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Protein Profiles of Red Bigeye Flesh (*Priacanthus Macracanthus*)

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ABSTRACT

Red bigeye fish with less than 15 cm length are categorized as low-value fish. The aim of this study was to determine the protein profiles of red bigeye flesh. Red bigeye flesh was analyzed for proximate analysis, LC-QTOF-MS analysis and Fourier transform infrared FTIR. It was found that red bigeye flesh contained 17.21% crude protein, 1.82% crude fat, 1.34% ash content and 3.42% carbohydrate. LC-MS/MS analysis revealed 83 unique proteins, including troponin 33, myosin 23, actin 19, tropomyosin 6, and parvalbumin 2. Regulatory proteins gave the largest portion of 51%, followed by enzymes at 28.92%, and unreviewed proteins at 20.48%. Red bigeye flesh proteins exhibited higher levels of post-translational modifications (PTMs) mainly oxidation and mutation. FTIR spectra analysis of red bigeye flesh protein exhibiting symmetrical bending stretches within amide regions A, B, I, II, III, and VII. This study shows that red bigeye flesh proteins is a promising source of functional food.

1. Introduction

Red bigeye fish (*Priacanthus macracanthus*) inhabits Malaysian waters. According to a study Jabbar *et al.,* (2017) [1], the size distribution of *P. macracanthus* spans from 5 to 34 cm, with a mean falling between 21 to 22 cm. In Malaysia, red big eye fish with lengths between 20 to 40 cm are preferred for surimi production, while with less than 15 cm length are classified as low-value fish. These low-value fish, also referred to as trash fish, which is normally less preferred by consumers and has negligible commercial value. Despite being considered low in value, these fishes are rich in protein and peptides.

Red bigeye fish is common in the Malaysian diet, often being sold fresh at seafood markets and restaurants. It is a popular ingredient in local dishes such as fish curry, stir-fries, and grilled preparations. The significance of harvesting this species extends to various Asian communities, including Indonesia [1]. It is not only marketed fresh, dried, and salted, but is also a preferred choice for surimi production and a potential source of natural antioxidants derived from fish skin gelatin

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hydrolysate [2-4]. Red bigeye fish holds particular importance in surimi production due to its capacity to form robust gels and retain water effectively [5]. Within Asian countries, species belonging to the Priacanthidae family are economically valuable due to their high protein content. They are integral components in a range of typical Asian culinary products like noodles, fish balls, sauces, and surimi items [1]. The red bigeye fish's popularity for consumption stems from its firm flesh and mild flavor, offering a rich source of protein, beneficial omega-3 fatty acids, and essential minerals. However, scientific research on the nutritional and health advantages of this fish remains limited.

In Malaysia, the annual landing of red bigeye fish (*Priacanthus macracanthus*) in the Malaysian ocean was recorded as 15,708 tonnes in 2021, with a price of RM 5.41 per kilogram [6]. The composition of fish muscle includes myofibrillar protein, sarcoplasmic protein, connective tissue, stroma protein, polypeptides, nucleotides, and non-protein nitrogen compounds [7]. Food proteins are recognized as precursors of biologically active peptides [8]. It has been reported that fish proteins constitute approximately 50% to 70% of salt-soluble myofibrillar proteins, 20% to 50% of water-soluble sarcoplasmic proteins, and 3% of insoluble stroma [9].

Over the last two decades, parallel to the growth of web-based protein databases, both commercial and academic bioinformatics tools have emerged to process experimental MS data for peptide and post-translational modification (PTM) identification [10]. A notable example is PEAKS® studio, a commercial software that identifies peptides and PTMs by comparing MS/MS peptide fragmentation spectral data against specific databases or through de novo sequencing. This software includes a variety of enzymatic cleavage sites and over 300 PTM types [10]. However, limitations exist in setting cleavage site types and PTM varieties. PEAKS® studio provides extensive peptide data, including identification scores, protein scores, sequence coverage, peptide count, parent proteins, and PTM details. The challenge remains in the protein's unique nature, as improper search parameter settings can lead to missing numerous peptides, with the peptide identification potentially affected by their inherent composition.

To date, no study has been reported on the protein profiles of red bigeye flesh. This study aims to explore the protein profiles of red bigeye (*Priacanthus macracanthus*) flesh and their post-translational modifications (PTMs). This preliminary report offers an in-depth look at the protein makeup of red bigeye flesh, potentially contributing to the development of pharmaceutical products with significant health benefits.

