

# Sustainable Food Technology

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### Sustainability spotlight statement

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The review titled “Harnessing non-thermal techniques for the sustainable protein extraction from blue foods and their potential applications in circular food systems” advances sustainable food systems by exploring non-thermal, energy-efficient technologies for protein extraction, highlighting its role as a green technology, as it does not add any negative impact to the environment than thermal processing does. The techniques, such as ultrasound, pulsed electric field, high-pressure processing, and enzyme-assisted extraction, help in minimizing waste generation and reducing chemical usage while improving yield and quality. By valorising marine biomass and underutilized aquatic species, this work supports the transformation of low-value by-products into high-value food ingredients, strengthening the foundation of a circular bioeconomy. The sustainable extraction of marine proteins also fosters biodiversity preservation and responsible resource management. The study aligns directly with the United Nations Sustainable Development Goals (SDGs), particularly SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), and SDG 14 (Life Below Water), by promoting low-impact processing technologies that reduce environmental burdens. Furthermore, the proposed framework will serve as a basis for the exploration of the potential of integrating these eco-innovative technologies into industrial-scale applications, thereby reducing the dependence on land-based proteins and mitigating greenhouse gas emissions associated with conventional processing. The implementation of such sustainable extraction methods can contribute to food and nutritional security, promote circularity in marine-based industries, and establish a model for green innovation within the global food sector. Overall, this review highlights how non-thermal technologies can serve as transformative tools to achieve climate-resilient, waste-free, and resource-efficient food production systems.



# Harnessing non-thermal techniques for the sustainable protein extraction from blue foods and their potential applications in circular food systems

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## Abstract

Blue foods exhibit diverse group of aquatic resources and are essential for its nutritional profile including proteins and fatty acids and key micronutrients for human health. Numerous chemical and biological elements exist in marine habitats, which make the ocean a perfect source for high value compounds with multiple applications. Marine sources provide distinctive proteins, polysaccharides, along with micronutrients and bioactive compounds. Modern developments in non-thermal technologies facilitates environmentally friendly green and sustainable methods for the effective extraction of high value proteins from marine resources, which support environmental sustainable goals. The review focused on the optimistic effect of novel non-thermal treatments on protein from blue foods. It evaluates new processing methods that reduce waste and enhance protein extraction efficiency to produce functional products. The analysis identified the obstacles encountered during the production of marine proteins. The main goal is to reduce environmental effects by using unused marine biomass through energy efficient methods, which also generate less waste. Non-thermal processing methods enhance marine protein functionality and convert waste materials into food grade products to support circular bioeconomy models. These methods bring essential benefits to sustainable food systems by minimizing pollution and optimizing resource management. Sustainable marine protein extraction technologies support global biodiversity preservation, together with developing climate-resistant food innovations

**Keywords:** sustainable protein, non-thermal processing, marine bioresources, green technology, circular economy, environmental sustainability



## 35 1. Introduction

36 A prevalent perspective suggests that the production of aquatic sustenance, termed as blue  
37 foods, obtained from marine and freshwater environments through capture or cultivation, must  
38 increase in the upcoming decades to meet the growing demand driven by population growth.<sup>1</sup>  
39 The blue food protein sector is gaining increased focus within the food sectors due to its  
40 abundance of high-quality ingredients that boast exceptional nutritional value and beneficial  
41 properties for health. Blue foods, encompassing both wild and cultivated items from marine  
42 and fresh water environments. It have been recently gained attention from scientists,  
43 nutritionists, private and public organizations. This interest stems from their potential to play  
44 a role in alleviating global hunger and ensuring nutritional security for humanity.<sup>2</sup> On a global  
45 scale, "blue foods" encompassing fish, invertebrates, and algae obtained from marine  
46 ecosystems play a vital role in ensuring the food and financial stability of billions of  
47 individuals. Serving as a pivotal source of protein, fatty acids, and micronutrients, these blue  
48 foods are indispensable in addressing challenges related to malnutrition and diseases.<sup>3</sup> The  
49 worldwide edible meat consumption from these blue foods is on the ascent, with a per capita  
50 consumption of 20.2 kg in the year 2020. The edible meat consumption from blue food  
51 constitutes 17% of animal protein which represent only sea and surpasses 50% in numerous  
52 Asian and African nations. In many regions, blue foods are not only more accessible but also  
53 more affordable compared to other animal-sourced proteins.<sup>4</sup> As the expansion of aquaculture  
54 persists, there is a corresponding need for aquafeeds that are both environmentally sustainable  
55 and cost-effective. This demand is projected to rise to 87 million tonnes by the year 2025.<sup>5</sup>  
56 Consequently, their production is on the rise, making them one of the most traded commodities  
57 globally and contributing to the support of jobs, livelihoods, and income on a worldwide scale.<sup>4</sup>

58 Ironically, while certain marine resources face over-exploitation or inefficient utilization for  
59 human sustenance, other abundant marine food sources remain largely untapped or are utilized  
60 primarily for producing feed for aquaculture or terrestrial animals. In general, there is a  
61 substantial loss, typically exceeding 90 per cent of nutritional value, such as proteins, at each  
62 tropic level in the food chain. Therefore, utilizing unexploited or underexploited blue foods  
63 directly for human consumption presents an ideal approach to optimize the use of our natural  
64 global resources, simultaneously addressing the challenge of feeding a growing population  
65 with nutritious food.<sup>6,7</sup> The challenges of a growing global population and increasing demand  
66 for protein sources through light on the utilization of marine proteins presents itself as a



67 promising avenue for sustainable food production. Beyond their nutritional contribution, blue  
68 foods are increasingly recognized within global sustainability frameworks, particularly under  
69 the United Nations 2030 Agenda for Sustainable Development. Blue food systems directly  
70 contribute to Sustainable Development Goals (SDGs) 2 (Zero Hunger), 12 (Responsible  
71 Consumption and Production), 13 (Climate Action), and 14 (Life Below Water). Recent  
72 evidence suggests that strategic integration of blue foods into national dietary policies can  
73 simultaneously improve nutrition outcomes, reduce greenhouse gas emissions, and enhance  
74 livelihood resilience across coastal and low-income regions.<sup>8</sup> The Food and Agriculture  
75 Organization has further emphasized “blue transformation” as a pathway to reorient aquatic  
76 food systems toward sustainability, equity, and circular resource use.<sup>9</sup> Despite increasing  
77 production, blue food systems are characterized by significant inefficiencies, particularly  
78 during processing stages where 30–70% of biomass may become underutilized by-products  
79 such as heads, bones, skins, viscera, and shells. Conventional linear processing models often  
80 divert these residues toward low-value feed or landfill streams, resulting in nutrient losses and  
81 environmental burdens. Transitioning toward a circular bioeconomy framework, where  
82 protein, lipids, minerals, and bioactive compounds are sequentially recovered and valorised,  
83 can significantly reduce landfill burden, minimize nutrient loss, and improve overall resource  
84 efficiency.<sup>5,10</sup> Valorization of fish processing waste not only enhances economic returns but  
85 also contributes to climate mitigation through reduced waste decomposition emissions.

86 Several studies published in the past decade have concentrated on the beneficial applications  
87 of different aquatic species and their by-products mostly scales, bones, and skins to the  
88 production of collagen. These are the aquatic species' high-collagen materials. The primary  
89 sources of collagen in aquatic species (freshwater, deep-sea, and so forth) and processing  
90 circumstances (acid-aided, pepsin-aided, and so forth) were the primary sources of variation in  
91 aquatic species collagen.<sup>11</sup> The proteins from blue foods have a vital role as a functional and  
92 nutritional property in the food chain. The protein influences characteristics such as water  
93 absorption, oil absorption, foaming, emulsion, solubility, and texture of food.<sup>12</sup> Numerous food  
94 protein qualities can be improved or changed via the use of non-thermal technology. Protein  
95 coagulation, aggregation, or gelation can be modulated by these shifts created from non-  
96 thermal effects which enable varying degrees of unfolding and denaturation of the protein  
97 structure.<sup>13</sup> Marine-derived proteins are broadly classified into myofibrillar, sarcoplasmic, and  
98 stromal proteins, each contributing distinctly to the nutritional and techno-functional properties  
99 of blue foods. Myofibrillar proteins, primarily myosin and actin, play a crucial role in gel



100 formation, water-holding capacity, and textural attributes, whereas sarcoplasmic proteins  
101 influence solubility and emulsification behaviour. The functional performance of these proteins  
102 is governed by their structural organization, including primary amino acid sequence, secondary  
103 folding patterns, and higher-order conformations. Processing parameters such as pH, ionic  
104 strength, temperature, and mechanical stress can significantly alter protein structure through  
105 denaturation, aggregation, or unfolding, thereby influencing extraction efficiency and end-use  
106 functionality.<sup>13,14</sup> Therefore, maintaining structural integrity during extraction is essential to  
107 preserve both nutritional quality and techno-functional characteristics. Recent studies have  
108 emphasized that advanced and non-conventional extraction technologies can enhance protein  
109 recovery while minimizing excessive structural degradation, thereby improving functional  
110 performance in food applications.<sup>15</sup> Beyond the functionality of intact proteins, controlled  
111 hydrolysis of marine proteins leads to the generation of bioactive peptides with enhanced  
112 biological activities. These peptides are produced through specific enzymatic cleavage of  
113 parent proteins and have been reported to exhibit antioxidant, antihypertensive, antimicrobial,  
114 and immunomodulatory properties. The production of such peptides further expands the  
115 utilization potential of blue biomass within food, pharmaceutical, and nutraceutical sectors.<sup>14</sup>

116 Several approaches have been developed to modify food proteins to improve their functional  
117 characteristics. These techniques include chemical methods (such as controlled enzymolysis,  
118 and chemical modification), physical methods (such as ultrasound, high-pressure treatment,  
119 pulsed electric field, and solvent extraction method), and hurdle techniques.<sup>16,17,18</sup>  
120 Conventional protein extraction techniques, such as strong acid or alkali solubilization,  
121 although effective, often require high chemical inputs, extensive water usage, and subsequent  
122 neutralization steps that increase environmental load. In contrast, non-thermal technologies,  
123 including ultrasound, pulsed electric field (PEF), high-pressure processing (HPP), and enzyme-  
124 assisted extraction, offer promising green alternatives. These approaches enhance cell  
125 disruption efficiency while operating at lower temperatures, thereby reducing energy demand  
126 and preserving protein functionality.<sup>14,15</sup> Moreover, enzyme-based and physical extraction  
127 methods minimize hazardous effluent generation and align more closely with sustainable  
128 processing principles. Such technologies, therefore, represent critical enablers in transitioning  
129 from linear extraction models toward environmentally responsible protein recovery systems.

