Utilizing bull's eye fish processing frame waste to produce edible proteins and quality assessment of the recovered proteins

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Abstract

With an aim to utilize the waste generated from Bull's eye (*Priacanthus hamrur*) fish processing, proteins were extracted from this waste using the pH shift method. The properties of extracted proteins were studied in detail. During the protein solubility measurement, maximum solubilisation was found at pH 3.0 (13.10 mg/mL) on the acidic side and pH 11.0 (14.25 mg/mL) on the alkaline side with a total yield of 51.62 ± 0.23 and 45.42 ± 0.29 (%), respectively. The variables tested in this study showed a significant effect on protein solubility (*p* < 0.05). The protein content of the isolates extracted from the waste was 23.80 ± 0.49 and 22.48 ± 0.39% for acid and alkali processed isolates, which was significantly higher than the mince (19.46 ± 0.67%). Processing of Bull's eye proteins caused a significant reduction in its pigments, lipids and myoglobin content (*p* < 0.05). Proteins processed using alkali had significantly higher values for foaming stability, water holding capacity, and emulsion capacity than proteins extracted using acid. An overall assessment indicated that protein isolates obtained using alkali extraction were better in terms of textural attributes, gelling ability and amino acid profile than protein extracted using the acid process.

Introduction

Globally the fisheries and aquaculture industry generate approximately 150.5 billion USD through fish trade and significantly contributes to the world economy, directly coming from total world fisheries and aquaculture production of 177.8 million tonnes. Out of the total production, 157.4 million tonnes were used for human consumption, whereas a portion used for non-food purposes was 20.4 million (FAO 2022). With the growing world population, interest in the animal protein source increased, consequently overexploiting fisheries resources, including low-value and smaller size fish species (Nolsøe and Undeland 2009; Kim and Venkatesan 2014).

The Bull's eye fish family (*Priacanthidae*) comprises around 17 species distributed mainly tropical and subtropical marine waters. In India, the fishery has been emerging significantly as non-conventional fishery resource with the increase in commercial deep-sea trawling activity. As per the latest estimate, the landing of Priacanthids in India in 2020 is 22,107 tonnes (ICAR-CMFRI 2022). Among these, *Priacanthus hamrur* (Bull’s eye) is the most commonly exploited species. It is mainly used for preparing surimi and surimi-based products (Benjakul et al. 2002), which could produce vast amounts of waste. Thus, it is crucial to advance a technology that effectively recuperates functional proteins from fish wastes to fulfil the nutritional demand and reduce the ecology-related pressure on seafood processing.

The fish processing industry generates a considerable range of by-products and wastes, viz., 9–12% (heads), 12–18% (viscera), 1–3% (skin and fins), 15–20% (filleting waste), 9–15% (bones), 5% (abdominal contents) reported by Rustad et al. (2011); Klomklao and Benjakul (2016), which are almost as high as 57% (w/w) of the total captured fish weight. Despite the rich nutritional profile, most of these by-products are discarded in land-fills or into water steams without prior recovery of the nutrients (Lee et al. 2016), which causes wastage of high value nutrients, severe environmental pollution and extra
operational costs. One of the most acceptable ways to use these processing wastes is to retrieve the nutrients from the wastes to use them for human edible purposes. This would address environmental pollution issues and reduce operational costs. The raw material type, source, and employed process would determine the functionality and characteristics of the retrieved nutrient. Therefore, it is vital to optimize raw materials quality, handling procedures and process parameters before applying them to food systems (Surasani 2018).

Over the years, science and technology have developed to solve the problem of environmental pollution and the loss of nutrients from seafood industry waste. Numerous attempts are being made in this area, but not a single one is good enough to address the existing problem because of many technical hurdles. In order to address solutions related to seafood processing waste, the method patented by Hultin and Kelleher (1999), known as isoelectric precipitation/pH-shift method/ acid-alkaline solubilization, which offered tremendous capability for protein isolation from nonconventional complex marine sources. The isolate obtained by this process is one of the food ingredients in producing a crumb with rich protein composition (minimum 90% dry basis). Much research has been conducted by following the method and stated that recovered protein from this technique has a higher yield (with good functionality) than conventional surimi production (Surasani 2018).