2. Methodology

2.1 Materials

Whole, fresh red bigeye fishes (*Priacanthus macracanthus*) were purchased from Fisheries Development Authority of Malaysia (LKIM) jetty, Pulau Kambing, Terengganu. Fifty kilograms of fresh fishes with less than 15 cm length were transported in ice to the laboratory. Then, the fish samples were cleaned by rinsing them with tap water. The fish samples were immediately degutted and washed with copious amounts of cool water. The temperature of fishes was maintained under 5°C along the process by using ice to cover them. The cleaned fish were filleted, homogenized using a food processor (Panasonic, Malaysia), and stored at -20°C until further use. All chemicals and reagents used in this research were of analytical grades. For further analysis, the frozen red bigeye fish was thawed and minced into smaller pieces beforehand.

2.2 Proximate Analysis

The proximate analysis of red bigeye flesh, including moisture, crude fat, crude protein, ash and carbohydrate content were determined using AOAC methods [11].

2.3 Determination of Protein Profiles of Red Bigeye Flesh

2.3.1 Protein extraction

The process of extracting protein from fish flesh involved a thermal method, as detailed out by Shaviklo (2013) [12]. Initially, the fish flesh was minced and combined with an equivalent amount of deionized water at a temperature of 23°C. This mixture was then homogenized using food processor (Kenwood, Malaysia) for a duration of 2 min, resulting in a thoroughly mashed flesh consistency. Afterward, the prepared sample was moved into a beaker containing 30 mL of deionized water. Subsequently, the sample was subjected to a temperature of 85°C on a hot plate stirrer (IKA, Malaysia) with continuous stirring for 60 min. Following this, the resulting mixture was transferred to a 50 mL Falcon tube and centrifuged at 2560 *× g* for 15 min at 4°C using a swing bucket centrifuge (Thermo Fisher, USA). The supernatant, obtained from this process, was collected, preserved at 80°C, and subsequently subjected to lyophilization using a freeze dryer (Labconco, USA).

2.3.2 Determination of soluble protein content

The Pierce™ 660 nm Protein Assay (Thermo Scientific, USA) was utilized to quantify the fish samples for soluble protein content. A standard curve was generated by diluting bovine serum albumin (BSA) to concentrations ranging from 0 to 2 mg/mL in deionized water. The analysis was done for three replicated and the control was bovine serum albumin.

2.3.3 In-solution trypsin digestion

The protocol for *in-solution* digestion was executed following the techniques outlined in previous study Kinter and Sherman (2005) [13]. Initially, a 100 µL portion of the protein sample solution, containing 1 mg of total protein, was introduced into a mixture of 6 M urea and 100 mM Tris buffer. Subsequently, a reducing reagent comprising 15 mg of dithiothreitol was dissolved in 375 µL of water was added, along with 125 µL of Tris stock, and gently mixed through vortex. This resulted in a final solution containing 200 mM dithiothreitol and 100 mM Tris in water, which was allowed to undergo reduction for 1 hour at room temperature. After the reduction step, 20 µL of an alkylating reagent composed of 18 mg of iodoacetamide was dissolved in 375 µL of water, along with 125 µL of Tris stock, was added. This led to a final concentration of 200 mM iodoacetamide and 100 mM Tris in the mixture, and the alkylation reaction proceeded for 1 hour at room temperature. To initiate digestion, trypsin stock solution (at a concentration of 200 mg/µL in a Tris solution) was added to achieve a sample-to-trypsin ratio of 50-to-1. The digestion process was carried out overnight at 37°C. After digestion, 2 drops of concentrated acetic acid were incorporated, and finally, the mixture underwent concentration using a vacuum concentrator.

2.3.4 Liquid Chromatography-Quadrupole-Time of Flight-Mass Spectrometry (LC-QTOF-MS)

The Liquid Chromatography-Quadrupole-Time of Flight-mass spectrometry (LC-QTOF-MS) analysis was performed following the protocol outlined in previous study Kinter and Sherman (2005)

[13]. Peptides were reconstituted in 50 µL of a solution containing 0.1% formic acid in deionised water and subsequently filtered using 250 µL polypropylene LC vials from Agilent (USA) with 0.2 µm RC-membrane filters from Sartorius (Germany) using a syringe filter. For analysis, 25 µL of the prepared sample was loaded onto a Liquid Chromatography-Quadrupole-Time of Flight-mass spectrometry (LC-QTOF-MS) system through an auto sampler. The chromatographic separation was executed using a Hypersil Gold C18 column (C18, 2.1 x 150 mm, 3 µm particles) from Thermo Scientific (USA) with two distinct mobile phase buffers. Mobile phase A was composed of 90% (0.1% formic acid in deionised water) while mobile phase B contained 10% (0.1% formic acid in acetonitrile). The separation was carried out at a flow rate of 15 µL/min. A mass range of m/z 100-2000 was obtained for the spectrum of MS and MS/MS studies. During the analysis, a maximum of four of the most abundant precursors were selected per cycle for fragmentation.