130 Although several studies have investigated protein extraction from marine organisms, existing  
131 literature predominantly focuses on yield optimization and functional characterization, with



132 limited integration of sustainability metrics, circular bioeconomy frameworks, and residue  
133 valorization strategies. Furthermore, comparative evaluation of conventional versus non-  
134 thermal extraction technologies from an environmental perspective remains  
135 underdeveloped.<sup>14,19</sup> There is a pressing need to critically examine how advanced extraction  
136 technologies can contribute to circular food systems while maintaining techno-functional  
137 performance. Therefore, this review aims to critically evaluate conventional and non-thermal  
138 protein extraction techniques from blue foods; analyse their sustainability implications,  
139 including energy demand and environmental footprint; explore circular bioeconomy  
140 integration through residue valorization pathways; and assess future opportunities within  
141 sustainable food systems.

## 142 2. Protein source from Blue-foods

143 The vitality of marine organisms as an originator of distinct proteins, fats, micronutrients, and  
144 bioactive substances continues escalating. Compounds obtained from marine organisms have  
145 emerged as a rich reservoir for exploring new peptides, although they remain relatively obscure  
146 due to the challenges associated with their retrieval. Marine peptides, constituting 8% of all  
147 bioactive marine compounds, play a significant role. A notable proportion of novel compounds  
148 have been identified from marine invertebrates, marine algae, and marine vertebrates (Fig.1.)  
149 in this section.

### 150 2.1. Algae-based sources for protein compounds

151 Most green and red macroalgae species have considerably higher protein content when  
152 compared with terrestrial plant protein sources such as soybeans, cereals, and nuts.<sup>20</sup> On top of  
153 their nutritional properties, the proteins and peptides derived from the marine algae have  
154 significant value because of their pharmaceutical, nutraceutical, and cosmeceutical properties  
155 like antioxidant property, immune modulatory property, anti-hypertensive property, and  
156 hepato-protective properties.<sup>21</sup> Brown seaweed has low protein content (3 to 15% of dry  
157 weight), whereas green and red seaweed demonstrate modest and high protein content of 9-26  
158 and 47 per cent of dry weight respectively. A few examples are *Undaria pinnatifida*, *Ulva*  
159 *pertusa*, *Ulva lactuca*, *Porphyraezoensis*, *Palmariapalmata*, *Laminaria digitata*, *Ulva*  
160 *rotundata*, *Ulva armoricana*, *Ulva rigida*, *Chondrus crispus*, *Ulva ohndi*.<sup>22,23</sup>

161 Microalgae act as an important source of bioactive molecules. They are loaded with  
162 carbohydrates, lipids, proteins, and minerals. Among microalgal species, some of the  
163 prominent classes are Cyanophyceae (blue-green algae), Bacillariophyceae (comprising



164 diatoms), Chrysophyceae (encompassing golden algae) and Chlorophyceae (green algae) (24)  
165 Compare with macroalgae, microalgae have abundant number of proteins, which comprises  
166 around 70% of the biomass dry weight in some notable species.<sup>25</sup> Out of 50,000 species, only  
167 a few species of microalgae are considerably safe for human consumption such as *Spirulina* or  
168 *Arthrospira platensis*, *Chlorella* or *Chlorella vulgaris*, *Dunaliella*, *Nostoc*, and  
169 *Aphanizomenon*.<sup>26</sup> Microalgae act as an exceptionally prominent source of proteins for human  
170 consumption since the amino acid profile almost matches as that of human body.

## 171 2.2. Marine animal sources for protein compounds

172 The marine animal sources include vertebrates and invertebrates like sea urchins, sea  
173 anemones, starfish, cuttlefish, crabs, prawns, squid, octopus, jellyfish, and different variants of  
174 fish viz. carp, tilapia, marine eel fish, amur sturgeon, red drum fish, big eye snapper and giant  
175 croaker.<sup>27,28,29</sup> Nowadays, marine sources are scrutinized as one of the safest sources of protein.  
176 It also aids in reducing the risk of diseases like BSE (Bovine Spongiform Encephalopathy),  
177 TSE (Transmissible Spongiform Encephalopathy), and FMD (Foot and Mouth Disease) which  
178 may be caused due to the consumption of land-based animal-derived protein. These marine  
179 animals have high amounts of collagen which assists with the valorization of the fish waste.<sup>27</sup>

180 The aromatic (tryptophan, tyrosine, and phenylalanine) and nucleophilic amino acids with  
181 sulphur side chains (cysteine and methionine) are more efficient in terms of bioactivity of fish  
182 hydrolysates because of their ability to easily give away hydrogen atoms and interact with free  
183 radicals.<sup>30</sup> The most remarkable bioactivities of fish-based hydrolysates are determined by their  
184 amino acid composition, as well as structural and conformational properties.<sup>31</sup> Taurine, a  
185 conditionally essential amino acid, is particularly intriguing. Taurine's potential has been  
186 tailored for use as a therapeutic agent against congestive heart failure and a variety of other  
187 conditions, including blood pressure reduction, improved cardiac performance, and blood  
188 cholesterol reduction.<sup>30</sup>

189 Fish proteins are high in amino acids and peptides, making them highly digestible, especially  
190 in balanced proportions. The digestibility of amino acids and proteins is critical to the  
191 bioavailability and therapeutic efficacy of fish products.<sup>32</sup> Protein hydrolysis can produce  
192 bioactive peptides, particularly those derived from various fish species. The protein in oily and  
193 white fish undergoes breakdown into polypeptides, amino acids, and peptides. Bioactive  
194 chemicals are so named because the vast majority of them have bioactive properties. Bioactive



195 chemicals are additional nutritional ingredients found in foods that occur naturally in small amounts. These compounds have been found to be beneficial to human health.<sup>33</sup>

197 They can be transported through the intestinal enterocytes before reaching circulation, where  
198 they have beneficial biological effects. Peptides can deliver a wide range of biotechnological  
199 products with enhanced bioactivity, including antibacterial, antioxidant, and antihypertensive  
200 properties.<sup>34</sup> Lysine and methionine are two essential amino acids found in large amounts in  
201 fish protein. Angiotensin I converting enzyme (ACE) is a type of inhibitory peptide found in  
202 fish, originally in sardine flesh. Similarly, fatty fish have around 20% protein, but their water  
203 (62-70%) and oil levels are much higher (10-18%).<sup>35</sup>

### 204 **3. Extraction of protein from Blue-foods**

#### 205 **3.1. Conventional techniques for protein extraction**

206 Conventional protein extraction methods include the pH shift process and solvent extraction.  
207 Acid and alkali solubilization methods are used for protein extraction under the pH shift  
208 process (Table.1). However, the most common extraction technique is the conventional  
209 techniques discussed in the following section (Fig.2).