This powder form offers several benefits, including practical proposal and safety during storage, and it does not call for any special storage conditions, which could be used to develop value-added products in the food system as intermediate product integration (Shaviklo et al. 2012). Despite several findings on recuperating and characterizing the protein isolates from fishery waste, its skilful application in the food sector is minimal (Shaviklo et al. 2016; Surasani 2018). With this background, the following study was conducted to optimize the pH-based solubilization and recovery of Bull's eye proteins from its processing waste and characterize the obtained isolates.

Materials And Methods

Raw material

Bull's eye (*Priacanthus hamrur*) fish (average size of 15–20 cm and 200 g weight) were procured from the major fish landing centre in Mangalore, India and transported aseptically to the laboratory in an insulated ice box and stored in ice until further used. All the preparatory and processing steps, including raw material processing, were performed at a temperature < 5°C.

Preparation Of Protein Isolates

Protein isolates were prepared as per the methodology given by Surasani et al. (2017a). The ground waste was added with cold deionized water at ratio of 1:9 (1:9 w/w; 4°C), followed by high-speed homogenization at 10000 rpm for 60 s (2 × 30 s) (Ultra-Turrax, T 25, Janke and Kunkel GMBH and Co., Staufen, Germany). The prepared homogenate was adjusted to the desired pH level using diluted NaOH
and HCl with simultaneous mixing using an overhead stirrer. Then, the homogenate was subjected to two stages of centrifugation (Thermo Sorvall Legend XTR, Thermo Fisher Scientific, USA) at 10,000×g for 20 min, followed by the resulting sediment (isolate) was packed in a zip-lock bag and kept on ice in a cold room at 4°C overnight.

Protein Solubility

Solubility curve

A pH series between 3.0 and 11.0 were adjusted to the homogenate with an interval using diluted acid and alkali (2M), followed by its protein determination using Biuret method (Robinson and Hogden 1940).

Solubility after isoelectric precipitation

Protein solubility post-isoelectric precipitation was tested using two pHs having the highest solubility (3.0 and 11.0) with a precipitation pH of 5.5. Protein solubility after the second centrifugation cycle was assessed using Biuret method.

Protein recovery and preparing isolates

Protein recovery (theoretical and practical) was calculated as per the methodology described by Surasani et al. (2017a).

Characterization of isolates

The compositional analysis of raw material and Bull’s eye protein isolate was done using standard protocols described by AOAC (2000). Colour values (L*, a* and b* values) and the overall whiteness of the samples were determined according to the methodology of Chaijan et al. (2010). Total lipid content was determined as per the standard protocol given in AOAC (2000). Total pigment content was determined as per the method described by Rawdkuen et al. (2009). The total myoglobin content of samples was determined as per the procedure used by Chaijan et al. (2006). Surface active properties such as foaming capacity (FC), foaming stability (FS) (Sathe et al. 1982), emulsification capacity (EC), water and oil holding capacity (WHC and OHC) (Foh et al. 2011) were determined using standard methods.

Protein gel preparation was done as per the procedure given by Kristinsson and Ingadottir (2006). Gel quality analysis i.e. folding test (Kudo et al. 1973), gel strength (Reddy 2016), and expressible moisture (Feng and Hultin 2001), was performed as per the standard protocols. Amino acid profiling was done using a standard protocol as described by Bidlingmeyer et al. (1984). The amino acid content of Bull’s eye protein was measured and expressed as g/ 100 g protein.

Statistics Analysis
All experiments were performed in triplicate unless otherwise stated. Results were expressed as mean ± standard deviation. All the results were subjected to a one-way analysis of variance (ANOVA), and significant difference was measured with tukey posthoc (p < 0.05) using IBM SPSS (Version 20.0) statistical software package program.