2.3.5 Proteins and peptides identification

Utilizing PEAKS Studio Version 7.5 from Bioinformatics Solution in Waterloo, Canada, both de novo sequencing and database matching were conducted. For the database matching step, the UniProt database from March 2016 was employed. The LC-QTOF-MS raw data was imported into the PEAKS software to facilitate de novo sequencing and obtain peptide sequences. The analysis parameters were configured following the guidelines outlined in reference by Kwan *et al.,* (2016) [14]. In essence, fixed modification parameters for post-translational modifications encompassed Oxidation, Carbomethylation, and Acetylation, while the maximum allowable missed cleavages was set to 2. The chosen database was focused on Bony fish (*Osteichthyes*). The precursor mass tolerance was established at 50 ppm, with monoisotopic mass values for fragment ions at 0.8 Da. To ensure robust results, a minimum False Detection Rate (FDR) of 1% and a De-novo Confidence Rate exceeding 20% were employed as criteria for accepting identified proteins. Furthermore, we required an Average Local Confidence (ALC) value greater than 15% was required to maintain confidence in the results obtained from the analysis.

2.4 Fourier Transform Infrared (FTIR) Spectroscopy

For the assessment of the structural configurations of red bigeye flesh, FTIR technique was employed. The functional groups inherent to red bigeye flesh was performed following the methodology outlined in Nurul *et al.,* (2015) and Bunaciu *et al.,* (2014) [15,16]. Prior to analysis, the sample holder, featuring a plate composed of zinc selenite (ZnSe) crystal, was meticulously cleaned using acetone. Subsequently, a background spectrum was gathered without a sample, spanning a resolution range of 650 to 4000 cm^{-1}, and encompassing thirty-two scans. For the analysis, the sample was placed onto the aforementioned plate and subjected to examination within the Thermo Nicolet 380 Spectrometer, housed by Fisher Scientific Inc. in the USA. Spectra were recorded spanning 500 to 4000 cm^{-1} at a data acquisition rate of 2 cm^{-1} per data point. The process of background subtraction was executed using Opus software provided by Fisher Scientific Inc. Each analysis was repeated three times to ensure reliability and consistency.

3. Results and discussion

3.1 Proximate Composition of Red Bigeye Flesh

Table 1 shows the proximate analysis of raw bigeye flesh. Crude protein of raw bigeye flesh was 17.21%. The value is comparable to other fish muscles such as from black tilapia (*Oreochromis*

placidus) (20.28%) and Catfish (*Tachusurus filiceps*) (17.7%)(Kasran *et al.,* 2023; Venugopal and Shahidi 1996) [17,18]. Nurhasan *et al.,* (2010) [19] reported that protein from raw swamp eel, walking catfish and snakehead murrel was 19.7%, 19.0% and 18.6%, respectively. Consequently, the substantial protein content in raw bigeye was valuable protein supplement for human dietary needs as well as functional food.

Table 1
Chemical composition of red bigeye flesh

Component (%)	Red bigeye flesh
Crude Protein	17.21±0.03[b]
Crude Fat	1.82±0.01[d]
Moisture	76.12±0.08[a]
Ash	1.34±0.01[d]
Carbohydrate	3.51±0.01[c]

*Means with different letter was significant difference ($p<0.05$)

Crude fat content red bigeye flesh (1.82%) is within a similar range with findings by Nurhasan *et al.,* (2010) [19] reported that crude fat from raw swamp eel (0.8%), walking catfish (3%) and snakehead murrel (0.4%). For black tilapia flesh the crude fat content was 0.58% (Kasran *et al.,* 2023) [17]. The lower fat content in red bigeye has the potential to enhance product stability by reducing susceptibility to lipid oxidation, thus extending shelf life [20]. Oxidation, a process affecting lipids, proteins, and carbohydrates in foods, is primarily responsible for compromising food quality through rancidity and a decrease in shelf life.