##### 210 **3.1.1. pH shift processing for extraction of protein**

211 pH shift process is the optimistic method in which the protein is extracted by altering the pH  
212 values more than its isoelectric point. In that condition, the protein will have a strong positive  
213 charge or negative charge, this will result in the repulsion of the charge causing the unfolding  
214 of the protein structure.<sup>36</sup> When the pH is modulated to the neutral pH, the proteins will get  
215 folded back into a molten state and there is an improvement in the functional properties.<sup>37</sup>  
216 Because of the unfolding and folding action, multiple subunits of proteins will get dissociated,  
217 increasing the solubility. Generally, food proteins are treated either with acid or alkali to attain  
218 extreme pH conditions, which creates a structural change in proteins.<sup>16</sup>

##### 219 **3.1.2. Acid extraction for isolation of protein**

220 The collagen fibres are less soluble in the aqueous medium when compared with the acidic  
221 medium. The acid extraction includes treating the fishes and seaweeds with an acidic medium  
222 generally 0.5 M of Acetic acid, hydrochloric acid, lactic acid, citric acid, etc.<sup>16</sup> where the  
223 collagen obtained through this treatment is often called Acid Soluble Collagen (ACS).<sup>27</sup> Under  
224 acidic conditions, the bond between the collagen molecules is cleaved, which improves the



225 extraction of collagen (Fig.3). When compared with the inorganic acids, the organic acids  
226 cleave the crosslink of the collagen molecules, yielding a higher extractability of the protein.<sup>27</sup>  
227 Under these conditions, there is repulsion between the tropocollagen molecules because of the  
228 positively charged collagen molecules, this phenomenon results in better solubilization.<sup>38</sup>

229 According to Pal & Suresh (2016), acid extraction can be conducted under high temperatures  
230 using 6 M hydrochloric acid for a period of 18 to 48 hours to get a resultant collagen from the  
231 fish.<sup>39</sup> Another study states that the fish is minced, and homogenized with cold water for 1  
232 minute, and then, the pH is adjusted to 2.5 using 2 M HCl. Followed by, the mixture is kept at  
233 4<sup>0</sup>C and then it is centrifuged to get the soluble proteins. The yield of collagen is maximum  
234 (90 %) when the Baltic cod is treated with lactic acid or acetic acid when compared with HCl  
235 and citric acid which is around 18 and 60 per cent respectively.<sup>27</sup> The protein extraction of  
236 seaweed (*Ascophyllum nodosum*) was achieved by dissolving the seaweed in the distilled water  
237 and incubating it at 4<sup>0</sup>C for 16 hrs. Further, the mixture is centrifuged to obtain pellets, which  
238 will be treated with HCl of different molarity in a 1:15 solid-to-solvent ratio. Later, the mixture  
239 was stirred and centrifuged to obtain the proteins

240 The interaction behaviour of collagen molecules was studied, where the aggregated state of  
241 collagen molecules and the acid concentration are inter-relatable. When the concentration of  
242 acid is increased, there is a better recovery of proteins. The rheological behaviour also depends  
243 on each other. The viscosity of collagen tends to decrease by increasing the acid concentration,  
244 resulting in the collagen's increased ability to flow.<sup>40</sup> Protein extracted by this method can be  
245 potentially used in commercial applications.

### 246 3.1.3. Alkali extraction for isolation of protein

247 In brief, alkali extraction is similar to acid extraction where the homogenized fishes are treated  
248 with high pH and it is accompanied by centrifugation to remove the higher-density particles  
249 from the solution. Then the proteins are allowed to precipitate by adjusting the pH to 5.5 and a  
250 second centrifugation is done to get the pure form of protein.<sup>41</sup> The recovery of sarcoplasmic  
251 and myofibrillar protein is higher in terms of the alkali shift process, these proteins will be  
252 washed off in the surimi process.<sup>42</sup> The alkaline pH provides higher solubilization of proteins  
253 than acidic pH so, the protein yield in alkali extraction is higher than that of the acid  
254 extraction.<sup>12</sup>



255 Several studies have concealed that the recovery of protein and solubilization is high in the pH  
256 above.<sup>11</sup> It clearly shows that the recovery of protein and the solubilization of protein mainly  
257 depends upon the pH value. If the pH value is higher, the consistency of the muscle will get  
258 lower, so that the phospholipid membranes will get separated leaving the protein during the  
259 first centrifugation which will increase the solubility of the protein.<sup>43</sup>

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#### 260 **3.1.4. Solvent extraction for isolation of protein**

261 The solvent used in the extraction will vary based on the target compound to get an efficient  
262 yield. This extraction may require a long duration depending upon the diffusion rate of  
263 solvents.<sup>44</sup> Based on the solvents, different solvent extractions are available. Among these  
264 processes, the Canadian process seemed to be effective which implies the extraction through  
265 isopropanol in a 2:1 solid-liquid ratio, yielding 19.94 percent. The defatted sample leads high  
266 quality of fish protein. Since proteins like insoluble collagen have hydrophobic and disulfide  
267 bonds, they rarely dissolve in an aqueous medium. By using high-reactive solvents like  
268 hydrogen peroxide, ethylene dichloride, and sodium hypochlorite, the amino acid profile may  
269 alter, resulting in the formation of toxic peptides and choline derivatives.<sup>45</sup>

270 A concept called Deep Eutectic Solvent Extraction (DES) is applied in the extraction of  
271 proteins, where two different solvents are used together, a hydrogen bond donor and a hydrogen  
272 bond acceptor respectively.<sup>40</sup> According to Bai, (2017), the extraction yield was maximum  
273 when the extraction was done with choline chloride and oxalic acid among six different  
274 methods.<sup>46</sup> In further studies, it was found that the extraction yield of protein would be higher  
275 by increasing the amount of oxalic acid and keeping the choline chloride constant. It can be  
276 used as a sustainable extraction of collagen.<sup>40</sup>

#### 277 **3.2. Advanced techniques for protein extraction**

278 The sustainable alternate protein from blue foods can be extracted using advanced and eco-  
279 friendly techniques like enzyme-assisted, sonication, pulsed electric field, and high pressure  
280 processing (Table.1). The influence of advanced techniques on protein extraction efficiency  
281 has been discussed in the following sections.

##### 282 **3.2.1 Enzyme-Assisted Extraction Technique for proteins from blue foods**

283 Enzyme-assisted extraction utilizes specific proteases or cell wall-degrading enzymes to  
284 hydrolyse structural components, thereby improving protein solubilization under mild



285 conditions. The efficiency of enzymatic extraction depends on enzyme specificity, enzyme-to-  
286 substrate ratio, pH, temperature, and reaction time.<sup>19</sup> Enzymatic hydrolysis of fish protein and  
287 other marine foods has attracted greater attention recently as a technique for producing high-  
288 quality FPH (Fish Protein Hydrolysates) that may be a desirable raw material for making  
289 bioactive peptides to treat various diseases and disorders. FPH obtained by this method  
290 provides a wide range of applications and nutrition such as dietary supplements,  
291 pharmaceuticals, animal nutrition, and cosmetics.<sup>47</sup> The protease enzymes have been used to  
292 hydrolyse fish. Proteins like Neutrase, Flavourzyme, Papain, Alcalase, and Bromilane have  
293 been used commercially.

294 For instance, demonstrated that enzyme-assisted extraction methods successfully extract  
295 bioactive compounds from seaweeds and microalgae used in the nutraceutical industry.<sup>48</sup>  
296 Similarly, accomplished the extraction and identification of proteins from the red seaweed  
297 *Palmaria palmata*, which resulted in better yield and improved functional properties.<sup>49</sup>  
298 Enzyme-assisted methods have been used to extract materials from fish and fish by-products.  
299 Wang, (2020) developed antioxidant-rich protein hydrolysates from cobia liver through  
300 enzymatic hydrolysis.<sup>50</sup> The successful extraction of protein and fish oil from Baltic herring by  
301 employing proteolytic enzymes.<sup>51</sup> Thus, the extracted proteins from fish backbones through a  
302 combination of steam explosion methods and enzymatic hydrolysis. Fish backbone proteins  
303 increased through the combined use of steam explosion and enzymatic hydrolysis.<sup>51,52</sup>

304 Tran, (2023) demonstrated that Basa fish skin collagen extraction through enzymatic  
305 hydrolysis maintained the bioactivity and structural integrity of collagen peptides.  
306 Appropriate enzyme choice alongside processing duration and conditions determines both  
307 product quantity and protein quality.<sup>53</sup> The enzyme-assisted extraction method presents  
308 multiple benefits over traditional solvent-based techniques by speeding up processing times  
309 and reducing harmful chemical use while enhancing environmental sustainability. Enzyme-  
310 assisted extraction has become a leading method to maximize blue food resources and fulfil  
311 the rising demand for functional and bioactive protein ingredients.

### 312 3.2.2. Ultrasound-Assisted Extraction Technique for blue foods

313 Ultrasound waves are mechanical waves that can travel through solid, gaseous, and liquid  
314 media by rarefaction and compression. When the pressure generated by this expansion is  
315 greater than the liquid's tensile strength, it causes negative pressures in liquids that eventually  
316 result in the creation of vapor bubbles. Bubble cavitation is the implosive collapse of vapor



317 bubbles. Macro turbulence, high-velocity inter-particle collisions, and micro-porous structure  
318 are produced by cavitation, which enhances matrix permeability and facilitates optimal solvent  
319 permeation into the cell's matrix, enabling target chemicals to interact with the solvent and  
320 streamlining the extraction process.<sup>54</sup>

321 Ultrasonic wave vibration creates cavitation. A phenomenon of dissipation of energy that  
322 spreads throughout the medium through the development and ensuing disintegration of vapor  
323 bubbles. Cavitation additionally raises the kinetic energy of the particles within the treated  
324 substance (Fig.4.). As a result of the ultrasonic energy's shock effect during the extraction  
325 process, high temperature offers enough energy for a reaction.<sup>55</sup> Numerous intra- and  
326 intermolecular covalent cross-links involving lysine and hydroxylysine residues, ester bonds,  
327 and other interactions with saccharides must be broken during collagen extraction. Mass  
328 transportation in wet processes is improved by ultrasonication.

329 Among all the proteins, collagen is the most abundant structural protein which accounts for  
330 about 25-30 percent and is found in the animal body. Collagen consists of a large molecule that  
331 has a triple helical structure with each polypeptide chain mostly containing repeating units of  
332 glycine, proline, and hydroxyproline.<sup>56</sup> It has been observed that conventional methods are  
333 prolonged processes and collagen residue tissue will be more, in addition to that, a high amount  
334 of insoluble collagens will be left behind.<sup>56</sup> The ultrasonication technique is non-destructive  
335 and non-invasive, the good quality of protein can be obtained without interrupting the  
336 molecular structure of collagen.<sup>57</sup> To extract collagen from various marine sources such as  
337 clown feather back, golden carp, sea bass, soft-shelled turtle, and grass carp calipash the  
338 ultrasonication method has been used.

