years, demand for consumption of marine foods, and especially fish, has
11 substantially increased worldwide. The majority of collagen available is sourced from
12 mammalian-derived products. Although fish derived gelatine is a viable alternative to mammalian
13 sourced gelatine, there are some challenges related to the use of fish gelatine including odour,
14 colour, gelling and film forming properties as well as consistency in gelatine amino acid
15 composition. Chemicals used for pre-treatment, as well as extraction conditions such as
16 temperature and time, can influence the length of polypeptide chains that result and the functional
17 properties of the gelatine. Compared to mammalian sources, gelatines derived from fish show
18 notable differences in physical and chemical properties, and great care should be paid to
19 optimization of the production process in order to obtain a product with the best properties for
20 intended applications. The focus of this review is to explore the feasibility of producing gelatine
21 sourced from marine processing by-products using different pre-treatment and extraction
22 strategies with the aim of improving the techno-functional properties of the final product and
23 improving the clean-label status of gelatines. The bioactivities of gelatine hydrolysates are also
24 discussed.

25 **Keywords:** gelatine; marine; by-products; fish; industry; extraction

26

27 **1. Introduction**

28 In recent years, demand for consumption of marine foods, and especially fish, has substantially
29 increased worldwide. This increase can be mainly attributed to the recognition of fish as important
30 in human health [1]. Another important factor is globalization of world food trade which has
31 resulted in lower prices and better accessibility of marine commodities around the world. Fish
32 consumption worldwide has seen an annual increase at an average rate of 3.2% since the early 1960s
33 [2], and this trend is likely to follow the growing global demand, driven by the increase in human
34 population and consumer purchasing power. Production of gelatine is becoming an increasingly
35 interesting perspective of adding economic value to by-products generated by the fishing industry.

36 The majority of collagen available is sourced from mammalian-derived products including pig skin,
37 cattle hide and cattle bones. Hayatudin [3] reports that approximately 41% of the gelatine produced
38 in the world is sourced from pig skin, 28.5% from bovine hides and 29.5% from
39 bovine bones. The production of fish-derived gelatine currently accounts for only 1.5% of total
40 annual gelatine production worldwide, which is estimated to be around 270, 000 metric tonnes [4].

41 The European Union has introduced the Common Fisheries Policy (CFP). This current policy
42 stipulates that between 2015 and 2020 catch limits should be set that are sustainable and which can
43 maintain fish stocks in the long term. The CFP has four principle policy areas: 1) fisheries
44 management, 2) international policy 3) market and trade policy and 4) funding policy. An important

45 part of the fisheries policy is related to the discards and landing obligation. Discarding is the practice
46 of returning unwanted catches to the sea (either dead or alive), due to lack of market demand,
47 undersized fish samples or because of the catch composition rules. The aim of the CFP is to first
48 gradually and then completely eliminate the practice of wasteful discarding. This should be attained
49 through the implementation of the landings obligation for all common fisheries from 2015 to 2019.
50 The landing obligation requires all catches of regulated commercial species on-board to be landed
51 and counted against quota, with undersized fish specimens that cannot be marketed for direct
52 human consumption, and obligation of certain protected species to be returned back to the sea. By
53 2019 all species subject to TAC (Total Allowance Catch) limits and Minimum Conservation
54 Reference Sizes in the Mediterranean will be subject to the landing obligation [5].

55 1.1 Opportunities for by-catch utilization

56 By-products from fish and shellfisheries processing represent a serious environmental and economic
57 problem due to inadequate disposal options and/or costs associated with disposal at landfills.
58 Processing leftovers including bloodwaters, trimmings, fins, frames, heads, shells, skin, viscera, and
59 stickwater/effluent are currently used in Ireland for the production of fish meal, fish oil, fertilizer,
60 and animal feeds [6]. Another important source of by-products is the solid waste from surimi
61 processing, which can amount for 50 to 70% of the original raw material [7]. Boarfish (*Capros aper*)
62 and blue whiting (*Micromesistius poutassou*) are two pelagic species which represent specific
63 challenges for the fish processing industry. They are currently viewed as lower value species, due to
64 their small size which makes their processing demanding, although some advances have been made
65 in the field of production of blue whiting skinless fillets [8]. Another option for processing of these
66 species would be for production of surimi products, especially in the case of small specimens which
67 are unsuitable for machine filleting operations.

68 Boarfish (**Figure 1.**) [9] are relatively small, long-lived deep bodied fish growing up to 23 cm in total
69 length. They are usually orange to red in colour, with large eyes and a highly protrusible mouth, and
70 are known to inhabit shallow shelf seas to shelf slopes from 40-600 m. This is a mesopelagic shoaling
71 species distributed in the eastern Atlantic from Norway to Senegal including the Mediterranean [10].
72 Although it is considered a sub-tropical fish species, in recent decades boarfish has become very
73 abundant throughout its range, which may be explained by rising ocean temperatures due to climate
74 change [11]. Although the 2017 boarfish quota for Ireland is 36% lower than previous year's quota,
75 the allowed 18850 tonnes limit is still among the highest among European countries [12]. The main
76 utilization of landed boarfish in Ireland includes export to Denmark for production of fishmeal [13],
77 but other potential uses are also considered. The Irish Sea Fisheries Board (Bord Iascaigh Mara, BIM)
78 currently recommends use of Boarfish for direct human consumption, with marketing options either
79 in the form of commodity products including 20 kg blast frozen blocks of mince or as a headed and
80 gutted product suitable for frying [14]. Other authors have recently discussed alternative means of
81 boarfish biomass exploitation, which include hydrolysis of its proteins to obtain protein
82 hydrolysates and extraction of valuable peptides and biomolecules [15, 16]. However, large-scale
83 production of gelatine from boarfish by-products is not sufficiently researched as an option for
84 valorisation of this biomass currently.



85

86 **Figure 1.** Boarfish (*Capros aper*) [9]

87 The focus of this review is to explore the feasibility of producing gelatine sourced from marine
88 processing by-products specifically from blue whiting and boarfish processing by-products
89 including skins and bones.