**Results And Discussion**

**Optimization of process parameters on bull's eye protein solubility and recovery**

**Effect of ph on protein solubility and recovery**

The solubility of proteins at different pH differed significantly (p<0.05). The protein solubility was maximum at pH 3.0 (13.10 ± 0.11 mg/mL) and pH 11.0 (14.25 ± 0.23 mg/mL). Generally, the higher protein solubility is achieved at pH 2.0 and 13.0, but the actual protein recovery percentage was maximum at pH 3.0 and 11.0 in the current study. Surasani (2018) explained that the feasibility of any protein extraction process depends on the yield and protein recovery. The study conducted by various researchers has documented that the maximum solubility of fish protein is in the pH range of 2.0-3.0 (acidic side) and 11.0 -13.0 (alkaline side), while the least solubility is in the pH of 5.0-6.0.

According to Kristinsson et al. (2005), maximum protein solubility is essential to isolate protein from contaminants, while minimum solubility is essential in precipitation to achieve higher recovery of solubilised proteins. Proteins surface charges turn more positive and negative, thus increasing solubility due to increased electrostatic repulsion above and below the isoelectric pH (Kelleher and Hultin 1999).

Bull's eye frame waste protein isolate with a higher yield (36.44 ± 0.39%) was obtained in the acidic solubilisation process than in the alkaline solubilisation process (30.22 ± 0.59%) (p<0.05) during the pH shift method. In this process, theoretical recovery was more than the actual recovery with all the pHs ranges used. A similar pattern was observed during recovery through the second centrifugation step, and total process yield were 51.62 ± 0.23% and 45.42 ± 0.29% for pH 3.0 and 11.0, respectively (Table 1). Several factors influence the recovery yield of protein during the pH shift method such as different raw materials and processing methods (Undeland et al. 2002; Kristinsson and Ingadottir 2006; Nolsøe et al. 2007). The higher solubility of proteins was observed on the acidic side and larger sediment formation on the alkaline side (Nolsøe and Undeland 2009), which supports the findings of the current study. Our findings are also in line with the study by Surasani et al. (2017a, b) on protein recovery from Pangas and Rohu processing waste.

**Effect of extraction time on solubility and recovery**

The effect of extraction time (5–120 min) on protein solubility and recovery of protein (%) obtained by the pH shift technique are given in Fig 1 (B). The protein solubility and recoveries at different extraction period were significantly different (p<0.05). Maximum protein solubility (20.13 ± 0.16 mg/mL) and protein recovery (59.44 ± 0.35%) was recorded at 60 min, and a further increase in extraction time caused
a decrease in solubility. The variation in protein solubility with different extraction times could be due to irregular diffusion of non-protein substances of homogenate that was observed in the present study and was well confirmed by the findings of previous researchers (Kahn et al. 1974; Montecalvo et al. 1984; Surasani 2020a, b, c).

**Effect of extraction temperature on protein solubility and recovery**

The extraction temperature strongly influences the protein solubility and recovery (p<0.05). Protein solubility and recoveries improved from 9.59 ± 0.33 to 16.70 ± 0.44 mg/mL as temperature increased from 4 to 50 °C as described in Fig 1 (C). Surasani et al. (2017a,b) reported a similar pattern during their work on proteins from processing waste of pangus and rohu. In contrast, a reverse trend was observed by Kahn et al. (1974); Montecalvo et al. (1984) during the recovery of proteins from flounder and squid.

**Effect of homogenate to solvent ratio on solubility and recovery**

The effect of homogenate weight to volume of solvent ratio on Bull’s eye frame waste protein solubility is given in Fig 1(D). Protein solubility increased with an increase in weight of homogenate to volume of solvent, significantly (p<0.05) i.e. 1:20 to 1:3 (10.49 ± 0.39 to 24.90 ± 0.85 mg/mL). These findings are in agreement with the observations made by a group of the researcher (Montecalvo et al.1984; Surasani et al. 2018a,b) who worked on protein extraction from fishery waste, stated that high ratios resulted in high viscous of thick solutions and low ratios of dilute solutions of large volumes, might cause trouble during handling. Based on the observations in this study, it is more advantageous to use the weight of homogenate to solvent ratio of 1:6 by for good protein recovery with easy of handling.