339 Moreover, ultrasonication reduces the size of collagen particles despite increasing their pH and  
340 salt-induced solubility.<sup>58</sup> Even though ultrasonication is a favourable and propitious technology  
341 to enhance the quality and amount of resulting collagen, the amplitudes and extraction times  
342 need to be carefully regulated. The use of ultrasonication in collagen extraction can shorten  
343 extraction times and improve the quality and quantity of collagen recovered. Low-intensity  
344 ultrasound alters the state of bacteria and promotes their development by causing repairable  
345 damage and stable cavitation.<sup>59</sup> Furthermore, water molecules may be broken down more easily  
346 by ultrasound, which can potentially generate free radicals and damage bacterial DNA and  
347 enzymes.<sup>60</sup>



348 Even though ultrasonication has many advantages, prolonged use of the technology results in  
349 a cavitation effect, which raises the temperature, shear force, and pressure of the medium and  
350 breaks hydrogen bonds and van der waals interactions in polypeptide chains, and this  
351 denaturizes proteins and enzymes.<sup>54</sup>

### 352 **3.2.3. HPP Treatment for Protein Extraction from Blue Foods**

353 A non-thermal technology called high-pressure processing (HPP) has been adopted to alter or  
354 improve the protein characteristics in a variety of foods.<sup>61</sup> It is one of the novel technologies  
355 and is considered an environmentally friendly technique.<sup>62</sup> HPP is a technique where high  
356 pressure is carried out on the sample up to 1000 MPa in the presence or absence of heat. It has  
357 been reported that when HPP treatment is implemented on the desired marine food product,  
358 there is a significant increase in the yield of gelatin protein, and it also shortens the extraction  
359 time.<sup>13</sup> This is because, during pre-treatment, the pressure ruptures the bond between the  
360 secondary, tertiary, and quaternary structures of the gelatin protein, which aids in the release  
361 of more peptide bonds soon after thermal hydrolysis.<sup>63</sup> The protein coagulation, aggregation,  
362 or gelation can change as a result of the modifications, which allow changing the protein  
363 structure via unfolding and denaturation to varying degrees (Fig.5).<sup>13</sup> According to several  
364 studies, myosin starts to unwind at pressures of about 50 MPa. The tertiary and secondary  
365 structures of myosin also tend to unfold when high pressure (100MPa) is applied.<sup>64</sup> These  
366 pressure levels may trigger the myosin's solubilization and denaturation, which are essential  
367 for the formation of protein gelation. However, the amount of pressure given to the meat  
368 product will determine how much of the protein unfolds.<sup>65</sup>

369 HPP has the ability to break down the cell wall of both gram-positive and gram-negative  
370 bacteria to facilitate more amount of protein yield through extraction. The interaction of side-  
371 to-side chains through covalent and non-covalent bonding promotes aggregation and gelation,  
372 when HPP is introduced to myosin. Gels are subjected to pressure treatments that encourage  
373 the formation of non-covalent linkages.<sup>13</sup> The development of a uniform and smooth gel  
374 structure contributed to the growth of the myosin matrix, which was significantly enhanced by  
375 the use of 300 MPa. Additionally, modifications to the structure of myofibrillar proteins may  
376 have an impact on the quality of meat products and the textural characteristics and properties  
377 of gels.<sup>66</sup> HPP enhances the gelling property in marine foods.

### 378 **3.2.4. Pulsed electric field for protein extraction from blue foods**



379 The pulsed electric field can facilitate the extraction of protein from blue foods by inducing  
380 electric currents with high voltage from an electric field within micro to milliseconds.<sup>67</sup>  
381 Through reversible or irreversible electroporation (Fig.6), the electric current will enter the cell  
382 wall, resulting in the release of intracellular components, including protein, DNA,  
383 carbohydrates, lipids, and micronutrients. Critical influencing factors include electric field  
384 strength, pulse duration, number of pulses, and conductivity of the extraction medium.<sup>68</sup>

385 According to Buchmann, (2019) found the interrelationship between the strength of the electric  
386 field and the concentration of protein being extracted from *Chlorella vulgaris* treated in 20  
387 kVcm<sup>-1</sup> electric field strength.<sup>69</sup> As an instance from the study, the protein concentration was  
388 0.55±0.01g L<sup>-1</sup> in electric field strength of 10 kV cm<sup>-1</sup> and increased to 0.80±0.04 g L<sup>-1</sup> in 20  
389 kV cm<sup>-1</sup>, resulting in the average protein extraction increased with the electric field strength.  
390 Some studies showed that the PEF is not feasible yet for the extraction of selectively proteins  
391 alone, because it will not completely disintegrate the cell wall.<sup>70</sup> Polikovsky, (2016)  
392 demonstrated the extraction of selective proteins through PEF where the yield of specific  
393 proteins is greater. By allowing the extraction of intact cytosolic proteins, there was a notable  
394 increase in protein extraction of *C.Vulgaris* and *Nannochloropsis salina*.<sup>71</sup>

### 395 **3.2.5. Supercritical fluid (SCF) extraction for protein isolation from blue foods**

396 Supercritical fluid extraction is a green technology that utilizes carbon dioxide which can act  
397 as a fluid at a certain temperature and pressure above its critical point.<sup>72</sup> Jafari, (2020) found  
398 that the yield of protein from the cod skin was 13.8 per cent through SCF which was relatively  
399 higher than the acid and pepsin-aided AcOH extraction (5.72 and 11.14% respectively).<sup>40</sup>  
400 Considerably, Supercritical fluid extraction is used in the selective extraction of bioactive  
401 proteins and peptides from the plant substrates whereas, the extraction of protein from seaweed  
402 is limited by SCF, and presently studies are focusing in this direction for better extraction and  
403 characteristics.<sup>67</sup>

### 404 **3.2.6. Hurdle technology for synergistic effect in protein extraction**

405 PEF and Enzymatic extraction: In the combination of PEF and enzymatic extraction, PEF could  
406 reduce the foaming properties and enhance the emulsifying properties and the solubility of the  
407 protein from the Abalone viscera, resulting in a promising yield. A study showed that the yield  
408 of combined treatment is higher than the pure enzymatic extraction.<sup>73</sup>



409 PEF And Mechanical Press: In this extraction process, PEF is coupled with a mechanical press,  
410 where 200 pulses having a field strength of 1kV/cm were applied to *Ulva ohndi*. The duration  
411 and repetition of pulses were 50 $\mu$ s and 3Hz respectively. It was done concurrently with the  
412 mechanical pressing at 107,682 N/m<sup>2</sup>. Around 14.94 per cent yield of protein was obtained  
413 through this extraction.<sup>23</sup>

414 PEF and Hydraulic Pressure: According to Polikovsky, (2016), the biomass of *Ulva* sp., was  
415 submerged in deionized water and 75 pulses with a field strength of 2.964 kV/cm were applied  
416 to the solution with a pulse repetition rate of 0.5Hz and 5.70 $\mu$ s of pulse duration. The extraction  
417 was combined with hydraulic pressing for 5 min under the pressure of 450 N/cm<sup>2</sup>. The total  
418 protein concentration of the extract after treatment is 59.13 $\pm$ 3.82  $\mu$ gmL<sup>-1</sup> which is higher than  
419 the untreated sample.<sup>21</sup> The advanced non-thermal approach leads optimistic nutritional and  
420 functional characteristics (Table 2) can be utilized for enhanced protein extraction from blue  
421 foods. The hybrid techniques pave a new direction for researchers to obtain synergistic effects  
422 in blue foods.

#### 423 **4. Factors affecting protein from blue foods and their mitigations**

##### 424 **4.1. Impurities on protein purity**

425 Even though Algae are rich in protein, they also tend to gather toxic heavy metals like mercury,  
426 lead, arsenic, and cadmium. The appearance of lead and mercury is under the legal limit for  
427 safe human consumption, whereas arsenic and cadmium presence are above the legal limits.  
428 There is a chance of protein contamination with heavy metals when extracted from algae. There  
429 is an acceptable level of heavy metal in the case of microalgae.<sup>68</sup>

##### 430 **4.2. Food allergenicity as a barrier for protein consumption**

431 Protein consumption faces significant obstacles because food allergies trigger immune  
432 responses that can be either IgE-mediated or non-IgE-mediated. The release of histamine  
433 through IgE-mediated allergies produces symptoms spanning the skin and the respiratory,  
434 digestive, and circulatory systems.<sup>74,75</sup> Food allergies affect 2.5% of people worldwide, while  
435 fish represents one of the main allergenic sources. The WHO/IUIS has recognized 12 fish  
436 proteins as allergens, which include parvalbumin along with tropomyosin and aldolase A.  
437 People who develop fish allergies typically have symptoms that last throughout their lives,  
438 although the severity changes over time. The fish species cod, salmon, and mackerel contain  
439 parvalbumin, which serves as a primary allergen. Protein structures undergo changes due to



440 processing methods like drying, heating, and smoking, which can influence their potential to  
441 cause allergic reactions.<sup>76</sup>

442 The effects of processing methods on allergenicity depend on the specific protein matrix  
443 structure, alongside detection techniques and antibody specificity. Experimental findings from  
444 ELISA and Western blotting indicate that thermal processing alters IgE protein binding  
445 patterns. IgE binding to heated whiff parvalbumin vanishes rapidly during digestion, but  
446 remains in some cases after extended heating periods.<sup>77,78</sup> Allergenicity varies based on  
447 demographic factors such as age, gender, region, and ethnicity, which show increased  
448 prevalence in Asian populations as well as females and adult individuals.