90 2. Properties and applications of marine-derived gelatine

91 Gelatine is a soluble protein compound obtained by partial hydrolysis of collagen, the main fibrous
92 protein constituent in bones, cartilages and skins of animals [7]. Collagen is the most abundant
93 protein in mammals and is the major protein constituent of skin, cartilage tissues, blood vessels and
94 teeth. It is found with other proteins such as elastin and proteoglycans around the cells in tissues
95 where it forms the extracellular matrix. The collagen molecule is a triple helix, with three α -chains
96 that adopt a three-dimensional structure suitable for intramolecular hydrogen bonding [6]. It
97 contains all of the 20 natural amino acids, with a particularly high percentage of glycine,
98 hydroxyproline, and proline. Collagen mostly consists of tri-peptides with frequent repetitions of
99 the sequence –Gly-Pro-X or Gly-X-Hyp and the distribution of polar and non-polar amino acid
100 residues at the X position determines the order of aggregation of the molecule. Denaturation causes
101 total or partial separation of the collagen chains due to destruction of the hydrogen bonds, causing
102 loss of the triple-helix conformation, and following denaturation, the polymers adopt a coiled form
103 [17]. Gelatine stability is thought to be influenced by the proportion of total amino acids and these
104 can vary depending on the source of collagen. The manufacture of gelatine includes treatment of
105 raw animal hides with dilute acid or alkali, which causes a partial cleavage of the crosslinks in
106 collagen structure, resulting in formation of “warm-water-soluble collagen”, i.e. gelatine [18]. It is
107 known that various marine processing by-products, such as fish skin, bones, scales, surimi
108 production discharge waste and squid skin represent a good source of gelatine [17,19–21].

109 In general, gelatine is used in the food, pharmaceutical and photography industry for a number of
110 applications including jelly production, encapsulation, and fruit juice clarification, dairy processing,
111 soup manufacture, photography and others. Typical applications of gelatine, depend on the gelatine
112 type, and some are shown in **Table 1** [22]. Its great versatility enables use in both the food and
113 pharmaceutical industry. In the food industry, gelatine is considered an essential ingredient, and can
114 also be considered a “clean label” product, since:

- 115 • Gelatine is not chemically modified and has no, possibly harmful, by-products of chemical
116 modification
- 117 • it does not contain and is not made of any genetically modified organisms

- 118 • Is not a food additive and therefore does not require an E-number
- 119 • It is considered Generally Recognised As Safe (GRAS)
- 120 • It does not cause any known allergies
- 121 • It has been consumed for more than 2000 years and is known for generations [23]

122

123 **Table 1:** Usage of gelatine depending on type [22]

Type of gelatine	Typical usage
Food grade	Confectionary, gelatine desserts, gelatine in meats, clarification of beverages and juices, special dietary uses
Pharmaceutical	Gelatine capsules (hard and soft type), tablets and tablet coating, suppositories, gelatine emulsions, microencapsulation, absorbable gelatine sponge and films, plasma substitute, pastilles and troches, bacterial growth media
Photographic	Photographic emulsions
Other (technical)	Coating and sizing, paper manufacture, printing processes, colloidal applications, matches, coated abrasives, adhesives, films and light filters, cosmetics, microencapsulation

124

125 *2.1 Legislative and safety considerations of marine gelatine production*

126 Although production and use of gelatine is a highly regulated field, additional challenges may lie
 127 ahead due to changes in consumer trends in recent times. Edible gelatine must meet the
 128 requirements laid by the Food Hygiene Regulation (EC) No 853/2004 (also Commission Regulation
 129 (EU) 2016/355 of 11 March 2016 amending Annex III to Regulation (EC) No 853/2004) and is
 130 additionally subject to European food regulations [23]. Pharmaceutical gelatine, in addition to these
 131 requirements, must also comply with the stringent requirements of the pharmacopoeias. The
 132 Regulation (EC) No 853/2004 prescribes the necessary critical points of control during gelatine
 133 manufacture. It addresses all aspects, from the raw materials to the delivery of the final product:
 134 origin, transport and storage of raw materials, manufacturing conditions, chemical requirements for
 135 gelatine and collagen peptides as well as packaging, storage and transport. The important safety
 136 parameters, such as levels of heavy metals and toxic contaminants and microbiological safety are
 137 covered by this regulation and complementary regulations, such as (EC) No. 2073/2005 [24].
 138 Additional requirements apply for gelatine that is used for pharmaceutical purposes; these are laid
 139 down in specific regulations. Gelatine is a well-known material with an excellent safety record and is
 140 GRAS for human use [25]. Other chemicals typically used for gelatine production are known and
 141 approved food additives which do not possess chronic toxicity and include: hydrochloric acid
 142 (E507), citric acid (E330), sodium hydroxide (E524) and calcium hydroxide (E526). Some of the
 143 enzymes which can be used for gelatine production, such as proteases from *Aspergillus oryzae*, are
 144 also included in the list of approved food additives under Regulation (EC) No 234/2011 [24].

145 Additionally, since the process of gelatine manufacture includes washing of the material after every
146 treatment step, as well as purification of the gelatine solution itself, these chemicals and enzymes are
147 removed from the final product.

148 Fish and fish products are known to be a common source of allergic reactions in consumers. The
149 Regulation (EU) No 1169/2011 on the provision of food information to consumers has entered into
150 application on 13 December 2014. Under this regulation, the obligation to provide nutrition
151 information, as well as stating the possible food allergens is mandated. Fish and fish products
152 (except fish gelatine used as a carrier for vitamin or carotenoid preparations and fish gelatine or
153 Isinglass used as fining agent in beer and wine) must be declared if present in the food. Fish allergy
154 is a pathophysiological immune response to specific fish proteins, mediated by IgE-type antibodies.
155 Humans can become sensitized by allergen exposure via the gastro-intestinal tract during ingestion,
156 which is the major route of sensitization, or via the respiratory system by fish aeroallergens or skin
157 contact [26]. Parvalbumins are recognized as the most important group of fish proteins with allergic
158 potential, but other proteins, such as collagen, transferrin, fish enolases and aldolases have also
159 shown allergic potential. Parvalbumins are highly stable, low-molecular-weight proteins (10–12
160 kDa), which are mostly found in fish muscle, but their content is significantly lower in pelagic fish
161 compared to warm water and freshwater species, since the highest concentrations can be found in
162 white muscle tissue [26]. Also, during the recent years, 50 kDa enolases and 40 kDa aldolases were
163 identified as important fish allergens in cod, salmon, and tuna [27]. Fish collagen was identified as
164 an allergen during the early 2000s, which may be a limiting factor for consumption of fish derived
165 gelatine in sensitive populations. The T-cell epitopes present in collagen are likely to be resistant to
166 digestion by proteolytic enzymes, potentially inducing sensitization [28].

