

Quantification of Water-Soluble Protein from Snakehead Fish (*Channa striata*) by Bradford UV-Vis and FTIR Profiling

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ABSTRACT

Snakehead fish (*Channa striata*) is widely recognised as a nutritionally valuable aquatic resource and is frequently associated with protein-rich traditional preparations. This study aimed to quantify the water-soluble protein fraction of *C. striata* using Bradford UV-Vis spectrophotometry and to characterise the corresponding functional groups by Fourier Transform Infrared (FTIR) spectroscopy. The fish flesh was processed to obtain an aqueous (water-soluble) protein isolate, and protein concentration was determined using the Bradford method with UV-Vis measurement at 595 nm. Functional-group profiling of the isolate was subsequently evaluated by FTIR to verify spectral features consistent with protein/peptide structures. The Bradford assay indicated a protein level of 460.6 ± 0.958 mg/g, corresponding to 46.06% within the analysed fraction. FTIR analysis revealed characteristic protein-related bands, including Amide A at 3266.43 cm^{-1} , Amide I at 1635.92 cm^{-1} , and Amide III at 1318.95 cm^{-1} , supporting the presence of peptide bonding patterns in the isolate. Collectively, these findings demonstrate that *C. striata* yields a measurable water-soluble protein fraction quantifiable by Bradford UV-Vis and confirmable at the functional-group level by FTIR, providing a practical analytical basis for further standardisation and quality-oriented studies.



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ABSTRAK

Ikan gabus (*Channa striata*) dikenal luas sebagai sumber pangan bernilai gizi tinggi dan sering dikaitkan dengan pemanfaatan tradisional berbasis kandungan proteinnya. Penelitian ini bertujuan untuk mengukur kadar protein fraksi larut air dari *C. striata* menggunakan spektrofotometri UV-Vis metode Bradford serta mengkarakterisasi gugus fungsionalnya melalui spektroskopi Fourier Transform Infrared (FTIR). Daging ikan diproses untuk memperoleh isolat protein berbasis air (water-soluble protein isolate), kemudian kadar protein ditetapkan dengan metode Bradford melalui pengukuran UV-Vis pada panjang gelombang 595 nm. Selanjutnya, profil gugus fungsional isolat dianalisis menggunakan FTIR untuk mengonfirmasi ciri spektral yang konsisten dengan struktur protein/peptida. Hasil uji Bradford menunjukkan kadar protein sebesar $460,6 \pm 0,958$ mg/g, yang setara dengan 46,06% pada fraksi yang dianalisis. Analisis FTIR memperlihatkan pita khas protein, meliputi Amida A pada $3266,43\text{ cm}^{-1}$, Amida I pada $1635,92\text{ cm}^{-1}$, dan Amida III pada $1318,95\text{ cm}^{-1}$, yang mendukung keberadaan pola ikatan peptida pada isolat. Secara keseluruhan, hasil ini menunjukkan bahwa *C. striata* menghasilkan fraksi protein larut air yang dapat dikuantifikasi secara praktis dengan UV-Vis metode Bradford dan dikonfirmasi secara kimiawi melalui FTIR, sehingga relevan sebagai dasar analisis lanjutan untuk standardisasi dan pengkajian mutu.

Kata Kunci: *Channa striata*; Protein larut air; Metode Bradford; Spektrofotometri UV-Vis; Spektroskopi FTIR.

1. Introduction

Protein is a key macronutrient required for growth, tissue regeneration, and physiological regulation through its structural and functional roles in biological systems. In food and nutritional sciences, protein quantification is therefore essential not only to describe composition, but also to support quality evaluation and analytical standardisation of protein-containing materials. Freshwater fish is widely utilised as a dietary protein source, and comparative profiling has shown that protein characteristics may differ among species, including snakehead fish (*Channa striata*), toman (*Channa micropeltes*), and betutu (*Oxyeleotris marmorata*), indicating that matrix and species variability must be considered when interpreting analytical results [1].

Snakehead fish (*C. striata*) is widely consumed in Indonesia and is frequently associated with protein-rich preparations in community practice. Protein analysis has also been conducted in processed snakehead fish products, such as *pekasam*, reinforcing that processing and handling conditions can influence measurable protein outcomes and their nutritional interpretation [2]. For routine laboratory determination, UV-Vis spectrophotometry is commonly applied in protein analysis of aquatic and food matrices because it is operationally practical and analytically feasible for concentration measurement when appropriate calibration and sample handling are implemented [3].