**Effect of centrifugation speed on protein solubility and recovery**

Centrifugation speed significantly affected protein solubility and recovery yields, illustrated in Fig 1(E). With the change in centrifugation speed from 2000 to 8000 rpm, protein solubility was improved from 17.81 ± 0.68 to 21.27 ± 0.83 mg/mL, respectively. Solubility was minimum at low centrifugation speed, which could be due to insufficient centrifugal force that could not separate soluble protein from insolubilized matter, causing a heavy fluid-gel-like debris sediment. Kain et al. (2009); Surasani et al. (2017a, 2020a) also performed similarly to produce the same result with their study process variable effect on protein recovery from rohu fish.

**Effect of stirring on protein solubility and recovery**

Based on statistical analysis results, notable changes (p<0.05) were observed with protein solubility, increasing from 15.67 ± 0.55 to 21.59 ± 0.47 mg/mL with an increase in stirring time as shown in Fig 1(F). However, theoretical and actual recovery were significantly different in all the conditions (p<0.05). The current finding suggests that stirring time slightly influences the protein solubility statically, but differences were insignificant. Surasani et al. (2018a) reported stirring time effect on protein solubility was non-significant.
Characteristics of protein isolates

Proximate composition

The composition of raw material and its quality determine the percentage of protein recovery and characteristics. The raw material nutritional components such as moisture, protein, fat, and ash were 73.66 ± 1.32, 19.46 ± 0.67, 1.52 ± 0.38 and 2.49 ± 0.41 %, respectively, used for the protein isolation (Table 2). The protein content of isolates extracted using acidic and alkaline processes was 22.48 ± 0.39 and 23.80 ± 0.49 %, respectively. Surasani et al. (2017a, b) reported that protein content of 9.9 and 8.15 for rohu and pangas processing waste to protein isolates were 17.86 and 20.45 %, respectively. Baraiya et al (2020) reported that the protein content of isolate obtained from Pacu fish (Freshwater species) on the acidic side was 21.01 %, and the alkaline side was 22.87 %. Surasani (2018) explained that the higher water percentage of acid-processed isolates might be the formation of the unique protein unfolding-refolding pattern as pointed to pH 2.5 vs pH 10.8 before the transition to pH 5.5.

Lipid composition is another noteworthy feature of protein isolate, which got reduced significantly during acidic (0.24 ± 0.11 %) and alkaline (0.25 ± 0.12 %) processing from counterpart Bull’s eye filleting waste a lipid content of 1.52 ± 0.38 %. Rohu and pangas protein isolates containing lipid concentration were reduced from 2.9 and 14.17 % processing waste to 0.24 and 0.78 % (p<0.05), respectively (Surasani et al. 2017a, b, 2018a). Earlier documents suggested that the lipids can be deposited in the bottom sediments during centrifugation, which can be removed as a pellet followed by a surfaced layer as some neutral lipids are segregated into this layer (Nolsøe and Underland 2009; Vareltzis et al. 2012).

Colour and whiteness

Colour is one of the essential characteristics of understanding the comparably individual processing effects in which whiteness often determines its application in the market (Tabilo-Munizaga and Barbosa-Canovas 2004). Lightness (L*). The overall whiteness value was better in acidic-based protein isolate 54.02 ± 1.02 than in alkaline-based protein isolates 48.85 ± 1.23 (Table 2) (p<0.05). Current findings are supported by the results of Surasani et al. (2017 a, b), who reported higher lightness values for acid-treated isolates (52.70) than alkaline-treated isolates (41.42) during rohu waste processing. The acid-treated protein isolate had a higher lightness value in the present study due to effectively eliminating pigments, myoglobin, haemoglobin, and melanin thus improving whiteness (Panpipat and Chaijan 2016). The Maillard reaction could be the reason for less whiteness value in alkaline treated protein isolate, which also causes increased b* value (yellowness discolouration). These interpretations were supported by Kristinsson and Hultin (2003); Kristinsson and Liang (2006) during acid/alkaline aided protein isolate extraction.