167 2.2 Comparison of fish and mammalian gelatine

168 Physical and chemical properties of mammalian gelatines have been extensively researched and
169 although fish-derived gelatines have also been extensively studied, the majority of results have been
170 published recently [17,21]. Fish derived gelatine is a viable alternative to mammalian sourced
171 gelatine. However, there are some challenges related to the use of fish gelatine and these include
172 odour, colour, gelling and film forming properties as well as consistency in gelatine amino acid
173 composition. Compared to mammalian sources, gelatines derived from fish show notable
174 differences in physical and chemical properties, and great care should be paid to optimization of the
175 production process in order to obtain a product with the best properties for intended applications.
176 Gelatine is rated based on parameters including solubility, transparency, colour, odour and taste,
177 and functional properties including rheology, moisture, ash, protein, pH, setting point and time,
178 melting point and time, gel strength and viscosity. Physical and chemical properties of gelatine are
179 mostly influenced by the animal species from which they are derived. It is known that, in general,
180 fish based gelatines have lower melting temperatures and strengths compared to their commercial
181 pig skin and bovine counterparts [29]. Warm water fish gelatine is reported in the literature to have
182 better functional properties than cold-water fish gelatines [30,31]. The principal reason for these
183 differences is that, in general, fish gelatines have a lower content of imino-acids (hydroxyproline and
184 proline) than mammalian gelatines. Therefore, gelatine with low levels of imino acids tends to have
185 lower gel strengths and melting points. The molecular weight distribution is also important in
186 determining the gelling behaviour of gelatine. Muyonga et al. [32] reported that the content of
187 hydroxyproline and proline is approximately 30% in mammalian gelatines, 22-25% in warm water
188 fish gelatines and only around 17% in cold water fish gelatines (such as cod). Relative lack of these
189 amino acids is partially compensated for by higher concentrations of serine and threonine. For this
190 reason, gelatines obtained from cold water fish act as viscous liquids at room temperature, limiting
191 their use in food industry [30]. Higher amount of hydrophobic amino acids can, however be a
192 potential advantage in certain scenarios. Avena-Bustillos et al. [30] have investigated water vapour
193 permeability of cold- and warm-water fish skin gelatines films and compared them with different
194 types of mammalian gelatines. Films obtained from cold-water fish species (Alaskan Pollock and

195 salmon) gelatines showed lower water vapour permeability compared to warm water fish and
196 mammalian gelatines. The authors concluded that, although physical properties of these gels were
197 inferior, the lower water vapour permeability of fish gelatine films can be useful particularly for
198 applications related to reducing water loss from encapsulated drugs and refrigerated or frozen
199 foods. However, a contrasting report has been published by Atma [31] on the comparison of amino
200 acid and proximate composition in several warm water fish species. Among the investigated fish
201 species, King weakfish and Lizard fish were found to have the highest hydroxyproline and protein
202 content, which did not correspond to their respective gel strengths. The author has concluded that
203 imino acid content may not be the main factor influencing gel strength in all cases, and that multiple
204 other factors, including other amino acids, extraction conditions and molecular weight distribution
205 may also play an important role in gelatine production.

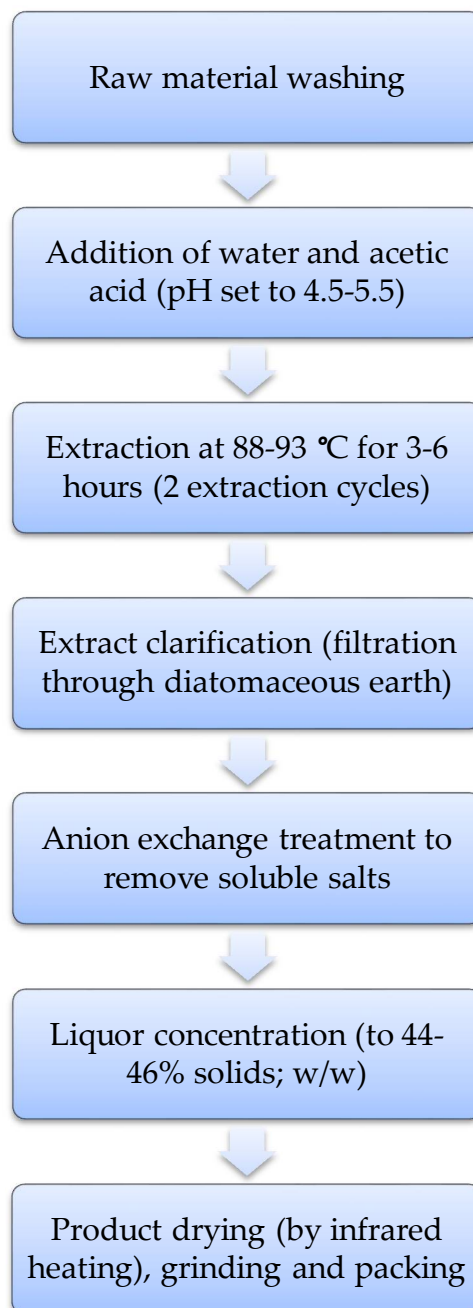
206 The most widespread single use of gelatine in the food industry is in water gel desserts, due to its
207 unique melt-in-the-mouth property [7,21]. Fish based gelatines have a disadvantage in this regard
208 due to their lower gel strength and melting temperature. For this reason, numerous attempts have
209 been made to improve their gel-forming and viscoelastic properties. This can be overcome by
210 increasing gelatine concentrations or by using gelatine mixtures (of cold and warm-water fish).
211 Zhou & Regenstein [33] have compared different textural properties of gelatine desserts obtained
212 from cold- (Alaskan pollock) and warm-water (tilapia) fish species with commercial
213 mammalian-based gelatines. Gel strength and rheological properties of cold-water fish gelatines
214 were less desirable compared to pure pig skin and tilapia gelatines, but mixtures of said gelatines
215 exhibited much improved properties. The authors concluded that desserts made from fish gelatines
216 would be more similar to desserts made from high bloom pork skin gelatine by a) increasing the
217 concentration of gelatine or b) by using gelatine mixtures. In addition, the gel desserts made from
218 fish gelatines melted at lower temperature, which may accelerate the flavor release in such food
219 products. Although cold-water fish gelatines tend to possess lower gel strength compared to
220 warm-water fish gelatines, cold maturation time should also be considered when creating gelatine
221 based products. Gómez-Guillén et al. [29] have reported on the importance of prolonged maturation
222 at low temperature in the case of hake gelatine. They concluded that longer maturation time might
223 be required to allow growth of existing nucleation sites within gelatine, since cold-water fish
224 gelatine possesses a lower percentage of β - and γ - components compared to individual α -chains as
225 found in hake gelatine.

226 The gelling temperature of cold-water fish gelatine is usually below 8-10 °C, which enables it to be
227 used as a base for light-sensitive coatings, since it is a good medium for precipitation of silver halide
228 emulsions at lower temperature than warm-blooded animal gelatine [34]. On the other hand, this
229 limits the use of such gelatines as gelling components in food production. Despite being a
230 techno-functional disadvantage, lower melting and setting points of fish gelatine may be useful in
231 development of certain food products, due to a better release of aromas and imparting stronger
232 flavour [35]. Absorption of ingested fish collagen is up to 1.5 times more efficient, indicating its
233 superior bioavailability over bovine or porcine types. Due to its more efficient absorption, it is
234 considered to be the best source of collagen for pharmaceutical applications [36].