In addition to quantitative determination, chemical characterisation is often required to support quality-oriented interpretation of extracted fractions. FTIR spectroscopy has been reported for profiling snakehead fish extracts prepared using different extraction approaches, demonstrating that FTIR can provide complementary information through functional-group signatures consistent with protein/peptide structures, particularly via amide-related regions [4]. Protein-focused studies on other fish matrices further support the need to interpret protein data in relation to the specific fraction produced and the analytical basis used (e.g., whole tissue versus isolate), because these choices can substantially affect the magnitude and meaning of reported values [5]. Importantly, when the scientific discussion includes albumin-associated fractions, methodological clarity is essential because albumin determination is

analytically distinct from general “total protein” assays and typically relies on more selective measurement principles [6].

Based on this rationale, the present study aimed to quantify the water-soluble protein fraction obtained from snakehead fish (*Channa striata*) using UV-Vis spectrophotometry and to characterise protein-consistent functional groups using FTIR spectroscopy as a complementary confirmatory approach.

2. Methods

Study design and analytical workflow

This laboratory-based experimental study quantified the water-soluble protein fraction obtained from snakehead fish (*Channa striata*) using UV-Vis spectrophotometry with the Bradford assay and subsequently characterised protein-consistent functional groups using Fourier Transform Infrared (FTIR) spectroscopy. The Bradford assay was selected because it is widely applied for quantitative determination of soluble proteins using BSA calibration in diverse biological matrices [11], [12], while FTIR was used as a complementary tool to confirm functional-group features consistent with protein/peptide structures based on established infrared spectroscopy principles [14]-[16].

Materials and reagents

Materials included snakehead fish (*Channa striata*), distilled water, bovine serum albumin (BSA) as the protein reference standard, Bradford reagent, and filter paper. (*If Biuret reagent is not used analytically, it should be removed from the materials list to avoid inconsistency.*)

Sample collection and preparation

Snakehead fish samples were collected from Lake Limboto, Limboto District, Gorontalo Regency, Gorontalo, Indonesia. The fish were cleaned by removing non-edible parts, and the edible flesh was washed and cut into pieces (approximately 2-3 cm) prior to isolation.

Preparation of aqueous (water-soluble) protein isolate

An aqueous extraction approach was used to obtain the water-soluble protein fraction from snakehead fish, consistent with reports describing extraction of snakehead fish into protein-rich preparations and the importance of controlling pre-analytical conditions [8]. Briefly, 300 g of fish flesh was weighed and mixed with 750 mL of distilled water. The mixture was blended for 15 min until homogeneous, then kept at refrigerated temperature for 30 min. The homogenate was centrifuged at 4500 rpm for 30 min to separate the soluble fraction from insoluble residues; such centrifugation parameters are known to influence filtrate recovery and downstream analytical outcomes [9]. After centrifugation, the upper supernatant (water-soluble fraction) was carefully collected using a Pasteur pipette and filtered through filter paper. The resulting isolate was stored at freezer temperature until analysis. Because fish protein isolates may exhibit different physicochemical profiles depending on species and isolation conditions, all quantitative interpretation in this study was restricted to the defined water-soluble fraction produced by this procedure [10].

Determination of total water-soluble protein by Bradford UV-Vis spectrophotometry

Total soluble protein concentration was determined using the Bradford method with BSA calibration, following widely used quantitative practice for soluble protein determination [11], [12]. A BSA stock solution was prepared by dissolving 100 mg BSA in 100 mL distilled water to obtain 1000 ppm. Working standards of 60, 80, 100, and 300 ppm were prepared by dilution of the stock solution.

For each standard and sample, 2 mL of solution was mixed with 8 mL of Bradford reagent, vortexed, and incubated for 10 min at room temperature. Absorbance was measured at 595 nm using a UV-Vis spectrophotometer against a reagent blank. A calibration curve was constructed using least-squares linear regression, and sample concentrations were calculated from the regression equation and expressed as BSA-equivalent soluble protein, incorporating any dilution factor applied. This reporting approach is consistent with the principle that Bradford UV-Vis yields a quantitative estimate of soluble protein relative to a reference standard rather than a protein-species-specific measurement, particularly in complex biological extracts [13].