Lipid, Pigment and Myoglobin content

The protein isolated recuperated through the acidic/alkaline aided method significantly reduced total lipid, myoglobin, and pigments than mince raw meat (Table 2). Among the two isolate groups, acidic
processed isolates had lower lipid content than alkaline ones. The same group of parameters, such as myoglobin and pigment, followed the same declined trend with the reduction from 285.87 ± 6.90 to 28.95 ± 1.77 mg/g and 180.97 ± 3.75 to 32.09 ± 1.23 ppm, respectively. The reported lipid reduction was 50 to 90 % in catfish, sardine and tilapia protein isolate (Kristinsson et al. 2005; Batista et al. 2007). Myoglobin content was decreased significantly in the acid/alkaline method compared to traditional surimi processing (Rawdkuen et al. 2009). The elimination of myoglobin was observed to a greater extent in sardine and mackerel muscles during alkaline processing, and efficiency was determined by fish species, muscle tissue type, the season of storage, and the washing method (Chaijan et al. 2006).

**Functional properties**

Functionality is a total of functional features that determine protein-based product integration. The alkaline-treated isolates were superior to acid-treated isolates in terms of FS, EC, WHC and OHC, whereas FC was higher in acid processed isolate (68.30 ± 1.14 mL/100 mL) compared to alkali processed isolate (56.50 ± 1.34 mL/100 mL). The current finding agrees with the statement of Surasani (2017a, b), who stated that the differences might be due to the differences in hydrophobic residues of proteins. Hultin and Kelleher (1999) stated that the protein structure gets altered on the lower WHC because the protein structure is sensitive to extreme pH, linked to intermolecular repulsions, and appears in the water-holding capacity transformation.

**Properties of protein gels**

Except for the colour values, all the textural attributes, i.e. expressible moisture, folding test, and gel strength, were poorer in gel produced from acidic processed isolates compared to gels made of alkaline extracted isolates (p<0.05) (Table 3). Protein isolate gels had a similar range of results to raw protein isolates. Acidic extracted isolates had high L* values, low a* and b* values than alkaline extraction (p<0.05). The whiteness value of protein isolates was (57.44 ± 1.23), relatively higher in acidic ranged protein isolates than alkaline ranged protein isolates (51.26 ± 0.75). Using the pH shift technique, Paker et al. (2015) also revealed identical results during the proteins from silver carp.

The expressible moisture and gel strength were higher in alkaline processed isolates than the acidic processed isolate. In contradiction to the folding test, all specimens were in the best condition by receiving scores of 5 (out of 5) in gel produced from protein isolates. Surasani (2020b) remarked that gels prepared from acid-processed protein isolates were weaker than those from alkali extracted isolates during their investigations on rohu and pungas. According to Chaijan et al. (2006), gelling properties differ due to the structure and effectiveness of gels influence on the process of protein bonding. The acidic aided method triggered poor networking of protein gels because the acidic pH causes denaturation of excessive protein. Raw material (fish species), extraction mode, and extraction time can determine the protein's gel formation capability (Kristinsson and Liang 2006; Nolsøe et al. 2007).

**Amino acid profiling**
Amino acid-containing to the homogenate was detected in protein isolate obtained through the pH shift method, indicating recovery of all amino acids same as in homogenate protein (Fig 2). The study suggested that the acidic/alkaline solubilisation method could not adversely influence amino acid recovery. It is observed that alkaline processed isolates revived more amino acids from homogenate, which might be due to the connective tissues that are in the more extractable form during alkaline processing (Batista1999). The amino acid composition of the sample group in the present study is identical to the work done on same species group (Dileep et al. 2011; Binsi et al. 2009; Surasani et al. 2017a, b). However, fish species, the way the pH method is employed and solubilised pH range determine the overall composition of amino acids and quality of protein isolate. Above all, the acid/alkaline solubilisation produced amino acid concentrations are above the range suggested for adult consumption by FAO/WHO/UNO.