235 Religious concerns and disease outbreaks including bovine spongiform encephalopathy (BSE) have
236 resulted in a desire for gelatine replacement hydrocolloids and alternatives to mammalian sourced
237 gelatine. Although the physical properties of most of the cold-water fish sourced gelatines are not
238 ideal compared to mammalian gelatines (pig skin, cattle hide) their advantage is almost universal
239 acceptability in terms of religious beliefs [37]. The Gelatine Manufacturers of Europe (GME) is an
240 association of European gelatine and hydrolyzed collagen manufacturers and was founded in 1974.
241 The eleven leading gelatine and collagen peptide manufacturers in Europe belong to GME. They
242 account for more than 98% of the European and approximately 33% of worldwide gelatine/collagen
243 peptide production [23].

244 3. Production strategies for gelatine

245 Industrial production of gelatine is a well-known process, and in general, includes raw material
246 washing, pre-treatment, extraction and purification followed by drying and packing of the final
247 product. Although the parameters of the steps vary greatly between manufacturers, the choice of
248 raw material dictates the pre-treatment procedure and influences the complexity of production.
249 Unlike bovine and porcine sources, fish skins used for industrial production of gelatine are often not
250 subjected to harsh pre-treatment, due to weaker bonds in this type of collagen. Simplified scheme of
251 fish gelatine production is shown in **Scheme I**. [38].



252

253 **Scheme I:** Basic steps of fish gelatine production process [38]

254 To properly assess the economic feasibility of industrial-scale fish gelatine production, numerous
255 factors, such as raw material availability and price, production costs and final product price margin,
256 need to be accounted. Although fish gelatine amounts to only a fraction of worldwide gelatine

257 manufacturing, the high quantities of by-products generated by fisheries represent a potentially
258 lucrative opportunity for its market increase. Recent work of the Trash2Cash project (2011-2015) in
259 Denmark has undertaken considerable research concerning the economic feasibility of gelatine
260 production from fish sources [39,40]. Findings from this project show that the market for fish
261 gelatine and fish collagen hydrolysates is small, (2000 to 3000 tons per year), and that prices of final
262 products vary from 10 to 15 € per kg, depending on traceability, degree of hydrolysis, taste and
263 purity [39]. As a part of the same project, financial and economic aspects of construction of a fish
264 gelatine plant have been evaluated. Using a “greenfield” model (model which assesses costs of
265 constructing a plant from nothing at starting point - i.e., “green field”) estimates of investments,
266 operating costs and revenues were made [40]. In general, the estimation showed that, when major
267 equipment and variable costs are taken into account, the final revenue would operate with a
268 financial margin of almost 50%, provided that the operation of the plant is at full capacity. This
269 operating revenue is estimated with fish gelatine prices set between 10-12 €/kg, in the case that the
270 raw material (fish skin) costs are 2.25-2.5 DKK (0.30-0.34 €) per kilogram and yield of produced
271 gelatine is 10% [40]. These estimations indicate that market prices of raw material and produced
272 gelatine have the most pronounced influence on the final operating revenue. However, the expected
273 yield of gelatine extraction can also be a major factor for considerations since it is dependent on
274 multiple variables, such as raw material quality, composition and origin. Having this in mind,
275 careful optimization of production steps (pre-treatment, extraction) has to be taken into account for
276 future production planning.

277 During gelatine production, the insoluble native collagen must be pre-treated before it can be
278 converted into a form suitable for extraction [7,21]. This is routinely done by heating in water at
279 temperatures higher than 45 °C. A chemical pre-treatment is intended to break non-covalent bonds
280 in order to disorganize the protein structure, and produce adequate swelling and collagen
281 solubilisation [7,17]. Since gelatine is obtained by denaturation of collagen, its properties are greatly
282 influenced not only by the species or tissue from which it is extracted, but also by the extraction
283 process, which may depend on pH, temperature, and time during both the pre-treatment and
284 extraction processes [21,29].

285 3.1 Pre-treatment and extraction strategies

286 Differences in the available literature are seen between different pre-treatment procedures regarding
287 the same type of fish material (skin, bones, offal). In general, during the production of gelatine, the
288 pre-treatment steps are important for weakening the chemical bonds between collagen chains and
289 make it more suitable for subsequent extraction. There are two main pre-treatments used in the
290 gelatine industry today: a) Acid pre-treatment, which is done by treatment of the material with
291 diluted acids. It is suitable for materials with less cross-linked collagen, like pig skin, and results in
292 the so called type A gelatine (with isoelectric point at pH 6–9) [41]. Acid pre-treatment is also
293 necessary in the case of gelatine production from bones, where it ensures the removal of bone
294 mineral components prior to extraction; b) Alkali pre-treatment, which is achieved by soaking of the
295 treated material with diluted alkali solutions (NaOH, KOH, Ca(OH)₂). It is commonly used as a
296 pre-treatment of materials with highly cross-linked collagen, such as bovine hides. Gelatine obtained
297 by this type of pre-treatment is called type B, with an isoelectric point at pH 5 [41]. Various types of
298 pre-treatment and extraction strategies for gelatine isolation from marine/freshwater sources are
299 shown in **Table 2**.

300

301

302

303

304 Table 2: Examples of gelatine pre-treatment and extraction strategies

Authors/year	Material	Pre-treatment	Extraction
Alfaro et al. (2014) [42]	African catfish (<i>Clarias gariepinus</i>) skin	NaOH at various concentration and time range (0.15-0.35% (w/v) and 40-120 min); Sulphuric acid at various concentration and time range (0.08-0.35% (w/v) and 40-120 min); Citric acid at various concentration and time range (0.6-1.4% (w/v) and 40-120 min)	Water at various temperature and time range (33-67 °C and 4-14h)
Chandra and Shamasundar (2015) [43]	Swim bladders of catla (<i>Catla Catla</i>)	0.15% NaOH (w/v) for 40 min; sulphuric acid (0.15%, v/v) and citric acid (0.5%, v/v) for 40 min (x2)	Water, 45-50 °C for 17h
Giménez et al. (2005) [44]	Dover sole (<i>Solea vulgaris</i>) skin	a) Acetic acid 0.05M b) Lactic acid at various concentrations (0.01, 0.025, 0.05M)	Water, 45 °C overnight
Haddar et al. (2012) [45]	Tuna (<i>Thunnus thynnus</i>) head bones	Alkaline protease from <i>Bacillus mojavensis</i> , 50 °C for 4 h; 0.4M HCl for 7.5h; 0.9% Ca(OH) ₂ (w/v) for 144h	Water, 75 °C for 4h
Jaswir et al. (2009) [4]	Skins of several marine species (kerapu (<i>Epinephelus sexfasciatus</i>), jenahak (<i>Lutjanus argentimaculatus</i>), kembung (<i>Rastrelliger kanagurta</i>), kerisi (<i>Pristipomodes typus</i>)	0.2% NaOH (w/v) for 40 min; sulphuric acid (0.2%, v/v) and citric acid (1%, v/v) for 40 min (x2)	Water, 45 °C for 18h
Jongjareonrak et al. (2006) [41]	Brownstripe red snapper (<i>Lutjanus vitta</i>) and bigeye snapper (<i>Priacanthus macracanthus</i>) skin	0.2 M NaOH (3 x 30 min); 0.05 M acetic acid for 3h	Water, 45 °C for 12 h