FTIR analysis for functional-group profiling

FTIR spectroscopy was used to characterise functional groups consistent with protein/peptide structures, referencing established infrared spectroscopy interpretation frameworks [14], [15] and protein-focused FTIR literature describing the diagnostic value of amide-related regions [16]. The isolate was prepared using a KBr pellet method; after drying, spectra were recorded over the range 4000–800 cm^{-1} . Instrument acquisition parameters (e.g., resolution and number of scans) were kept constant across measurements. Spectral interpretation focused on principal peak positions (cm^{-1}) and their assignment to protein-associated bands (notably amide-related features).

Analytical performance and statistical presentation

Analytical performance of the Bradford UV-Vis assay was assessed through evaluation of the calibration model and measurement repeatability. Calibration linearity was examined using least-squares regression of the BSA standard curve, and the regression equation together with the coefficient of determination (R^2) was reported. Repeatability was evaluated from triplicate measurements ($n = 3$) and expressed as mean \pm standard deviation (SD); where appropriate, relative standard deviation (%RSD) was also calculated to describe measurement variability. FTIR measurements were performed on three independently prepared isolate aliquots ($n = 3$) under consistent acquisition conditions, and spectra were compared to confirm reproducibility of the principal protein-associated bands prior to reporting representative peak positions

3. Results and Discussion

Protein Isolation of Snakehead fish (*Channa striata*)

The aqueous isolation procedure in this study was intended to generate a defined water-soluble protein fraction from snakehead fish (*Channa striata*) as the analytical matrix for Bradford UV-Vis quantification and FTIR profiling. Previous comparative work has shown that protein-related characteristics differ among freshwater fish species, including *C. striata*, and that the magnitude of “protein content” is strongly dependent on the analytical basis (whole tissue versus isolated fractions), which justifies reporting the present results strictly for the soluble fraction produced by the described workflow [1]. Protein measurements in processed snakehead fish products further indicate that handling and processing can influence measurable protein outcomes, reinforcing the need to describe fraction recovery and procedural context in a transparent manner [2]. UV-Vis spectrophotometry has been widely used for protein determination in aquatic and food-related matrices, supporting its practicality for quantitative analysis of the recovered soluble phase [3].

Complementarily, FTIR-based profiling has been reported for snakehead fish extracts obtained under different extraction methods, demonstrating that infrared spectra can provide supporting chemical information through functional-group patterns consistent with protein/peptide structures [4]. Protein-oriented studies in other fish

matrices also emphasise that reporting should clarify whether values represent whole tissue or an extracted fraction, because isolate-type preparations may yield higher apparent protein concentration relative to the original matrix [5]. Because the discourse on snakehead fish is often linked to albumin-associated preparations, it is analytically important to distinguish total soluble protein quantification from albumin-specific determination, since albumin attribution requires selective measurement principles beyond general protein assays [6]. In this context, FTIR offers confirmatory value for functional-group signatures (e.g., amide-related bands) and is commonly used for profiling natural matrices when chemical class confirmation is needed [7]. The isolation logic used here is aligned with the general concept of producing protein-rich preparations from snakehead fish, where the supernatant is treated as the soluble protein-containing phase suitable for downstream analysis [8]. Furthermore, centrifugation parameters can influence recovery volume and clarity of the supernatant/filtrate, thereby affecting downstream analytical outcomes if the recovery basis is not explicitly reported [9]. Finally, since fish protein isolates can differ in physicochemical behaviour depending on extraction and processing conditions, reporting the recovery profile supports interpretability and reproducibility across laboratories [10].

In the present work, recovery is reported as filtrate/supernatant volume (mL) and, when expressed as a percentage, calculated on a volume basis relative to the initial extraction solvent volume (v/v). Using the recorded filtrate volume around 230 mL, recovery corresponds to approximately 30.7% v/v relative to the initial 750 mL extraction volume. The recovery profile is summarised in **Table 1**.

Table 1. Recovery profile of the water-soluble protein isolate from *Channa striata* (n = 3)

Parameter	Replicate 1	Replicate 2†	Replicate 3†	Mean ± SD
Fish flesh mass (g)	300	300	300	300 ± 0
Distilled water volume (mL)	750	750	750	750 ± 0
Solvent-to-sample ratio (mL/g)	2.50	2.50	2.50	2.50 ± 0.00
Filtrate volume after filtration (mL)*	230	228	232	230 ± 2
Filtrate recovery (% v/v of 750 mL)**	30.67	30.40	30.93	30.67 ± 0.27

Note:

* Filtrate volume refers to the filtered supernatant (water-soluble fraction) used for Bradford UV-Vis and FTIR.