#### 449 **4.3. Economic perspective on commercial usage**

450 The commercialization of microalgae to extract proteins was found to be a challenge.<sup>79</sup> The  
451 cost of microalgae production will vary depending on the upstream process and downstream  
452 processes. Some studies showed that microalgae can be cultivated in wastewater, but that will  
453 result in the contamination of the produce, and it can't be used in foods.<sup>80</sup> To get better growth  
454 of microalgae, photobioreactors can be used, but the operating costs for this reactor are high.  
455 The development of some robust processes is needed for the commercialization of  
456 microalgae.<sup>81</sup>

#### 457 **4.4. Environmental Sustainability**

458 To enhance transparency in the environmental sustainability discussion, the carbon footprint  
459 considerations presented in this review follow a cradle-to-gate system boundary. This includes  
460 raw material acquisition from blue food sources, pre-treatment processes, non-thermal  
461 extraction stages, and subsequent drying operations, while excluding downstream processes  
462 such as packaging, distribution, retail handling, consumer use, and end-of-life disposal. Such  
463 boundary definition aligns with standardized environmental assessment approaches applied in  
464 food system evaluations.<sup>82</sup> The functional unit was defined as 1 kg of isolated protein produced,  
465 ensuring comparability with food-system-level sustainability metrics reported for blue foods.<sup>2,8</sup>

466 Carbon emission estimations were primarily based on reported energy consumption associated  
467 with non-thermal technologies including ultrasound, pulsed electric field, and high-pressure  
468 processing. Emissions were conceptually calculated by multiplying energy demand (kWh) by  
469 corresponding carbon intensity factors of electricity, consistent with carbon footprint  
470 frameworks used in food basket and resource assessment studies.<sup>82</sup> Assumptions include



471 average industrial electricity consumption, partial water reuse during extraction, and solvent  
472 recovery efficiencies where applicable. Capital equipment manufacturing and transportation  
473 emissions were excluded due to limited reporting across extraction studies. By explicitly  
474 defining system boundaries, functional unit, and calculation approach, the environmental  
475 discussion is aligned with broader sustainability assessments of blue foods and global food  
476 systems, thereby improving clarity, transparency, and methodological consistency.<sup>2,8</sup>

## 477 **5. Applications of protein from blue foods**

478 The aquatic ecosystem is still unexploited reservoir of many nutritional and functional  
479 components. Though, the marine protein (collagen) plays a vital application in pharmaceutical,  
480 food, and cosmetic and other domains. The food-based application of protein from blue foods  
481 has been highlighted in this review.

### 482 **5.1. Taylor-made foods / Functional foods from blue foods**

483 The algal proteins are incorporated into foods due to their nutritional and sensorial properties  
484 and also to overcome the challenges of the low dispersibility of proteins. The algal proteins are  
485 used in the formulation of Yogurt with *A. plantensis* and pasta with *C. vulgaris* and *A.*  
486 *maxima*.<sup>83,84</sup> The microalgal proteins have foaming, emulsifying, and stabilizing properties,  
487 which shows the advantages over plant proteins.<sup>81</sup>

488 Some studies showed that bread can be enriched with fish proteins through different  
489 formulations. The acceptability rate was also high and this showed that there was not much  
490 difference in the sensorial properties of bread.<sup>85</sup>

### 491 **5.2. Nutraceuticals from aquatic organisms**

492 Natural bioactive compounds with therapeutic benefits, called nutraceuticals, are becoming  
493 more popular as synthetic drug substitutes because disease rates continue to rise. Shrimp  
494 processing generates 40–50% biomass waste, which is considered valuable among aquatic  
495 sources. Shrimp waste contains high levels of astaxanthin (C<sub>40</sub>H<sub>52</sub>O<sub>4</sub>), which acts as a  
496 powerful antioxidant while giving shrimp meat its red color and making up 65–98% of its total  
497 carotenoid content.<sup>86</sup> Each year, India generates over 15,000 tonnes of shrimp waste, which  
498 represents a significant potential for resource recovery.<sup>87</sup>

499 Feed microalgae-derived β-carotene or zeaxanthin undergoes oxidative conversion to create  
500 astaxanthin, which holds substantial commercial importance in both food and pharmaceutical



501 sectors.<sup>88</sup> The process of extracting astaxanthin from crustacean waste achieves sustainable  
502 waste management goals while meeting increasing market needs for natural dietary  
503 supplements. Product consistency and global market needs present major challenges to scaling  
504 shrimp-waste-based nutraceutical production.

### 505 **5.3. Valorization of biowaste from marine sources**

506 Generally, macroalgae are used as a feasible feedstock for biomass. The challenging one is the  
507 fractionation of biomass; some sustainable processes and methods are needed to upscale it.  
508 Without the fractionation, the algae will become waste. To reduce that, PEF coupled with  
509 mechanical pressing is applied to the macroalgae (*U. ohnoi*) for the extraction of a wholesome  
510 amount of proteins.<sup>23</sup>

511 The optimization of low-value foods and underutilized foods is necessary to avoid wastage.  
512 The underutilized fish can be used to extract the proteins. Through this, the underutilized fish  
513 will be converted into valuable ones. Further, the fish proteins can be incorporated into the  
514 foods.<sup>89</sup>

### 515 **5.4 Sustainable animal feed and aquafeed**

516 Blue food-derived protein hydrolysates, which are typically by-products, present nutritious and  
517 sustainable feed options for animals and aquaculture that yield better results. Protein  
518 hydrolysates contain abundant bioavailable amino acids and bioactive peptides that support  
519 animal growth performance and immunity while improving nutrient absorption. The process  
520 of enzyme-assisted hydrolysis produces fish protein hydrolysates from sardine by-products and  
521 marine wastes, which deliver high-quality feed supplements having minimal environmental  
522 impact.<sup>47</sup> These protein compounds exhibit functional advantages through their antioxidant and  
523 antimicrobial properties, which enhance animal well-being and lessen antibiotic dependence in  
524 feed.<sup>45</sup>

525 Aquaculture systems can use fish protein hydrolysates to replace traditional fishmeal either  
526 partially or completely, which helps to reduce the exploitation of wild fish stocks. For example,  
527 microalgal proteins extracted through non-thermal methods, such as pulsed electric field or  
528 enzymatic hydrolysis, show great potential for use in aquafeeds.<sup>81</sup> Alternative protein sources  
529 have exhibited excellent digestibility and palatability while offering a balanced profile of  
530 amino acids.



531 The residual biomass from blue food production, including seaweed residues and microalgal  
532 cell walls, can be transformed into feed components or bio-stimulants for use in integrated  
533 aquaculture systems.<sup>68</sup> The examined methods for transforming algal residues with high  
534 structural carbohydrate and protein content into viable dietary components for fish and shrimp  
535 following proper processing. Nutrient sources from these residues also support gut health and  
536 maintain microbial balance among aquatic organisms.

### 537 **5.6. Potential Medical Applications from Marine Sources**

538 Proteins, peptides, and amino acids obtained from marine sources possess enormous potential  
539 for advanced biomedical research, providing insight into the workings of both healthy and  
540 diseased human bodies. Moreover, they provide the potential to create highly targeted and  
541 effective medications to treat a range of illnesses. Consider collagen that has been taken from  
542 some marine mammals, for example. Tissue-like structures in 3D bioprinting are often built on  
543 collagen, a structural protein essential for many body tissues. These collagen scaffolds are very  
544 useful for tissue engineering applications, such as blood vessel, skin, and bone regeneration.<sup>90</sup>

545 Furthermore, collagen is an invaluable medical excipient due to its exceptional qualities, which  
546 include low antigenicity and biocompatibility. It has several uses, including drug transport  
547 capsules, bone tissue regeneration fillers, and deionized membranes in surgery. When  
548 introduced into the body, these membranes work well with host cells and tissues, reducing  
549 negative immune reactions.<sup>91</sup> Collagen is biodegradable and naturally absorbed by the body.<sup>92</sup>

### 550 **5.8. Innovative food products**

551 Marine proteins have become popular in the food industry due to their high nutritional  
552 value. Collagen from marine animals is used to make gelatin, a versatile Ingredient.<sup>93</sup> Gelatin  
553 is a major component in many delicious desserts, such as fudge, puddings, marshmallows, and  
554 salads. Algal proteins, rich in amino acids and readily digestible, have been incorporated into  
555 innovative food products.<sup>94</sup> These proteins are incorporated into vegan meat substitutes,  
556 protein-rich snacks, and dairy alternatives. Protein derived from edible microalgae like  
557 *Isochrysis galbana* and *Chlorella vulgaris* shows potential as food supplements and additives.  
558 Phycobiliproteins from *Porphyridium cruentum* (red algae) and *Synechococcus spp.* (blue-  
559 green algae) have been utilized as natural food colorants. These specialized protein groups have  
560 been integrated into various food products, including dairy, chewing gums, jellies, and ice  
561 sherbaths, to serve as colouring agents.<sup>92</sup>