Khiari et al. (2013) [46]	Mackerel (<i>Scomber scombrus</i>) and blue whiting (<i>Micromesistius poutassou</i>) bones	a) 0.1 N NaOH for 30 min; 0.25M HCl for 18h b) Flavourzyme/alcalase at an enzyme/substrate ratio of 0.1% (v/w) for 4h (50 °C); 0.25M HCl for 18h	Water, 45 °C for 18 h
Kittiphattanabawon et al. (2016) [47]	Clown featherback (<i>Chitala ornata</i>) skin	0.1 M NaOH for 2h; 0.05M acetic acid for 30 min	Water at various temperature and time range (45, 65, 85 °C and 6h and 12h)
Kołodziejska et al. (2004) [48]	Baltic cod (<i>Gadus morhua</i>) skin	No pre-treatment (only manual cleaning of material)	Water at various temperature and time range (30–60 °C and 15–120 min)
Muyonga et al. (2004) [32]	Nile perch (<i>Lates niloticus</i>) skin and bone	Skin: 0.01 M sulphuric acid (pH of 2.5–3.0) for 16h Bones: 3% HCl for 9-12 days	Three sequential extractions for 5 h, at 50, 60 and 70 °C; followed by boiling for 5 h
Nagarajan et al. (2012) [20]	Splendid squid (<i>Loligo formosana</i>) skin	0.05 M NaOH for 6h; 0.05 M phosphoric acid for 24h	Water, with different temperatures (50, 60, 70 and 80 °C)
Niu et al. (2013) [49]	Tilapia (<i>Oreochromis niloticus</i>) skin	0.3 M NaOH for 1h; HCl, citric and acetic acid at various concentrations (0.01–0.20 M)	Water, 50 °C for 3h
Norziah et al. (2009) [19]	Herring species (<i>Tenulosa ilisha</i>) skin	0.2 M Ca(OH) ₂ for 1h; 0.1 M citric acid for 3h	Water, 50 °C for 3h
Norziah et al. (2014) [50]	Ribbon fish (<i>Lepturacanthus savel</i>) surimi processing waste	0.2 M Ca(OH) ₂ for 1h; 0.1 M citric acid containing bromelain in various concentrations for varying times	Water, at different combinations of temperatures and durations
Shakila et al. (2012) [51]	Red snapper (<i>Lutjanus campechanus</i>) and grouper (<i>Epinephelus chlorostigma</i>) bones	0.2% NaOH (w/v) for 45 min; sulphuric acid (0.2%, v/v) and citric acid (1%, v/v) for 45 min (x2)	Water, 45 °C for 24 h
Shyni et al. (2014) [35]	Skins of dog shark	0.1 M NaOH for 2h;	Water, 45 °C for

	(<i>Scoliodon sorrakowah</i>), skipjack tuna (<i>Katsuwonus pelamis</i>) and rohu (<i>Labeo rohita</i>)	0.05M acetic acid for 24h	12h
Sinthusamran et al., (2014) [52]	Seabass (<i>Lates calcarifer</i>) skin	0.1 M NaOH for 3h; 0.05M acetic acid for 2h	Water at various temperature and time range (45, 55 °C and 3, 6 and 12h)
		0.2	
Zhou and Regenstein (2005) [53]	Alaska Pollock skin	NaOH/ Ca(OH) ₂ at various concentrations for 60 min; acetic, citric and sulfuric acid at various concentrations for 60 min	Water, 50 °C for 3h

305

306 3.1.1 Chemical pre-treatment

307 Chemicals used for pre-treatment as well as extraction conditions such as temperature and time can
308 influence the length of polypeptide chains and the functional properties of gelatine [48]. The degree
309 of collagen cross-linking in the raw material is a key factor in deciding the pre-treatment process
310 required for gelatine manufacture, and is highly dependent on a number of factors, such as collagen
311 type, tissue, animal species, age [54]. In the case of fish skins, acid pre-treatment may be considered
312 as sufficient, and numerous authors have used it as the only form of pre-treatment. Gómez-Guillén
313 et al. [7] have investigated chemical and physical properties of gelatine obtained from several
314 different marine species, under mild swelling conditions using 0.05M acetic acid as pre-treatment,
315 followed by extraction in distilled water at 45 °C overnight. Their results showed that gelatines from
316 flat-fish species (sole and megrim) possessed higher strength and thermostability than those
317 obtained from cold-water fish species (cod and hake). Lactic acid at concentration of 0.025M has
318 been found to be suitable for pre-treatment of fish skins instead of the commonly used acetic acid
319 [54]. Higher concentration of lactic acid (0.05M), however, increase the level of hydrolysis and
320 therefore adversely affected the gel strength and viscoelastic properties. Citric acid may also be used
321 for the manufacture of food-grade gelatine from fish skin since it does not impart undesirable
322 sensory properties (colour, odour) to the extracted gelatine. Gómez-Guillæn and Montero [55] have
323 investigated the influence of several organic acids on the properties of gelatine extracted from
324 megrim (*Lepidorhombus boschii*) skin. They concluded that, among all tested organic acids, acetic and
325 propionic acid extracts produced gelatine with the best properties including viscoelastic, setting
326 and melting temperatures and gel strength properties. Although pre-treatment with citric acid has
327 shown to produce the least turbid gelatine, its physical properties were inferior to other investigated
328 acids. The influence of different acid pre-treatments was also investigated by Niu et al. [49] on
329 gelatine obtained from tilapia (*Oreochromis niloticus*) skin. The authors concluded that the
330 concentration of used acid had significant influence on gelatine recovery, gelatine viscosity and
331 molecular weight distribution. Gelatine prepared using too low or too high a concentration (e.g.
332 0.01M or >0.05 M HCl or citric acid) yielded a product with a lower ratio of large molecule
333 components, such as β -chains, and exhibited lower viscosity.