The moisture content for red bigeye flesh was 76.12%. Studies conducted by Nurhasan *et al.,* (2010) [19] showed that moisture from raw swamp eel, walking catfish and snakehead murrel was 77.9%, 76.8% and 80.4%, respectively. Kasran *et al.,* (2023) [17] reported that the moisture for black tilapia flesh was 77.18% and the ash was 1.23%. The ash for red bigeye flesh was 1.34%. The elevated ash content is primarily attributed to the heightened mineral content [21]. The carbohydrate was 3.51% for red bigeye flesh. Kasran *et al.,* (2023) [17] reported that 0.73% of carbohydrate from black tilapia. As such, the chemical composition results showed the potential of red bigeye as an excellent source of high-quality protein, delivering essential components for human nutritional needs.

3.2 Protein Sequence of Red Bigeye Flesh

The red bigeye flesh sample gave a total soluble protein concentration of 23.72±0.015 mg/ml. LC-QTOF-MS analysis revealed that the protein sequence of red bigeye (*Priacanthus macracanthus*) flesh gave a total of 422 proteins. There were 83 proteins with unique peptides (troponin 33, myosin 23, actin 19, tropomyosin 6 and parvalbumin 2 with coverage of more than 9% in the BIOPEP database were identified as listed in Table 2. These pivotal proteins actively contribute to vital cellular processes. From 83 proteins, the highest was regulatory proteins 42 (51%), enzyme 24 (29%) and unreviewed 17 (20%) (Figure 1).

Table 2
List of protein detected in red bigeye (*Priacanthus macracanthus*) flesh

Protein name	Accession	Protein name	Accession
Myosin regulatory light chain 2, skeletal muscle isoform DTNB	A0A4U5VU95	Fast/white muscle troponin T adult isoform	A0SJB2
Troponin T, fast skeletal muscle isoforms-like	A0A671U8G8	Fast/white muscle troponin T larval isoform	A0SJB3
Troponin T, fast skeletal muscle isoforms-like	A0A671UB90	Troponin C, skeletal muscle	A0A3B4V4A2
Troponin T, fast skeletal muscle isoforms-like	A0A671U6S8	Troponin C, skeletal muscle	A0A3B4V2L3
Troponin T, fast skeletal muscle isoforms-like isoform X1	A0A6P7KCD4	Troponin C, skeletal muscle-like	A0A665VW78
Troponin T, fast skeletal muscle isoforms-like	A0A671U7R2	Actin, cytoplasmic 1	A0A4W4GQM7
Fast/white muscle troponin T embryonic isoform	A0SJB4	Actin, alpha skeletal muscle 2	A0A4W4E2R1
Troponin T, fast skeletal muscle isoforms-like	A0A671U7Q2	Beta-actin	K9MU88
Beta-actin	K9MSJ7	Actin, alpha cardiac	A0A4W4F961
Actin, alpha skeletal muscle 2	A0A4W4HEM3	Actin, alpha cardiac muscle 1-like	A0A4W4FWD5
Beta-actin	A0A161HAV8	Actin, alpha cardiac muscle 1 isoform X2	A0A6P7LHZ8
Actin, alpha cardiac	A0A3P9DP62	Beta-actin	A0A286LVJ2
Actin, alpha skeletal muscle 2	A0A4W4HAX2	Actin, alpha skeletal muscle 2	A0A4W4HAQ9
Actin alpha 2, smooth muscle	A0A4W4G3W9	Tropomyosin 1	A0A3B3R8P7
Actin beta	A0A4W4DM97	Myosin, light polypeptide 3, skeletal muscle	A0A7N6APB0
Actin beta	A0A3P9BIS7	Myosin light chain 3, skeletal muscle isoform	A0A3B5QNZ3
Actin, cytoplasmic 1	A0A4W4GQ52	Myosin, light polypeptide 3, skeletal muscle	A0A3Q1IN57
Beta-actin	A8CZW6	Myosin, light polypeptide 3, skeletal muscle	A0A7N6AV75
Actin, cytoplasmic 1	A0A4W4GNE6	Myosin light chain 3, skeletal muscle isoform	A0A3B5RBB9
Troponin I type 2 (Skeletal, fast)	A0A1A8FS83	Myosin light chain 3, skeletal muscle isoform	A0A3B5R1L0
Troponin I2, fast skeletal type	A0A3Q0S2Y5	Myosin, light polypeptide 3, skeletal muscle	A0A7N5ZVQ4
Troponin I, fast skeletal muscle-like	A0A672GEI3	Myosin, light polypeptide 3, skeletal muscle	A0A7N6ARR4
Troponin I, fast skeletal muscle-like	A0A3B3V3D3	Myosin light chain 3, skeletal muscle isoform	A0A3B3C1H6
Troponin I, fast skeletal muscle-like	A0A3B4ZK41	Myosin light chain 3, skeletal muscle isoform	A0A673C8Q4
Troponin I, fast skeletal muscle-like	A0A3Q3EFC9	Myosin light chain 3, skeletal muscle isoform	A0A3B3C3N2
Troponin I type 2 (Skeletal, fast)	A0A1A8I952	Parvalbumin	A0A4Z2C010
Troponin I, fast skeletal muscle-like	A0A3Q1CR89	Parvalbumin	A0A5C6NQR5
Troponin I, fast skeletal muscle-like	A0A3Q3F988	Troponin T, fast skeletal muscle isoforms-like isoform X1	A0A6P6PRS8
Troponin I2, fast skeletal type	A0A3Q0SBS4	Troponin T, fast skeletal muscle isoforms-like isoform X4	A0A6P6PTK7
Troponin I2, fast skeletal type	A0A3Q0SFS4	Troponin T3, fast skeletal type	A0A3Q3W1I1