**Conclusion**

The protein isolates extracted from Bull's eye filleting waste at pH 3.0 and pH 11.0 can be accomplished by the acid/alkaline solubilization method and produce good yield and recovery. Alkaline-aided processing is a suitable technique for protein recovery from marine fish processing waste with good functional properties. The retrieved protein isolate showed decent protein concentrate, good amino acid recovery, gelling and texture properties. The pH shift method could significantly reduce lipid, pigment and myoglobin content, followed by whiter isolates with more stability. This procedure enhances the colour and texture of isolates, especially in the instance of protein isolates by the alkaline method. This technology is well accepted for recovering proteins from marine fish processing waste. However, some setbacks can be curtailed by amending the process parameter such as pH, water ratio, centrifugation speed, etc. More research on this subject could offer in-depth knowledge with advanced analytical instruments. Accordingly, newer definite investigations should be encouraged better to understand protein isolate's process variable and efficiency.

**Declarations**

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**Ethical Approval**

Ethical approval was not required for this research.

**Funding**
Not applicable

**Authors Contributions**

Manjanaik Bojayanaik: Conceptualization, Methodology, Supervision, Reviewing and Editing, Baraiya Kirankumar Gopalbhai: Investigation and Writing- Original draft preparation, Taral Pravinkumar Vaghabhai: Data curation, Vijay Kumar Reddy Sursani, Krishnamoorthy Elavarasan, and Veena Shetty Alandur: Visualization and Validation. All authors read and approved the final manuscript.

**Consent to Publish**

All authors are agreed to submit research paper to ESPR

**Consent to Participate**

All author are agreed to participate

**Competing Interests**

The authors have no relevant financial or non-financial interests to declare

**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Tables**

**Table 1.** Recovery and process yields of protein from Bull's eye fillet frames during solubilization at pH 3.0 and 11.0.

<table>
<thead>
<tr>
<th>Recovery and Yields</th>
<th>Acid processed isolate (pH 3.00)</th>
<th>Alkaline processed isolate (pH 11.0)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical recovery after first centrifugation (%)</td>
<td>40.52 ± 0.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.02 ± 0.88&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Actual recovery after first centrifugation (%)</td>
<td>36.44 ± 0.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.22 ± 0.59&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Theoretical recovery after second centrifugation (%)</td>
<td>40.05 ± 0.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.11 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Actual recovery after second centrifugation (%)</td>
<td>44.43 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.61 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total recovery (%)</td>
<td>51.62 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.42 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Note: Different superscripts in small letters (a, b, c) indicate significant differences (p<0.05) amongst columns. Values are expressed as mean ± SD (n=3).
Process conditions: Temperature: 28° C; Homogenate weight to solvent ratio: 1:06; Centrifugation speed: 5000 rpm; Extraction time: 60 min.