334 In the case when fish skin is used as a material for gelatine extraction, it is known that combinations
335 of alkali and acid pre-treatments have positive effects on the final product properties, and this type
336 of pre-treatment has been patented by Grossman et al. [56]. Zhou and Regenstein [53] have shown
337 that combinations of acid and alkali pre-treatment had a positive impact on the yield and gel
338 strength of gelatine extracted from Alaska Pollock. Shyni et al. [35] have reported on physical and
339 chemical differences between gelatines extracted from skins of dog shark (*Scoliodon sorrakowah*),
340 skipjack tuna (*Katsuwonus pelamis*) and rohu (*Labeo rohita*). Their results show that dog shark skin
341 gelatine had highest yield and gel strength, as well as other physical and chemical properties
342 (molecular weight, viscosity, melting point, foaming properties, water holding capacity, odour,
343 colour and clarity) compared to tuna and rohu gelatine, which could be explained by its high content
344 of hydroxyproline. Alkali pre-treatment is useful for removal of non-collagen proteins and fats,
345 while subsequent treatment with diluted acids provides mildly acidic pH of the medium which
346 enables good yield of gelatine extraction [35,57]. Gómez-Guillén et al. [58] have reported that
347 application of high pressure (250 and 400 MPa) either during acid pre-treatment or during water
348 extraction enabled significant shortening of the duration of time required for those steps, obtaining
349 good yield of gelatine in only a few minutes. Other collagen-rich tissues in fish by-products may also
350 be a feasible source of gelatine, especially if their industrial output is sufficiently abundant.
351 Extraction of gelatine from swim bladders of catla (*Catla catla*) using mild pre-treatment with NaOH,
352 sulphuric and citric acid is reported by Chandra and Shamasundar [43]. The obtained gelatine in
353 their study had satisfactory yield (13.5% (w/w)) and good gel strength (264.6 g), indicating that fish
354 swim bladders can also represent an underused source for production of fish gelatine.

355 Besides from fish skin, gelatine can also be extracted from mineralized structures such as fins, scales,
356 and bones. Although fish bone and scale represent a valuable source of gelatine, additional
357 demineralization should be introduced prior to gelatine extraction due to the high mineral content of
358 these tissues. Diluted hydrochloric acid is most often used for bone demineralization [45,46,51],
359 although other compounds, such as EDTA, have also been used for this purpose [59,60]. Although
360 recoveries of gelatine extracted from bones and scales are usually lower in comparison to skin
361 gelatines of the same species, bones and scales are nevertheless an important sources due to their
362 high percentage in the total industrial output of fish by-product generated from surimi
363 production[7]. Therefore, care must be taken in order to optimize the pre-treatment methods for
364 such composite samples in order to obtain the highest yield of gelatine with the best properties.

365 3.1.2 Enzymatic pre-treatment

366 Treatment with proteolytic enzymes, either alone or in combination with other pre-treatments
367 (alkaline, acidic, etc.) is another option for improving extraction yield and quality of the obtained
368 product. Enzymes are catalyst biomolecules which can speed the rate of biological reactions by
369 catalyzing a transition state with a lower energy of activation. They can also hydrolyze the covalent
370 cross-links in the terminal regions of proteins and facilitate the transformation of collagen to gelatine,
371 while producing less waste compared to the chemical treatments [61]. Khiari et al. [46] have
372 compared properties of gelatine extracted from bones of mackerel and blue whiting obtained using
373 non-enzymatic (HCl) and enzymatic pre-treatment using Flavourzyme (fungal protease/peptidase
374 complex obtained from *Aspergillus oryzae*). They concluded that gelatine obtained by enzymatic
375 pre-treatment of bones showed significantly higher emulsifying activity (EAI) and stability (ESI)
376 indices in comparison to acid pre-treatment. Gelatin extraction from bigeye snapper (*Priacanthus*
377 *tayenus*) skin was developed by Nalinanon et al. [62], using a pepsin-aided process (big eye snapper
378 pepsin, BSP) in combination with a protease inhibitor (pepstatin A and soybean trypsin inhibitor).
379 The bloom strength of pepsin-treated gelatine was greater than the gelatine extracted from bigeye
380 snapper skin by the conventional process, which had a substantial degradation of gelatine
381 components, and soybean trypsin inhibitor added during the extraction process significantly
382 reduced the degradation of α - and β -chains in the gelatine. Since most proteolytic enzymes are
383 usually able to cause significant degradation of gelatine α - and β -chains, careful optimization of

384 pre-treatment conditions is required to avoid this. Zhang et al. [62] have investigated pre-treatment
385 optimization of grass carp fish (*Ctenopharyngodon idella*) scales by protease A2G enzyme utilizing the
386 response surface methodology (RSM). The resulting gel strength (276 ± 12 g) and viscoelastic
387 properties were comparable to porcine skin gelatine at lower temperatures, while the imino acid
388 content, gelling and melting points were lower. Since surimi processing wastes represent composite
389 material of skin, scale, bone and muscle, enzymatic pre-treatment may be a good solution for
390 removal of non-collagenous proteins prior to gelatine extraction. Enzymatic digestion can also be
391 used as part of the pre-treatment, to remove interfering tissues before a more conventional chemical
392 treatment is used. Haddar et al. [45] have used alkaline protease from *Bacillus mojavensis* in their
393 work on extracting gelatine from tuna (*Thunnus thynnus*) heads, where the enzyme was used to
394 obtain clean bone material before demineralisation with HCl and subsequent treatment with
395 $\text{Ca}(\text{OH})_2$.

396 3.1.3 Extraction of gelatine

397 After pre-treatment of fish skins, extraction of gelatine with water at various temperatures and time
398 lengths is the universally applied approach for obtaining gelatine. Karim and Bhat [17] and
399 Karayannakidis and Zotos [21] have reported on the various procedures employed for gelatine
400 pre-treatment and extraction. Most commonly, distilled water was used and the temperatures and
401 lengths of extraction show a high variability between different authors. The most often used
402 extraction temperature in various research papers is around $45\text{ }^\circ\text{C}$, with the time of the extraction
403 varying from 12 to 18h (or “overnight”) [43,48,51,54]. Multi-stage extractions and different
404 temperatures have also been reported [32,63–65]. Hou and Regenstein [63] have developed an
405 optimized method for pre-treatment and extraction of gelatine from Pollock skin. They concluded
406 that an extraction temperature of $50\text{ }^\circ\text{C}$ was optimal regarding the extraction yield. Besides from
407 pure water, some authors have reported successful gelatine extraction using mild acidic conditions
408 [66] and also with addition of mixtures of protease inhibitors [53]. Due to the low denaturation
409 temperature of fish collagen, the extraction temperature and time can have a significant influence on
410 the properties of the extracted gelatine, especially on the gel strength. Gel properties of gelatine from
411 clown featherback skin under different extraction temperatures (45 , 65 and $85\text{ }^\circ\text{C}$) and times (6 and
412 12 h) were investigated by Kittiphattanabawon et al. [47]. Their results indicated that, although yield
413 was highest at the highest extraction temperatures, by increasing temperature and prolonging
414 extraction time, band intensity of α -, β - and γ -chains decreased in the extracted gelatines. Similar
415 findings were reported by Alfaro et al. [42], where temperature, extraction time and concentration of
416 acid during pre-treatment were used to assemble a central composite rotational design (CCRD) in
417 order to elucidate its effect on gelatine viscosity. The strong influence of pre-treatment and
418 extraction conditions on the yield and properties of fish gelatine need to be taken into consideration
419 in an industrial setting, and usually a compromise between yield, desired properties and energy
420 efficiency needs to be considered for optimal production.