Troponin I, fast skeletal muscle-like	A0A6P7KJX5	Troponin T, fast skeletal muscle isoforms-like isoform X5	A0A6P6PPL6
Troponin I type 2 (Skeletal, fast)	A0A1A8CWY6	Myosin light chain 3, skeletal muscle isoform	A0A672LUI4
Troponin I, fast skeletal muscle-like	A0A3B5QM30	Fast skeletal muscle myosin light polypeptide 3	Q9I8U7
Troponin I, fast skeletal muscle	I3JXF9	Myosin light chain 3, skeletal muscle isoform	A0A672LUG5
Troponin I2, fast skeletal type	A0A3Q0S8S1	Myosin light chain 3, skeletal muscle isoform	A0A672LXS7
Troponin I, fast skeletal muscle-like	A0A3Q1BID1	Myosin light chain 3, skeletal muscle isoform	A0A672LSC4
Tropomyosin alpha-4 chain isoform X5	A0A1S3MAQ6	Myosin light chain 3, skeletal muscle isoform	A0A672LU25
Myosin heavy chain, fast skeletal muscle	A0A5C6NSV1	Myosin, light chain 1, alkali; skeletal, fast	A0A4W5MNG5
Myosin motor domain-containing protein	A0A3B4WJQ0	Myosin light chain 3, skeletal muscle isoform-like	A0A3B4CXZ2
Myosin heavy chain, fast skeletal muscle-like	A0A3P9NGH5	Tropomyosin 1	A0A3B3R8T2
Tropomyosin alpha-1 chain	A0A5A9PCD9		
Tropomyosin alpha-4 chain isoform X4	A0A6J2USB0		
Tropomyosin alpha-1 chain-like isoform X5	A0A1S3MLH4		

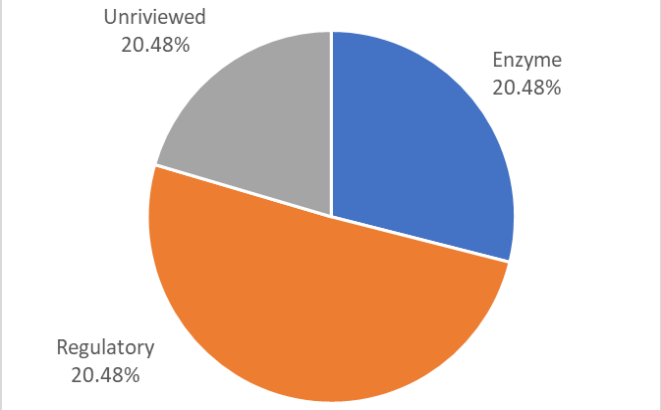

Fig. 1. Protein functional group

Post-translational modification (PTM) refers to the enzymatic or spontaneous covalent alterations of proteins after their synthesis, transforming polypeptide chains into mature proteins. These modifications, which can occur on amino acid side chains or at the protein's C- or N- termini, play a crucial role in cell signaling, such as in the conversion of prohormones to hormones [22]. PTMs greatly enhance the chemical diversity of the 22 amino acids in the human body by modifying existing groups or adding new ones like phosphate. Among these, phosphorylation is particularly noteworthy for its role in regulating enzyme activity, representing the most common form of post-translational change [23].