**Table 2.** Physicochemical attributes of Bull's eye fish raw material and the isolates obtained through pH shift processing
<table>
<thead>
<tr>
<th>Physicochemical attributes</th>
<th>Bull’s eye waste</th>
<th>Acid processed isolate (pH 3.0)</th>
<th>Alkali processed isolate (pH 11.0)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>73.66 ± 1.32^{Aa}</td>
<td>75.58 ± 1.04^{Aa}</td>
<td>74.76 ± 1.25^{Aa}</td>
<td>0.228</td>
</tr>
<tr>
<td>Protein</td>
<td>19.46 ± 0.67^{Bb}</td>
<td>22.48 ± 0.39^{Ab}</td>
<td>23.80 ± 0.49^{Ab}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat</td>
<td>1.52 ± 0.38^{Ac}</td>
<td>0.24 ± 0.11^{Bc}</td>
<td>0.25 ± 0.12^{Bc}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ash</td>
<td>2.49 ± 0.41^{Ac}</td>
<td>1.05 ± 0.18^{Bc}</td>
<td>0.98 ± 0.20^{Bc}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Color values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>50.64 ± 1.06^{Ba}</td>
<td>54.26 ± 1.02^{Aa}</td>
<td>49.53 ± 1.34^{Ba}</td>
<td>0.006</td>
</tr>
<tr>
<td>a*</td>
<td>6.41 ± 0.35^{Ac}</td>
<td>0.71 ± 0.15^{Bc}</td>
<td>0.96 ± 0.13^{Bc}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>b*</td>
<td>11.68 ± 0.36^{Ab}</td>
<td>4.61 ± 0.47^{Cb}</td>
<td>8.26 ± 0.52^{Bb}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whiteness</td>
<td>48.87 ± 0.91^{Ba}</td>
<td>54.02 ± 1.02^{Aa}</td>
<td>48.85 ± 1.23^{Ba}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Functional properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foaming capacity (mL/100 mL)</td>
<td>-</td>
<td>68.30 ± 1.14</td>
<td>56.50 ± 1.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Foaming stability (mL/100 mL)</td>
<td>-</td>
<td>58.15 ± 1.13</td>
<td>64.81 ± 1.99</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Emulsion capacity (mL/100 mL)</td>
<td>-</td>
<td>47.10 ± 2.50</td>
<td>67.07 ± 2.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water holding capacity (mL/g))</td>
<td>-</td>
<td>0.33 ± 0.11</td>
<td>1.49 ± 0.40</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>Oil holding capacity (mL/g))</td>
<td>-</td>
<td>0.85 ± 0.11</td>
<td>2.12 ± 0.32</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td><strong>Other properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipid content (%)</td>
<td>2.55 ± 0.42^{A}</td>
<td>0.84 ± 0.10^{B}</td>
<td>0.86 ± 0.11^{B}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total myoglobin content (mg/g)</td>
<td>285.87 ± 6.90^{A}</td>
<td>28.95 ± 1.77^{B}</td>
<td>26.31 ± 1.12^{B}</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Total pigment content (ppm) | 180.97 ± 3.75<sup>A</sup> | 32.09 ± 1.23<sup>B</sup> | 34.70 ± 1.09<sup>B</sup> | <0.001

Note: Different superscripts in capital letters (A, B, C) indicate significant differences (p<0.05) amongst rows and superscripts in small letters (a, b, c) indicate significant differences amongst columns. Values are expressed as mean ± SD (n=3).

**Table 3.** Properties of protein gels prepared using Bull's eye protein isolates obtained through acid and alkaline solubilization

<table>
<thead>
<tr>
<th>Gel properties</th>
<th>Acid processed isolate (pH 3.0)</th>
<th>Alkali processed isolate (pH 11.0)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour L*</td>
<td>58.43 ± 1.34</td>
<td>53.84 ± 1.05</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>a*</td>
<td>1.25 ± 0.40</td>
<td>2.67 ± 0.49</td>
<td>&lt;0.017</td>
</tr>
<tr>
<td>b*</td>
<td>9.02 ± 0.32</td>
<td>15.36 ± 0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whiteness</td>
<td>57.44 ± 1.23</td>
<td>51.26 ± 0.75</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Expressible moisture (g/100g)</td>
<td>18.76 ± 1.72</td>
<td>26.49 ± 2.66</td>
<td>&lt;0.013</td>
</tr>
<tr>
<td>Folding test</td>
<td>5.00 ± 0.00</td>
<td>5.00 ± 0.00</td>
<td>-</td>
</tr>
<tr>
<td>Gel strength (g.cm)</td>
<td>232.29 ± 2.09</td>
<td>238.12 ± 1.79</td>
<td>&lt;0.013</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SD (n=3). L*, lightness; a*, redness/greenness; b*, yellowness/blueness.

**Figures**
Figure 1

Optimization of different extraction variable on solubility of proteins from Bull’s eye fillet frame during alkaline solubilization.
Figure 2

Amino acid profiling of raw material and proteins recovered from Bull's eye fillet frames during solubilization at pH 3.0 and 11.0.


*Essential amino acid