421 3.2 Improving the properties of fish gelatine

422 Although there has been an increasing demand for fish gelatine due to its religious and safety
423 advantages over pig and bovine sources of gelatine, the main limiting factors of its widespread use
424 lies in its technofunctional properties –i.e., the lower gel strength and melting temperatures
425 compared to those for mammalian gelatines. This poses a challenge for commercial exploitation, and
426 various approaches have been proposed to date to overcome these issues. Ultraviolet (UV)
427 irradiation represents a physical, cost-effective, non-thermal, and environmentally friendly
428 technology that has received increased attention in the food sector during recent years. Bhat and
429 Karim [67] have investigated the effect of UV irradiation (at 30 and 60 minute interval lengths) on
430 the gel strength of fish gelatine granules. They concluded that the irradiated samples exhibited
431 significant improvements in the gel-strength, a reduction in viscosity, as well as changes in the
432 melting enthalpy. These results indicate the possibility of using simple UV radiation as a method to

433 improve cold fish gelatine properties. In their more recent work, Bhat and Karim [68] have also
434 investigated combination of UV irradiation and addition of sugars (ribose and lactose) on the
435 properties of fish gelatine based films. Their results indicated that films with added ribose showed
436 decreased solubility after UV treatment and exhibited higher swelling percentages than films with
437 added lactose. Otoni et al. [69] have also noted an improvement in functional properties of fish
438 gelatines from cold- (cod, haddock, pollock) and warm-water (tilapia) fish as a consequence of UVB
439 radiation exposure.

440 Gelling properties of fish based gelatines can be modified by use of various chemical agents which
441 induce molecular crosslinking, such as glutaraldehyde [70], as well as by creating mixtures with
442 various non-gelatine systems such as pectin [71]. Besides from natural polymers, several synthetic
443 polymers have been used to create gelatine hybrid hydrogels. Zohuriaan-Mehr et al. [72] have
444 reported a number of organic (PEG-dialdehyde, acrylamines, EDTAD, poly(acrylic acid)) and
445 inorganic (kaolin, silica gel) compounds which can affect gel strength, solubility, and
446 hydrophobicity of such composite hydrogels. Another means of improving gelling properties of fish
447 gelatine is to introduce enzymatic crosslinking using transglutaminase. This enzyme catalyses the
448 formation of crosslinking bonds between γ -amide groups of glutamine and ϵ -amino groups of
449 lysine. Baltic cod gelatine treated with transglutaminase was shown to be able to withstand heating
450 in boiling water for 30 minutes without melting [48]. As a collagen denaturation product, gelatine
451 contains many divalent metal ions such as calcium, copper, iron and zinc. These ions can form ionic
452 bonds with the gelatine carboxylic acid groups, thus influencing the organization of the gelatine
453 network. Removal of those metal ions by means of ion-exchange may improve further crosslinking
454 between gelatine molecules, as demonstrated by Xing et al. [73] who purified gelatine solutions
455 using Chelex resin to replace divalent metal ions with sodium ions prior to crosslinking by
456 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). On the other hand, the effect of different
457 salts on the rigidity or melting temperature of animal gelatines has also been researched previously
458 [74,75]. Koli et al. [75] have optimized a method for improving fish gelatine extracted from
459 Tiger-toothed croaker (*Otolithes ruber*), using combination of three co-enhancers (MgSO₄, sucrose,
460 and transglutaminase). By addition of co-enhancers at optimal concentrations in their experiments,
461 the gel strength and melting point were improved from 170 to 240.89 g and 20.3 to 22.7 °C,
462 respectively. Due to their better acceptability by consumers, natural compounds and extracts can
463 also be used to improve gelatine properties. Araghi et al. [76] examined the effects of natural
464 phenolic cross-linkers (ferulic and caffeic acid) on fish gelatines. In their study, caffeic acid had
465 notable effects in decreasing solubility, water vapour permeability, and oxygen permeability of fish
466 gelatine films. Natural phenolic compounds may therefore be used as a natural ingredient for
467 increasing safety of gelatine-based biodegradable packaging, by improving their barrier and
468 physicochemical properties. Another natural material, chitosan nanoparticles (CSNPs), with
469 excellent physicochemical properties, is known to be environmentally friendly, and bioactive, has
470 been researched for improving properties of fish gelatine based films. Hosseini et al. [77] have
471 created novel bio-nanocomposite films by addition of CSNP particles (created by ionic gelation
472 between chitosan and sodium tripolyphosphate) into fish gelatine film matrix. Newly created films
473 had significantly increased tensile strength and elastic modulus, and decreased water vapour
474 permeability compared to fish gelatine films.

475 With the exception of its inferior physical properties when compared to mammalian counterparts,
476 fish derived gelatine intended for food use often possesses undesirable sensory properties
477 characterized by an unpleasant "fishy" flavour [78]. Sae-leaw, Benjakul and O'Brien [79] have
478 investigated the effects of defatting and tannic acid incorporation during extraction on the properties
479 and fishy odour of gelatine obtained from seabass skin. They concluded that defatting by
480 pre-treatment with citric acid and isopropanol and subsequent incorporation of tannic acid during
481 the extraction prevented lipid oxidation and the subsequent development of volatile compounds
482 and fishy odours in the resulting gelatine. The intensity of fishy odour may also increase if the
483 storage of frozen raw materials is prolonged before processing, due to formation of volatile

484 aldehydes and alcohols [78]. Therefore, delays in processing should be avoided in order to minimize
485 formation of undesirable odour and further loss of technofunctional properties of gelatine.

486 4. Opportunities for novel applications of fish gelatine and collagen

487 Although gelatine has many applications in various industries, advances in food science, medicine
488 and material science have yielded a number of novel applications. Due to its versatile
489 physicochemical properties, high degree of biocompatibility and relatively low price, gelatine is an
490 ideal material for numerous applications.