** Filtrate recovery (%) = (filtrate volume / 750 mL) × 100.

Bradford UV-Vis calibration curve and assay linearity

Quantification of the water-soluble protein fraction was performed using the Bradford UV-Vis assay with BSA as the reference standard, because this method is broadly applied for soluble-protein measurement in complex matrices and is appropriate for routine laboratory quantification when calibration and blanking are controlled [11], [12]. In this study, absorbance readings were acquired at 595 nm after incubation, and measurements were performed against a reagent blank consisting of Bradford reagent and distilled water processed identically to standards and samples. This blanking step is essential because the Bradford dye system exhibits inherent baseline absorbance, and the blank corrects for background contributions unrelated to protein-dye complex formation; nonetheless, a non-zero intercept may still occur due to residual baseline offset and instrument response characteristics under the selected optical conditions [11]-[13].

Table 2. BSA standard solutions and Bradford UV-Vis absorbance at 595 nm

BSA concentration (ppm)	Absorbance (mean \pm SD)
60	0.454 \pm 0.003
80	0.486 \pm 0.003
100	0.512 \pm 0.004
300	0.726 \pm 0.004

The calibration dataset, summarised in **Table 2**, shows that across the working range of 60–300 ppm the BSA standards produced a proportional increase in absorbance, supporting linearity of the assay within this concentration domain. The calibration model was generated using least-squares regression, yielding a linear equation of the form $A = mC + b$, where A is absorbance at 595 nm and C is BSA concentration (ppm). The regression equation and R^2 value are presented in **Figure 1** and were subsequently used to calculate isolate protein concentrations as BSA-equivalent soluble protein, consistent with quantitative practice for Bradford-based protein determination [11]–[13]. Replicate measurements ($n = 3$) exhibited low dispersion across standards (Table 2), indicating acceptable repeatability of the calibration measurements.

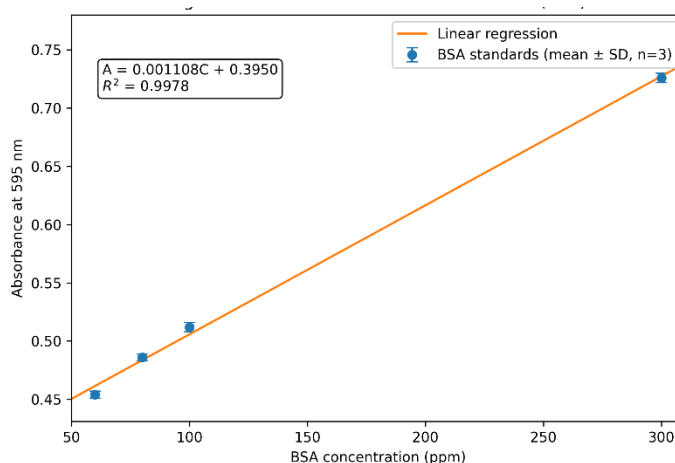


Figure 1. Bradford UV-Vis calibration curve using BSA standards (60–300 ppm) measured at 595 nm ($n = 3$), with linear regression $A = 0.00111C + 0.395$ and $R^2 = 0.998$.

Total water-soluble protein content of *Channa striata* isolate (BSA-equivalent)

Total soluble protein in the water-soluble isolate was quantified by Bradford UV-Vis using BSA calibration and is therefore reported as BSA-equivalent total soluble protein, not as an albumin-specific endpoint. This distinction is analytically important because the Bradford reaction measures dye-binding soluble proteins within the analysed fraction and does not selectively quantify albumin unless a selective albumin assay is applied [11]–[13]. Using the calibration model established in Section 3.2 (Figure 1), isolate protein concentrations were calculated as described in the *Methods* section (calibration back-calculation and unit normalisation), and the final results were expressed as mg/g and % (w/w) on the stated basis.

Triplicate measurements ($n = 3$) demonstrated high repeatability, yielding a mean protein content of 460.6 ± 0.958 mg/g, corresponding to 46.06% (w/w) in the analysed fraction, with low variability across replicates (%RSD \approx 0.21%). The triplicate

absorbance values, calculated BSA-equivalent concentrations, and final converted protein content are summarised in **Table 3**.