Post-translational modifications of red bigeye protein revealed oxidation and mutation was the highest 23% (Figure 2), which exert a notable impact on protein functionality. Regulatory proteins play a crucial role in governing diverse cellular processes through interactions with other molecules, primarily functioning within gene expression and signal transduction pathways. These proteins maintain equilibrium and operational integrity in organisms by finely tuning the actions of specific

target molecules, such as genes, enzymes, or other proteins. Their functional spectrum encompasses tasks like managing gene expression, orchestrating signal transduction, controlling the cell cycle, regulating metabolic processes, and guiding cell differentiation. A prime example of a regulatory protein is the lac repressor in bacterial systems, which oversees genes linked to lactose metabolism [24].

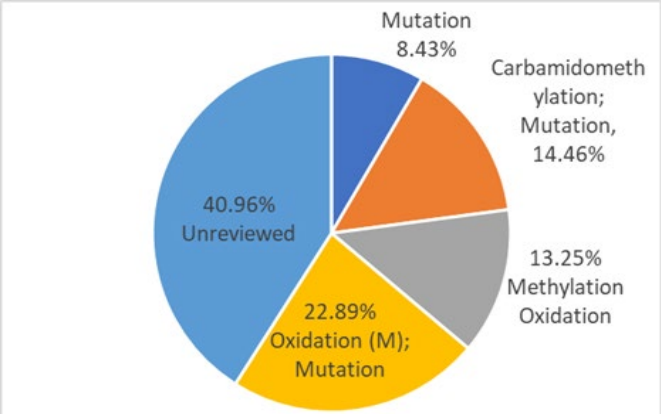

Fig. 2. Post-transtitional modification

Oxidation in peptides involves the chemical modification of certain amino acids, often leading to changes in the peptide's structure and function. In fish samples, this can be particularly relevant due to the susceptibility of fish proteins to oxidative processes. A notable study by *Goris et al.,* (2023) [25] explored the enzymatic conversion of trimethylamine (TMA) into trimethylamine N-oxide (TMAO) in marine by-products. This process is significant in mitigating the fishy odor commonly associated with these products. The study demonstrated the potential of an engineered flavin-containing monooxygenase (FMO) from *Methylophaga aminisulfidivorans*, which showed increased thermostability, making it more suitable for industrial applications in reducing TMA levels in salmon protein hydrolysates [25]. This research underscores the role of oxidation in altering peptide properties and improving the sensory qualities of fish-derived products.

Alterations in peptide sequences, such as mutations, can significantly impact their biological functionality, influencing their structure, stability, and interactions with other molecules. A key example of this is peptide methylation, which involves adding a methyl group to specific amino acids, thereby altering the peptide's activity, interactions, and stability. This modification typically occurs on the amino acid's side chain and is closely associated with cell proliferation, particularly within the cell cycle pathway [26]. Methylation PTMs have been linked to changes in fibroblast behavior, as Neary *et al.,* [27]. have shown, playing a role in the transformation of fibroblasts into pathological, scar-forming myofibroblasts. This transformation is a critical factor in initiating the inflammatory phase of wound healing and progressing to fibrotic diseases. The study by Neary *et al.,* [27] also underscores the potential of various components of the methylation process as therapeutic targets, suggesting that further research could yield significant insights. Additionally, this PTM is likely related to the previously mentioned histone proteins, contributing to enhanced wound healing.

Carbamidomethylation is a common post-translational modification, often observed in cysteine residues. It involves the addition of a carbamidomethyl group and is frequently used in proteomics studies to protect cysteine residues during analysis. In the context of fish peptides, carbamidomethylation could play a role in stabilizing peptides for analytical studies. This modification can affect the structural and functional properties of peptides. Further types of post-translational modifications include peptide bond cleavage, like the transformation of a pro-peptide into its mature form or the removal of the initiating methionine residue, some modifications are induced by

oxidative stress [28]. Frequently targeted residues for these modifications possess functional groups capable of acting as nucleophiles in reactions. These include hydroxyl groups of serine, threonine, and tyrosine; amine derivatives of lysine, arginine, and histidine; thiolate anions of cysteine; carboxylates of aspartate and glutamate; as well as N- and C-termini.