491 Tissue engineering has been an emerging field of modern regenerative medicine. Collagen,
492 primarily that of type I, has long been used in biomedical applications as a hemostatic agent to treat
493 tissue injuries. After discovery of its regenerative properties, it was applied in 3D cultures for use in
494 regenerative medicine [80]. Recently, scaffolds consisting of natural polymers, such as collagen and
495 gelatine, bioabsorbable synthetic polymers, such as polylactic acid and polyglycolic acid, and
496 inorganic materials, such as hydroxyapatite, as well as composite materials have been rapidly
497 developed [81]. In particular, collagen is the most promising material for tissue engineering due to
498 its biocompatibility and biodegradability. However, due to the low denaturation and melting
499 temperatures, collagen of most fish species is not suitable for such applications in its native form. For
500 this reason, cross-linking of collagen by chemical or physical means is often studied for biomedical
501 applications. Chemical treatments induce high strength and stability to the collagen matrix but they
502 can result in potential cytotoxicity or poor biocompatibility, whereas physical treatments, such as
503 UV irradiation may produce good stability and no cytotoxicity [81]. Nagai et al. [82] have prepared
504 elastic vascular grafts from salmon collagen using mixtures of acidic collagen solution and
505 fibrillogenesis-inducing buffer containing a cross-linking agent (water-soluble carbodiimide, WSC).
506 These grafts induced little inflammatory reactions after subcutaneous placement in rat tissues.
507 Collagen was also used as a matrix for research investigating the possibility of regeneration of dental
508 pulp after pulpectomy, using stem cells [83]. Furthermore, 3D printing processes have found
509 numerous applications, including biomedical. Fish gelatine, which is more soluble and remains
510 liquid at lower temperatures compared to mammalian gelatines is a good potential candidate for
511 use as a biological dye for use in 3D printing of tissue scaffolds [84]. Visser et al. [85] have created
512 reinforced gelatine methacrylamide (GelMA) hydrogels with poly(ϵ -caprolactone) (PCL) fiber
513 scaffolds using melt electrospinning direct writing as a form of 3D printing. The stiffness and
514 elasticity of the created structures have approached those of articular cartilage tissue.

515 Beside the use of gelatine in its native form, fish gelatine hydrolysates, obtained by enzymatic
516 hydrolysis, offer an interesting option for by-product utilization by the fish-processing industry.
517 Numerous companies worldwide offer fish gelatine/collagen hydrolysates for use in nutraceutical
518 and for cosmetic purposes. Although the EU Commission has yet to approve many of the health and
519 cosmetic claims, some manufacturers are already selling their products with certain claims
520 supported by current research. Considering the higher cost of fish-derived gelatine in comparison to
521 mammalian sources, production of bioactive products for specialized food and pharmaceutical use
522 may represent a good opportunity for increasing its economic value. Such hydrolysates, consisting
523 of various peptides, are relatively cheap and easy to produce, and many have shown to possess
524 proven health and functional (antioxidant, antihypertensive, immunomodulatory and
525 antimicrobial) benefits. Bioactive peptides from food proteins offer great potential for incorporation
526 into functional foods and nutraceuticals [15,86]. Some of these products, such as sardine muscle
527 hydrolysate, have already been approved by FDA and EFSA for use in human nutrition [15]. Lee et
528 al. [87] have investigated angiotensin I converting enzyme (ACE I) inhibitory properties of tuna
529 frame hydrolysates obtained by several proteolytic enzymes (alcalase, neutrase, pepsin, papain,
530 α -chymotrypsin and trypsin). Their results showed that peptic hydrolysate exhibited the highest
531 ACE-I inhibitory activity, and a potent ACE-I inhibitory peptide composed of 21 amino acids was
532 subsequently isolated. Antioxidant activity of a hydrolysate from Nile tilapia (*Oreochromis niloticus*)

533 skin gelatine was examined by Choonpicharn et al. [88]. Hydrolysates obtained by several enzymes
534 (bromelain, papain, trypsin, flavourzyme, alcalase and neutrase) showed varying levels of
535 antioxidant (ABTS radical scavenging, reducing power, ferrous ion chelating activity, inhibition of
536 linoleic acid oxidation) activity and also a significant degree of ACE-I inhibitory activity. Beside their
537 health benefits, fish gelatine hydrolysates also exhibit many useful techno-functional properties
538 which may be utilized by the food industry. Hydrolysate of shark skin gelatine was tested as a
539 cryoprotectant on surimi subjected to different freeze-thaw cycles by Kittiphattanabawon et al [89],
540 and the results indicated that gelatine hydrolysates with 10% degree hydrolysis was able to prevent
541 the denaturation of surimi protein compared to a commercial cryoprotectant. Nikoo et al. [90]
542 reported that a tetrapeptide isolated from Amur sturgeon skin gelatine showed antioxidative and
543 cryoprotective effects in Japanese sea bass mince subjected to repeated freeze-thawing cycles. Such
544 properties of gelatine hydrolysates have excellent potential for use by the food industry for
545 improving shelf-life and oxidative stability of food products and commodities. Antimicrobial
546 activity of fish gelatine hydrolysates has also been demonstrated by Hong et al. [91].
547 Alcalase-derived glycosylated hydrolysates of fish gelatine had antioxidative and antimicrobial
548 activity when incubated with *Escherichia coli* and *Bacillus subtilis*, indicating its potential for use as
549 an antimicrobial agent.

550 5. Conclusions

551 By-products from fish and shellfisheries processing represent a serious environmental and economic
552 problem due to inadequate disposal options and/or costs associated with disposal at landfills.
553 Processing leftovers including bloodwaters, trimmings, fins, frames, heads, shells, skin, viscera, and
554 stickwater/effluent are currently mostly used for the production of fish meal, fish oil, fertilizer, and
555 animal feeds [6]. Gelatine is used in the food, pharmaceutical and photography industry for a
556 number of applications including jelly production, encapsulation, and fruit juice clarification, dairy
557 processing, soup manufacture, photography and others. Typical applications of gelatine, depend on
558 the gelatine type, and its great versatility enables use in both the food and pharmaceutical industry.
559 Edible gelatine must meet the requirements laid by the Food Hygiene Regulation (EC) No 853/2004
560 (also Commission Regulation (EU) 2016/355 of 11 March 2016 amending Annex III to Regulation
561 (EC) No 853/2004) and is additionally subject to European food regulations [23].

562 Production of gelatine from fishery by-products requires careful selection and optimization of
563 pre-treatment and extraction steps in order to obtain optimum yield and physico-chemical
564 properties. Numerous chemical, physical and enzymatic pre-treatment steps have been reported in
565 the scientific literature, although current industrial scale production usually resorts to most
566 cost-effective simple procedures. Depending on the intended use, properties of the fish derived
567 gelatine may be further improved and modified using various chemical and physical processes
568 which can impact its physical properties, such as bloom strength, elasticity and solubility. Beyond its
569 well established uses in food and pharmaceutical industry, fish gelatine has a potential use in several
570 emerging fields, such as biomedical science (tissue engineering/3D printing), owing to its unique
571 properties, good biocompatibility and relatively low price. Beside the use of gelatine in its native
572 form, fish gelatine hydrolysates, obtained by enzymatic hydrolysis, offer an interesting option for
573 by-product utilization by the fish-processing industry. Such hydrolysates, consisting of various
574 peptides, are relatively cheap and easy to produce, and many have shown to possess proven health
575 and functional (antioxidant, antihypertensive, immunomodulatory and antimicrobial) benefits.
576 Numerous companies worldwide offer fish gelatine/collagen hydrolysates for use in nutraceutical
577 and for cosmetic purposes, although the EU Commission has yet to approve many of the health and
578 cosmetic claims. Based on the recent scientific advances in production and novel fields of potential
579 use, gelatine derived from marine products represents an interesting option for industrial processors
580 for adding economic value to fishery by-products in the future.

581

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587

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