Table 3. Bradford UV-Vis determination of total water-soluble protein in *Channa striata* isolate

Replicate	Absorbance at 595 nm	Calculated C (ppm, µg/mL)	Protein content (mg/g)	Protein content (% w/w)
1	0.52927	121.252	461.56	46.16
2	0.52899	121.000	460.60	46.06
3	0.52872	120.748	459.64	45.96
Mean ± SD	—	121.000 ± 0.252	460.6 ± 0.958	46.06 ± 0.10
%RSD	—	0.21	0.21	0.21

When comparing with published reports, it should be noted that many protein values for snakehead fish refer to whole tissue composition or processed products, which can yield different magnitudes than an isolated soluble fraction produced under defined extraction and centrifugation conditions [1], [2]. UV-Vis-based protein analyses across aquatic matrices likewise show that the reported “protein level” depends strongly on analytical basis and sample handling [3]. Therefore, the relatively high protein percentage observed here is most appropriately interpreted as a characteristic of the defined water-soluble isolate fraction, consistent with broader evidence that fish protein isolates differ in physicochemical profiles depending on isolation approach and species [10].

FTIR spectral characteristics of the isolate and functional-group assignment

FTIR spectroscopy was used as a complementary tool to verify that the recovered fraction exhibits spectral features consistent with protein/peptide structures, in line with prior FTIR profiling work on snakehead fish extracts and established infrared interpretation frameworks [4], [14], [15]. Spectra were acquired using the KBr pellet approach described in the *Methods* section and recorded over 4000–800 cm⁻¹. Measurements were performed on three independently prepared isolate aliquots (n = 3), and a representative spectrum is presented in **Figure 2**. The principal peak positions were reproducible across replicates, supporting the analytical consistency of the isolate’s spectral profile.

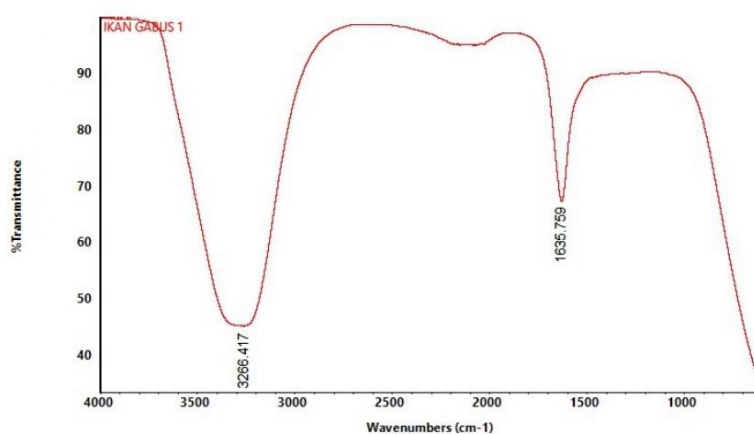


Figure 2. FTIR spectrum of the water-soluble protein isolate from *Channa striata* (KBr pellet method; representative spectrum from n = 3)

The key FTIR peaks and their functional-group assignments are summarised in **Table 4**. A broad band at 3266.43 cm^{-1} corresponds to Amide A, which is predominantly associated with N-H stretching coupled with hydrogen-bonding interactions and is frequently observed in protein-containing materials [14]–[16]. The strong band at 1635.92 cm^{-1} lies in the Amide I region and is dominated by C=O stretching vibrations of the peptide backbone, making it a widely accepted diagnostic region for confirming peptide-bonding patterns in proteins [16]. In addition, the band at 1318.95 cm^{-1} corresponds to Amide III, reflecting mixed vibrational contributions (primarily C-N stretching and N-H bending) that provide supporting evidence for protein/peptide structures when interpreted together with Amide I and Amide A [16], [20]. Collectively, the amide-related bands listed in Table 4 indicate that the isolate retains peptide-bonding features consistent with a protein-rich soluble fraction.

Table 4. Major FTIR peaks of the *Channa striata* water-soluble isolate and functional-group assignments.

Observed peak (cm^{-1})	Band assignment	Interpretation (protein-related)	Supporting references
3266.43	Amide A	N-H stretching; hydrogen bonding in peptide/protein systems	[14]–[16]
1635.92	Amide I	Predominantly C=O stretching of peptide backbone	[16]
1318.95	Amide III	Mixed modes (C-N stretching and N-H bending) supporting peptide structure	[16], [20]
1078.78	C-O stretching (carbohydrate/ether region)*	Possible contribution from polysaccharides/excipients or residual matrix components	[14], [15]

Note: *Reported as an additional feature in the fingerprint region; interpretation should be conservative because biological isolates may contain minor non-protein constituents

Although FTIR amide bands can be used to discuss secondary-structure contributions under rigorous spectral treatment, such interpretation typically requires deconvolution or second-derivative processing and careful fitting of the Amide I envelope rather than relying on single peak positions alone [18]. Therefore, in the present study, FTIR results are interpreted conservatively as functional-group confirmation consistent with protein-associated amide bands, not as definitive evidence of α -helix or β -sheet dominance, in line with methodological caution in protein secondary-structure assessment [19].