In Figure 2, the post-translational modifications for red bigeye protein revealed a higher percentage for oxidation and mutation (23%), carbamidomethylation and mutation (15%), methylation and oxidation (13%), mutation (8%) and unreviewed (41%). Unfortunately, most proteins remained unreviewed on the UniprotKB website. Moreover, the significant presence of essential proteins in red bigeye hydrolysate positions it as a promising protein source for developing functional food products. Mohanty, *et al.,* 2015 [29] reported that they identified 21 protein spots, representing 17 proteins (Myosin light chain, myosin polypeptide, heat shock, cytoskeletal proteins, tropomyosin and others), have been identified by MALDI-TOF/TOF-MS and LC-MS/MS using white muscle from freshwater catfish (*Rita rita*).

In a study on Snakehead fish (*Channa striata*) flesh proteins, Kwan & Mohd [30] have identified 53 unique proteins in the crude mucus sample and 120 in the fractionated sample. These included histones, ribosomal proteins, protein S100, heat shock protein, proteolytic enzymes, heparin cofactor II, and a group of uncharacterized proteins. Additionally, 39% of these proteins underwent post-translational modifications, with methylation, hydroxylation, acetylation, ubiquitination, and biotinylation being the detected modifications [30]. In their research, Ong *et al.,* [31] analyzed skin secretions from *H. erythraea* and identified 29 proteins. These were classified as antimicrobial proteins (AMPs), constituting 38% of the total, followed by hormones and enzymes (each 17%), unreviewed proteins (17%), structural proteins (7%), and regulatory proteins (4%).

3.3 Fourier Transform Infrared (FTIR) Spectrum

Fourier transform infrared (FTIR) spectrum analysis provided information on structure of red bigeye flesh as shown in Figure 3. Generally, red bigeye flesh show wide range of spectra exhibiting symmetrical bending stretches within amide regions A, B, I, II, III, and VII. Table 3 shows wave numbers (cm^{-1}) and types of amides found in red bigeye flesh. Peaks for red bigeye were Amide A (3274.35 cm^{-1}), Amide B (2965.03 and 2104.26A cm^{-1}), Amide I (1643.97 and 1633.36 cm^{-1}), Amide II (1453.37 and 1397.02 cm^{-1}), Amide III (1242.29,1171.45 and 1078.08 cm^{-1}) and Amide VII ranged from 409.95-456.34 cm^{-1}. Peaks at 3854.51, 3274.35, 2965.03 and 2104.26 cm^{-1} indicated the presence of N-H stretching (Amide A and B). Peaks at 1643.97 and 1633.36 cm^{-1} represented the C=O stretching (Amide I). Amide A indicates NH-stretching along with hydrogen bonding, while amide B suggests weak N-H stretching [32].