## 5.9. Residue Valorization and Circular Bioeconomy

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Protein extraction from blue biomass generates significant residual fractions which are still rich in lipids, minerals, collagen, chitin, and other bioactive compounds. These residues should not be considered as waste, as they possess high biological and commercial value. Effective utilization of these fractions plays a vital role in strengthening the circular bioeconomy model and improving sustainability of the blue food supply chain.<sup>5</sup> The lipid fraction remaining after protein extraction may contain long-chain omega-3 fatty acids such as EPA and DHA which are widely used in nutraceutical and functional food formulations. Tuna processing side streams have been successfully utilized for recovery of proteins, lipids, and minerals through integrated biorefinery approaches, thereby reducing environmental burden and enhancing economic return.<sup>95</sup> Similarly, invasive marine species such as blue crab (*Callinectes sapidus*) have been valorised into high-value nutritional ingredients and bioactive compounds, converting ecological challenges into sustainable opportunities.<sup>96,97</sup>

Collagen and gelatin extracted from fish skin, bones, and connective tissues have wide applications in food, pharmaceutical, and biomedical industries due to their functional and structural properties. Mineral-rich residues, particularly from bivalve shells and crustacean exoskeletons, are important sources of calcium carbonate and chitin. Valorization of shell waste has gained attention within circular blue bioeconomy strategies for applications in agriculture, environmental remediation, and material science.<sup>98</sup> Moreover, the remaining biomass after sequential extraction can be utilized as an animal feed ingredient or converted into bioenergy through anaerobic digestion, thereby reducing landfill accumulation and greenhouse gas emissions. Integration of protein extraction into a multi-product biorefinery framework enables the cascading utilization of marine biomass and supports sustainable, circular food systems.<sup>99</sup>

## 6. Practical and Industrial Challenges in the Application of Isolated Blue Food Proteins

Despite the promising functional and nutritional attributes of isolated proteins derived from blue foods, several practical and industrial challenges remain that may limit their large-scale implementation. One major limitation involves variability in raw material composition, which can significantly influence protein yield, purity, and techno-functional performance.<sup>81,100</sup> Seasonal variation, species diversity, and environmental conditions affect protein structure and extractability, thereby complicating process standardization. Furthermore, purification and isolation technologies often require multiple processing steps, including membrane filtration,



594 precipitation, and chromatographic separation, which increase operational costs and energy  
595 demand.<sup>101</sup> Although non-thermal techniques improve extraction efficiency, scale-up  
596 feasibility and equipment investment remain critical constraints.

597 In addition, structural modifications occurring during extraction and drying may alter  
598 solubility, emulsifying capacity, and gelation behaviour, potentially limiting functional  
599 performance in complex food matrices.<sup>102,103</sup> Sensory challenges, including off-flavors, color  
600 instability, and marine-associated aromas, can also affect consumer acceptance, particularly in  
601 mainstream food applications.<sup>104</sup> Regulatory considerations, allergenicity assessments, and  
602 labelling requirements further complicate commercialization pathways for novel protein  
603 ingredients. Therefore, while isolated blue food proteins demonstrate considerable potential for  
604 sustainable food system transformation, addressing techno-economic feasibility, sensory  
605 optimization, and regulatory compliance is essential for successful real-world adoption.

## 606 7. Conclusion, limitations, and Future Perspective

607 This review has explored the innovative techniques for protein extraction from blue foods  
608 based on the concept of non-thermal techniques, which are a safe, green approach, with less  
609 treatment time, and can enhance the extraction efficiency of protein with supreme quality. The  
610 protein from marine sources can be commercialized for its virtuous approach, specifically  
611 nutraceuticals and bioactive compounds. There is a projected substantial market for food  
612 products, supplements, and natural health products containing marine bioactive due to a  
613 multitude of possible health benefits. Despite providing a comprehensive overview of non-  
614 thermal techniques for sustainable protein extraction from blue foods, this review is limited by  
615 the availability of large-scale industrial data and standardized environmental assessment  
616 metrics across studies. Variations in reported extraction efficiencies, functional properties, and  
617 processing conditions restrict direct quantitative comparisons. Additionally, techno-economic  
618 feasibility and long-term storage stability remain underexplored in the current literature,  
619 highlighting the need for pilot-scale validation and integrated life cycle assessments in future  
620 research. The review directs future research and development on modified protein, or bioactive  
621 compounds, into foods for prominence, as these components have significant nutritional and  
622 functional benefits in addressing various health concerns. In the future, the commercialization  
623 process will gain advantages through the application of biorefinery approaches, which aim to  
624 create cost-effective and environmentally friendly extraction methods for generating bioactive  
625 compounds with well-measured beneficial effects. Innovative non-thermal technologies have



626 arisen as green approaches, promising, safe, and efficient methods for retrieving high-quality  
 627 nutritional components, specifically protein. These modified proteins have optimistic structural  
 628 and functional properties that can be commercially utilized as functional ingredients.

### 629 **Conflict of interest**

630 The authors stated that there is no conflict of interest.

### 631 **Abbreviations**

Abbreviate	Expansion
3D	Three Dimensional
ACS	Acid Soluble Collagen
BSE	Bovine Spongiform Encephalopathy
DES	Deep Eutectic Solvent
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FMD	Foot and Mouth Disease
FPH	Fish Protein Hydrolysates
HPP	High-Pressure Processing
HVAC	High Value Compounds
IUIS	International Union of Immunological Societies
PEF	Pulsed Electric Field
SCF	Super Critical Fluid
TSE	Transmissible Spongiform Encephalopathy
WHO	World Health Organisation

632



634 **Author contribution**View Article Online  
DOI: 10.1039/D5FB00784D635 **Nikashini Thirugnanam**<sup>†</sup>- conceptualization, design of methodology, data acquisition, data curation,  
636 writing original draft, review, and editing637 **Monisha Chandran**<sup>†</sup>- conceptualization, design of methods, writing original draft, review, and  
638 editing639 **Pathare Ashutosh Dattatrya**- conceptualization, design of methods, data acquisition, data curation,  
640 writing original draft641 **Loganathan Manickam**\*-conceptualization, design of methods, review and editing, supervision,  
642 project administration643 <sup>†</sup>- Equally contributed

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Table 1. Extraction of Protein from blue foods with different methods

Sources	Method of extraction	Extraction conditions	Yield (%)	Reference
<b>Algae</b>				
<b>Microalgae</b>				
<i>Aglaothamnion uruguayense</i>	Alkali extraction	Addition of 1mL 0.1N NaOH to the centrifuged pellet with occasional shaking	22.38	105
<i>Hilleasp.</i>	Alkali extraction	Addition of 1mL 0.1N NaOH to the centrifuged pellet with occasional shaking	25.04	105
<i>Amphidinium carterae</i>	Alkali extraction	Addition of 1mL 0.1N NaOH to the centrifuged pellet with occasional shaking	24.06	105
<i>Haematococcus pluvialis</i>	Ultrasonication	Frequency-20 kHz, 5 s cycles with 15 s of resting time for 30 min	8.5±0.0	106
<i>Porphyridium cruentum</i>	Ultrasonication	Frequency-20 kHz, 5 s cycles with 15 s of resting time for 30 min	67.0 ± 0.9	106





<i>Arthrospira platensis</i>	High pressure homogenization (HPH)	2700 bar pressure in two passes	78.0 ± 2.8	106
<b>Macroalgae</b>				
<i>Ulva ohnoi</i>	PEF coupled with a mechanical press	200 pulses with a field strength of 1kV/cm, repetition rate and pulse duration 3 Hz and 50 μs, and combined with mechanical pressing at 107,682 N/m <sup>2</sup>	14.94	23
<i>Ulva armoricana</i>	Enzyme assisted extraction	6% endoprotease enzyme, soaked at 50 <sup>0</sup> C for 3h	41.40	107
<i>Chondracanthus Chamissoi</i>	Enzyme assisted extraction	Cellulase enzyme, pH 4.5, for 12h at 50 <sup>0</sup> C, 1:10 enzyme-solvent ratio	36.10	108
<i>Gracilaria sp.</i>	Ultrasound-assisted alkaline extraction	Suspension of sample in 10% (v/v) NaOH, sonication for 2h, further filtration (0.45μm), and dialysis (2kDa) is done	86	109
<i>Ascophyllum Nodosum</i>	sequential acid-alkaline extraction	0.4 M HCl for 1h at 4 <sup>0</sup> C & 0.4 M NaOH for 1h at 4 <sup>0</sup> C	59.75	110

<i>Fucus vesiculosus</i>	HPP	600 MPa for 40 min, followed by filtration	23.7	111
<i>Chlorella vulgaris</i>	PEF with bead milling	14 pulses with a field strength of 20.6 kV cm <sup>-1</sup> and tip speed of 8 ms <sup>-1</sup>	~30 protein release	70
<i>Neochloris Oleoabundans</i>	PEF with bead milling	14 pulses with a field strength of 19.7 kV cm <sup>-1</sup> and tip speed of 8 ms <sup>-1</sup>	~50 protein release	70
<i>Ulva sp.</i>	Enzyme-assisted PEF extraction	Enzyme pretreatment with Cellulase Onzuka R-10, 30 pulses with the field strength of 1kV cm <sup>-1</sup> , pulse duration, and repetition rate is 30 μs and 5 Hz	19.6±0.33	112
<i>Gracilaria verrucosa</i>	Enzymatic extraction	Agarase + Cellulase, 2 h and 14 h incubation	63% - 2 h 21% - 14 h	113
<i>Chondrus crispus</i>	Enzymatic extraction	Carrageenase + Cellulase, 2 h and 14 h incubation	32% - 2 h and 14 h	113