Integrated interpretation, analytical scope, and study limitations

Taken together, the analytical workflow applied in this study provides a coherent, fraction-specific evaluation of the water-soluble protein isolate obtained from snakehead fish (*Channa striata*). The Bradford UV-Vis assay offered a practical quantitative estimate of total soluble protein in the recovered supernatant expressed as BSA-equivalent protein, while FTIR spectroscopy provided complementary chemical confirmation through protein-consistent functional-group features, particularly the characteristic amide-associated bands. Interpreted jointly, these results support the conclusion that the isolation protocol yields a soluble fraction that is measurably protein-

rich and displays infrared signatures consistent with peptide bonding patterns, thereby strengthening the quality-oriented interpretability of the isolate beyond reliance on a single analytical technique.

At the same time, the analytical scope of the present study must be stated explicitly to avoid over-interpretation. Neither the Bradford assay nor FTIR profiling can, on their own, identify albumin as a discrete protein entity within the isolate. The Bradford reaction reports total dye-binding soluble proteins relative to a standard and does not differentiate albumin from other proteins, whereas FTIR amide bands reflect general peptide-bonding features and cannot attribute peaks to a single named protein without orthogonal identification. Accordingly, any discussion of albumin in the context of snakehead fish should be framed as biological relevance and rationale, rather than as an analytically proven endpoint in this dataset. If albumin-specific quantification is required, a selective measurement principle must be applied (e.g., albumin-targeted colorimetric methods or other protein-specific analytical platforms), as illustrated by studies that explicitly perform albumin determination in food-based samples [6]. This distinction is also consistent with the broader biochemical literature describing albumin's physiological importance and medical applications, which motivates interest in albumin-associated preparations but does not substitute for albumin-selective measurement in an analytical study [17].

Several limitations should therefore be acknowledged. First, the isolate is derived from a complex biological matrix, and Bradford-based quantification may be influenced by matrix-associated interferents (e.g., non-protein constituents, turbidity, or residual components) that can affect dye binding and baseline absorbance, even when blanking is applied. Second, the BSA calibration in this study covered 60–300 ppm, which supports linearity within that working range but remains relatively narrow; expanding the number of calibration points and the concentration range could improve robustness for broader applications. Third, the centrifugation condition was reported in rpm; because rpm does not directly translate across rotor geometries, reporting relative centrifugal force (RCF, $\times g$) would strengthen inter-laboratory reproducibility. Fourth, FTIR in this work was applied primarily for functional-group confirmation, and more detailed acquisition parameters (e.g., spectral resolution, number of scans, and baseline correction approach) would further strengthen methodological transparency. Finally, if the scientific objective is to substantiate the isolate as “albumin-rich” in a protein-specific sense, future work should incorporate albumin-selective assays and/or orthogonal separation/identification methods to support compound/protein attribution beyond functional-group confirmation.

4. Conclusion

This study demonstrated that aqueous extraction of snakehead fish (*Channa striata*) produced a defined water-soluble protein fraction that could be quantified reliably using the Bradford UV-Vis assay and characterised by FTIR spectroscopy. Bradford UV-Vis analysis, reported as BSA-equivalent total soluble protein, yielded a mean protein content of 460.6 ± 0.958 mg/g, corresponding to 46.06% (w/w) in the analysed fraction, with low variability across triplicate measurements ($n = 3$). Complementarily, FTIR profiling of the isolate (KBr pellet method) revealed characteristic protein-associated bands, including Amide A (3266.43 cm^{-1}), Amide I (1635.92 cm^{-1} ; predominantly C=O stretching), and Amide III (1318.95 cm^{-1}), supporting the presence of peptide-bonding features consistent with a protein-rich soluble isolate. Collectively, these findings indicate that the combined Bradford UV-Vis and FTIR

approach provides a practical analytical framework for initial evaluation and quality-oriented characterisation of water-soluble protein isolates from *C. striata*. However, because the present methods quantify total soluble protein and provide functional-group confirmation rather than protein-specific identification, any albumin-related claims require additional selective assays or orthogonal protein identification techniques in future work.

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Conflicts of Interest:

The author declares that there are no conflicts of interest in this research.

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