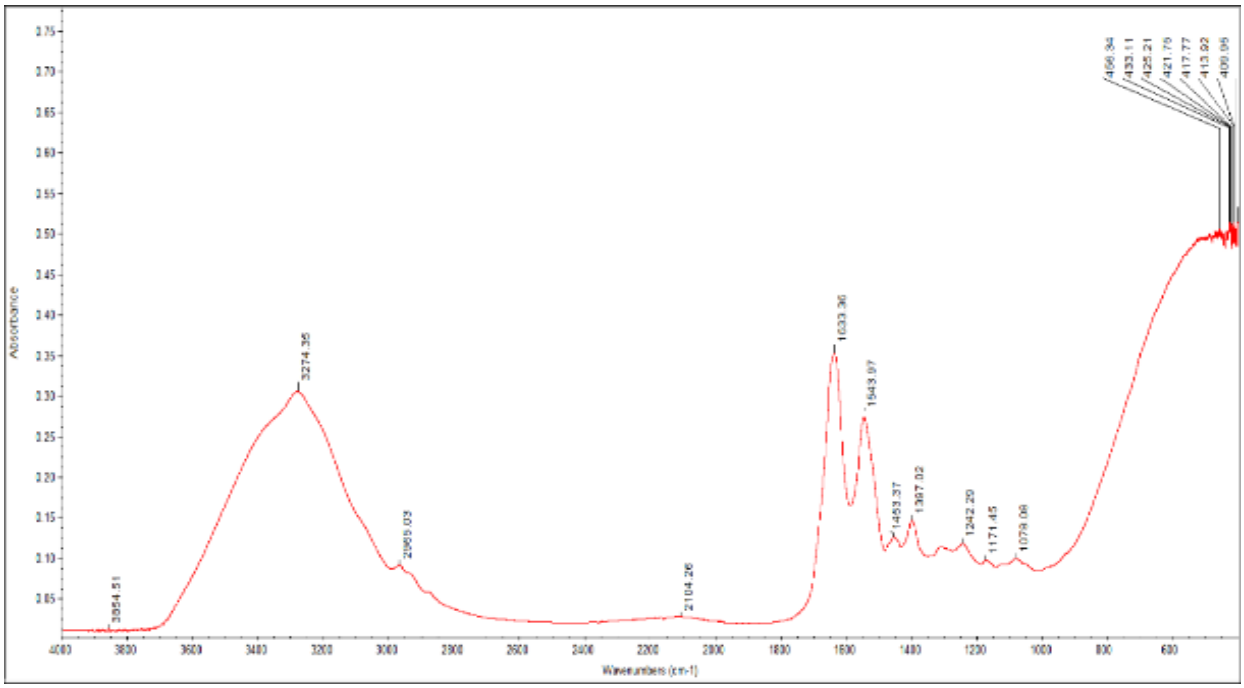

Fig. 3. Fourier transform infrared (FTIR) spectra of red bigeye flesh

Table 3
Wave number (cm^{-1}) value and types of amides in red big eye flesh

Wave number (cm^{-1})	Designation	Description
Red bigeye flesh		
3854.51, 3274.35	Amide A	NH stretching
2965.03, 2104.26	Amide B	NH stretching
1643.97, 1633.36	Amide I	C=O Stretching
1453.37, 1397.02	Amide II	CN stretching, NH bending
1242.29,1171.45,1078.08	Amide III	CN stretching, NH bending
409.95-456.34	Amide VII	Skeletal torsion

The peaks at 1453.37, 1397.02, 1242.29, 1171.45, 1078.08 cm^{-1} exhibited CN stretching and NH bending (Amide II and III). While the peaks at 409.95 and 456.34 cm^{-1} representing skeletal torsion (Amide VII). As per *Yakimets et al.,* (2005) [33], the emergence of peaks within the ranges of 1700–1600 cm^{-1} (eel) and 1560–1500 cm^{-1} (bovine) signified amides I and II, respectively. Peaks detected at 1162.99 cm-^{1} and 1031.62 cm^{-1} exclusively, in commercial bovine gelatin corresponded to the –OH stretching of tyrosine and the C–O of serine, respectively [34]. The amide I vibration primarily represents C=O stretching along with contributions from C-N stretching, C-C-N deformation, and in-plane N-H bending modes [35]. Absorption within the amide I region proves most valuable in analyzing secondary protein structures [36]. Conversely, Barth, (2000) explained that robust peaks at 1633.94 cm^{-1} and 1634.72 cm^{-1} correspond to arginine's C-N stretching. The amide II vibration mode arises from an out-of-phase combination of CN stretch and in-plane NH deformation modes of the peptide group [35, 37]. Generally, amide II peaks are more sensitive to hydration than to secondary structural changes [38].

These findings concerning red bigeye flesh bands is correlated with a study conducted by Chi *et al.,* (2014) [39], that identified similar bands (amide A (3433 cm^{-1}), amide B (2926 cm^{-1}), amide I (1641 cm^{-1}), amide II (1549 cm^{-1}) and amide III 1240 (cm^{-1}) of collagen hydrolysate from Spanish mackerel skin. Amide containing N-H dipoles facilitate their function as hydrogen bond donors, enabling them to dissolve in water and other protic solvents. This interaction could enhance protein solubility. Red

bigeye flesh had a wide range of amide A, B, I, II, III, and VII showed many protein component alignment with the findings of Li *et al.,* (2013) [40], where FTIR band positions of hydrolyzed collagen hydrolysates from *Sphyrna lewini, Dasyatis akjei and Raja porosa* identified the same region for five peaks (amide A, amide B, amide I, amide II and amide III) the collagen hydrolysates comprised of many protein components with a wide range.

4. Conclusions

Through LC-QTOF-MS analysis, 83 unique proteins (troponin 33, myosin 23, actin 19, tropomyosin 6 and parvalbumin 2) were successfully identified from the flesh of the red bigeye fish, with a notable prevalence of regulatory proteins. This study significantly advances our comprehension of the protein profiles of red bigeye. Analysis of FTIR spectra demonstrated the structural composition of red bigeye flesh symmetrical bending stretches within amide regions A, B, I, II, III, and VII. These outcomes of essential protein a promising potential for red bigeye flesh in functional food applications.

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Conflict of Interest

None of the authors of this study has any financial interest or conflict with industries or parties.

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