<b>Marine animals</b>				
Yellowfin Tuna <i>Thunnus albacares</i>	Acid extraction	0.5 M acetic acid in a sample-solvent ratio of 1:10 for 48 h at 4 <sup>0</sup> C	1.07	114
Grass carp <i>Ctenopharyngodon idella</i>	Acid extraction	0.5 M acetic acid in a sample-solvent ratio of 1:10 for 72 h at 4 <sup>0</sup> C	90	115
Catla <i>Catlacatla</i>	Acid extraction	0.5 M acetic acid in a sample-solvent ratio of 1:10 for 24 h at 4 <sup>0</sup> C	63	116
Rohu <i>Labeorohita</i>	Acid extraction	0.5 M acetic acid in a sample-solvent ratio of 1:10 for 24 h at 4 <sup>0</sup> C	46	116
Small spotted catshark <i>Scyliorhinus canicula</i>	Acid extraction	0.5 M acetic acid at 25 <sup>0</sup> C for 24 h	61.24	117
Thornback ray <i>Raja clavata</i>	Pepsin-aided acid extraction	0.5 M acetic acid in a sample solvent ratio of 1:10, followed by the addition of 5g of pepsin/g, T=4 <sup>0</sup> C for 18h	30.16	118

Red drum fish ( <i>Sciaenops ocellatus</i> )	Pepsin-aided acid extraction	15 l of 1 M acetic acid, 0.5% pepsin, t = 8 h	4.32±0.30	29
Mackerel	Isoelectric solubilization precipitation	0.2 M NaOH in 1:10 sample-solvent ratio	48.08±0.04	54
Starfish	A combination of Ultrasound and High-shear mechanical homogenization	0.1 M NaOH in 1:10 sample solvent ratio, 4000 rpm for 2.5 min, 70% amplitude for 10 min	3.37±0.10	56
Golden carp ( <i>Probarbus jullieni</i> )	Ultrasound assisted acid extraction	Acid pretreatment in 1:10 sample solvent ratio, 20 Hz frequency, 80% amplitude for 30 mins	81.53	119
Clown featherback ( <i>Chitala ornate</i> )	Ultrasound-assisted extraction	750 W, f = 20 kHz, 20,40,60,and 80% amplitude with different time intervals 10,20,30 min	27.18 to 57.35	120
Jellyfish ( <i>Acromitushardenbergi</i> )	Acid extraction	0.5M acetic acid (1:1000 w/v)	65.20±1.12	121





Table 2. Functional properties of modified Protein from blue foods using novel techniques

S.No	Treatments	Sources	Functional Property	Reference
1.	Ultrasound-assisted extraction	<i>Spirulina platensis</i>	WAC, OAC, EA, ES, FC, FS	122
2.	Osmotic shock + Ultrasound	<i>Chlorella vulgaris</i>	WAC, OAC, EA, ES, FC, FS	123
3.	Isoelectric precipitation	<i>Loligo vulgaris</i>	FC, FS, ES, FBC, WHC	124
4.	HPP	<i>Callinectes sapidus</i>	WBC	125
5.	Ultrasound-assisted extraction	<i>Dosidicus gigas</i>	FC, FS	126
6.	PEF	<i>Haliotis discus hannai</i>	FC, FS, EA, ES	73
7.	HPP	Blue crab	Gelation property	13
8.	Ultrasound-assisted extraction	<i>Dasyatis zugei</i>	Viscosity, RP, WHC, ES	57
9.	Ultrasound-assisted extraction	Tuna	WHC, ES, EA, Viscosity	58

Where WHC-Water Holding Capacity, WAC-Water Absorption Capacity, ES-Emulsion Stability, EA-Emulsifying Activity, RP-Rheological Property, FC-Foaming Capacity, FS-Foaming Stability, OAC-Oil Absorption Capacity, FBC-Fat Binding Capacity

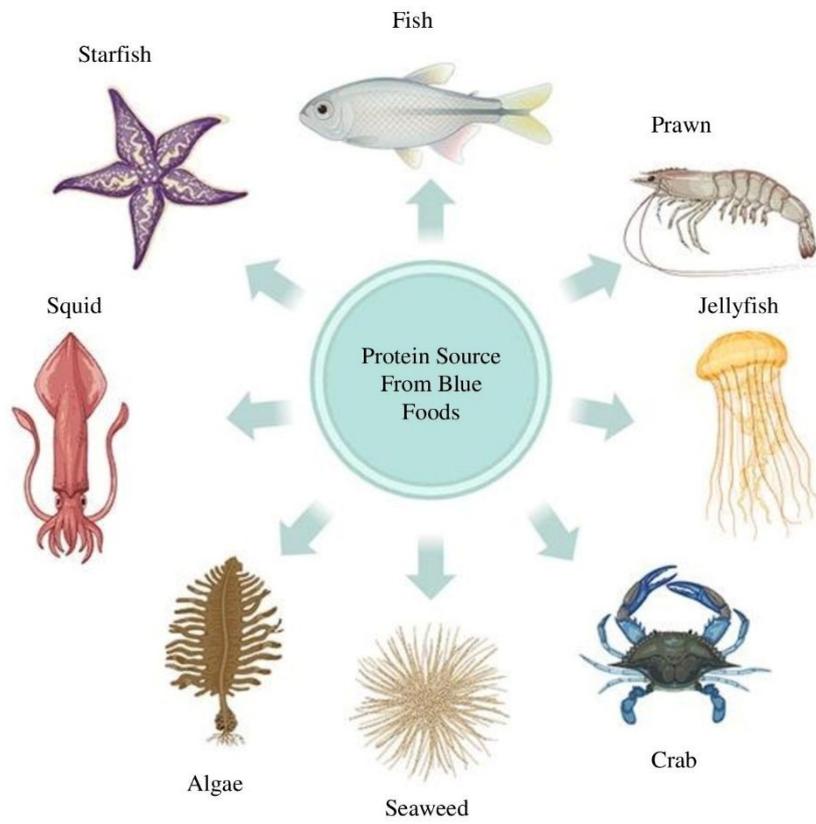


Fig.1. Protein sources from blue foods



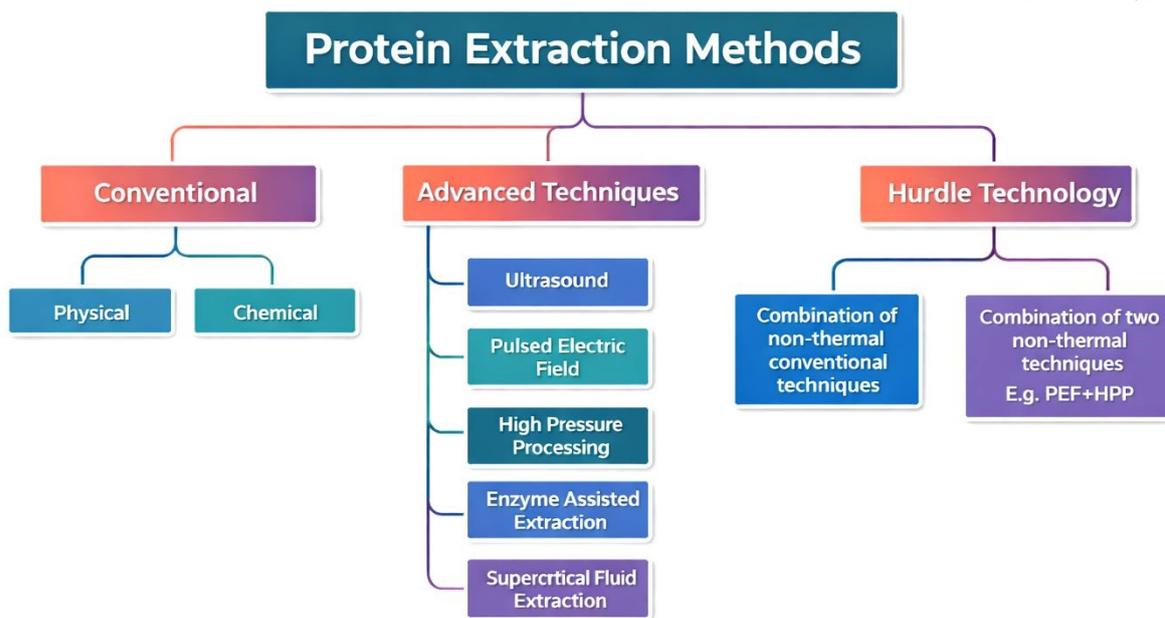


Fig.2. Protein extraction methods

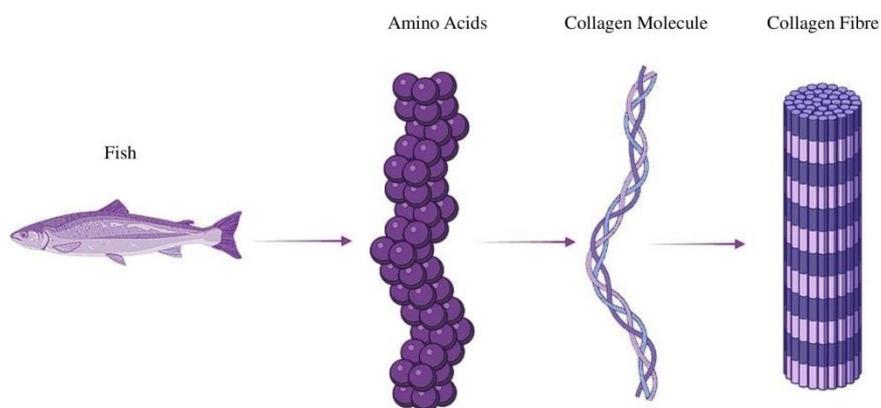


Fig.3. Collagen formation from Fish source



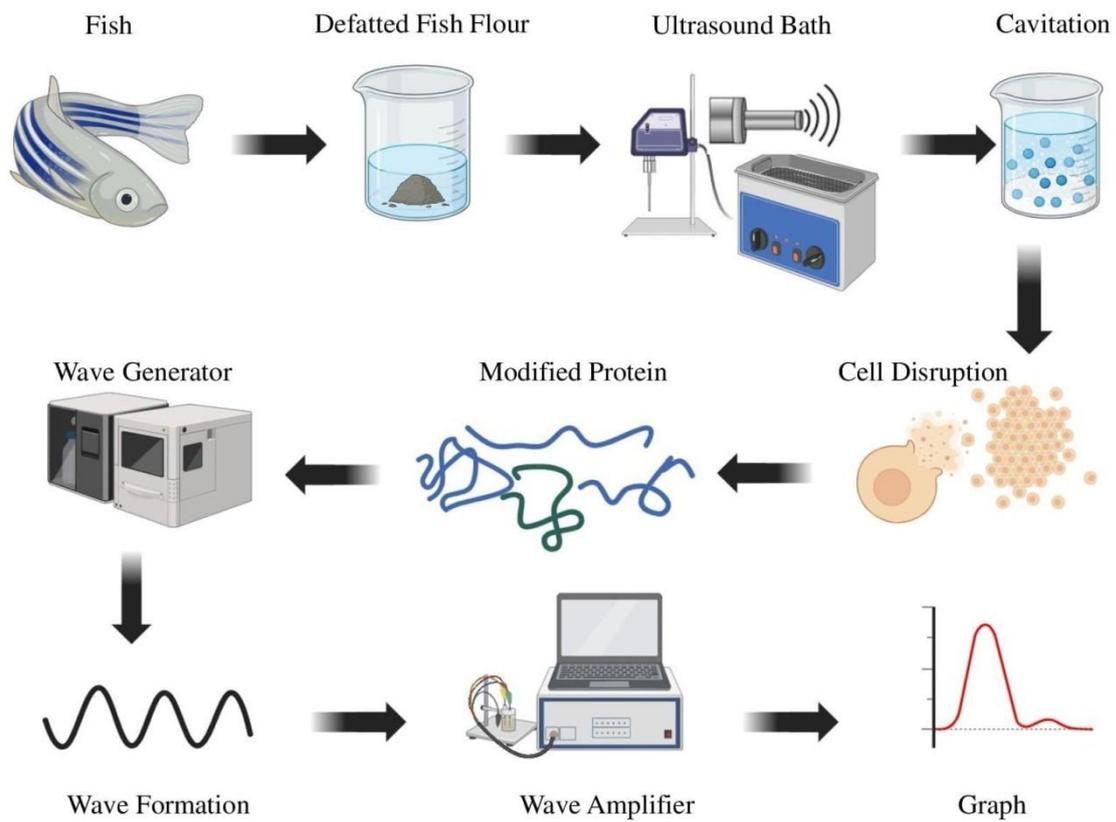


Fig.4. Protein extraction using ultrasound treatment



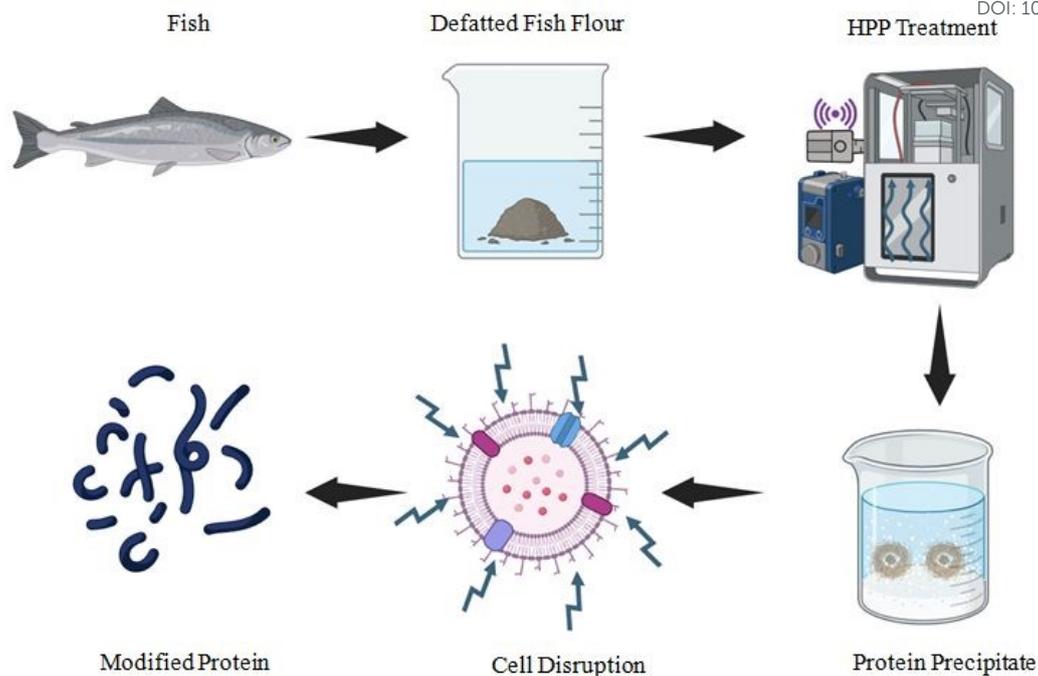


Fig.5. Cell disruption in protein molecule using HPP treatment

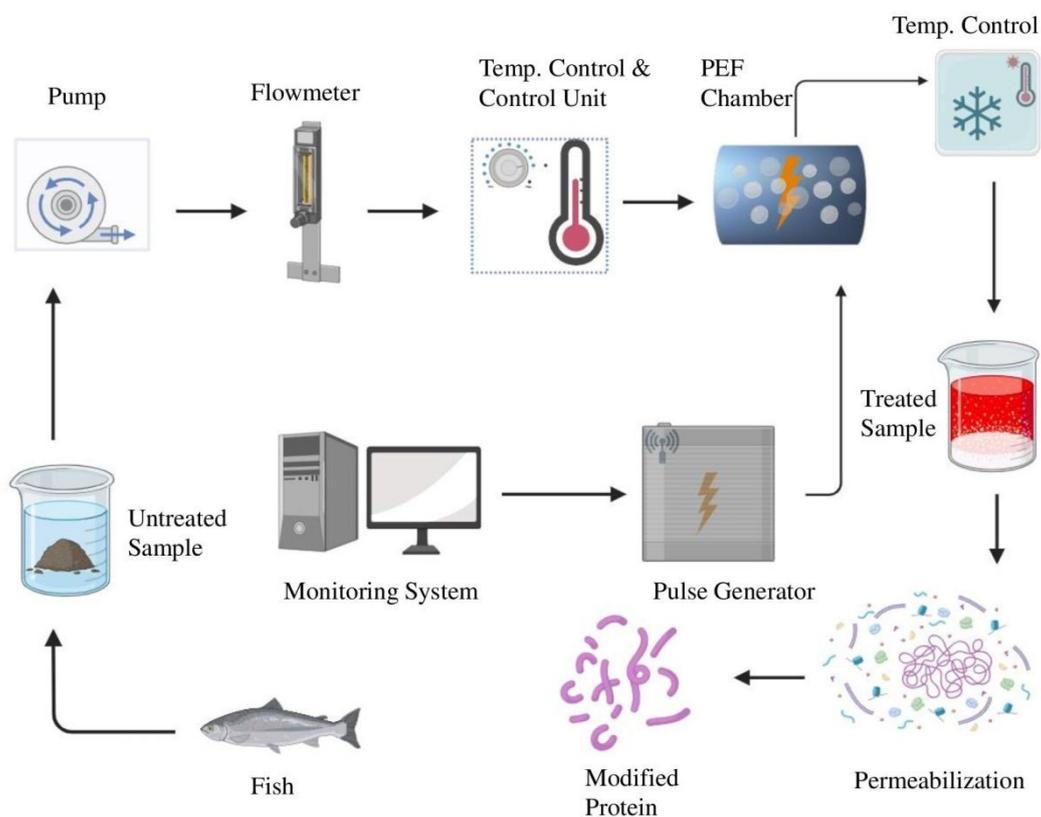


Fig.6. Cell permeabilization in protein for pulsed electric field



### Data Availability Statement

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Data sharing does not apply to this article as no new data were created or analyzed in this study. The manuscript is based entirely on published literature that is appropriately cited and available in the public domain.

