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Comparative solvent evaluation for protein extraction from *Channa striata* and development of a stable capsule formulation for nutritional supplementation

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ABSTRACT

Background: *Channa striata* is a protein-rich freshwater fish with therapeutic potential, yet systematic optimization of extraction methods and stable formulation development remain limited.

Objective: To compare protein extraction efficiency using three solvents (1% HCl, 0.9% NaCl, and distilled water) and develop optimized capsule formulations.

Methods: Protein was extracted at 1:4 (w/v) ratio and quantified using the Lowry method at 595 nm. The method was verified for linearity (R = 0.998), accuracy (98-102%), and precision (RSD <2%). Extracts were formulated into capsules at 0, 300, 400, and 500 mg (F0-F3) and evaluated for quality parameters and 28-day stability at -4°C, 24°C, and 40°C. Data were analyzed using one-way ANOVA with Duncan's post-hoc test.

Results: The 1% HCl yielded highest protein content (4.947 ± 0.071 mg/mL) and extraction efficiency (21.390%) compared to 0.9% NaCl and distilled water ($p < 0.05$). All formulations met pharmaceutical standards. Notably, F3 maintained protein retention >95% across all storage conditions (97.1-99.3%), while F0-F2 failed this criterion.

Conclusion: The 1% HCl demonstrated optimal extraction efficiency. F3 (500 mg) exhibited superior stability and dissolution characteristics, representing a promising candidate for *C. striata*-based nutritional supplements.

Keywords: capsule formulation, Channa striata, Lowry method, protein extraction, solvent optimization

Introduction

Channa striata, commonly known as striped snakehead, represents an economically important freshwater species extensively cultivated throughout Indonesia. National production data indicate substantial growth from 6,490 tonnes in 2015 to 21,987 tonnes in 2019, reflecting increased commercial interest in this species [1]. Lampung Province contributes significantly to this production, with documented harvest of 630,357 fish in 2020 [2]. beyond its economic value, *C. striata* has garnered attention for its nutritional composition, particularly its high protein content and bioactive compounds with potential pharmaceutical applications [3].

The protein content of *C. striata* reaches approximately 25.5% (w/w), exceeding that of most freshwater fish species by approximately 6.22% (w/w) [4]. This protein-rich composition includes substantial concentrations of essential amino acids, omega-3 and omega-6 fatty acids, and minerals including calcium, phosphorus, and zinc. Additionally, the skin and osseous tissues contain appreciable amounts of gelatin and collagen, compounds widely utilized in pharmaceutical and nutraceutical formulations [5]. The predominant protein in *C. striata* is albumin, which has been investigated for its role in cellular regeneration, tissue repair acceleration, and maintenance of oncotic pressure in vascular and interstitial compartments [6].

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These properties suggest potential applications in wound healing, postoperative recovery, and nutritional supplementation for vulnerable populations including malnourished individuals and children at risk of stunting.

Despite the established nutritional and therapeutic potential of *C. striata*, systematic investigation of optimal extraction parameters remains limited. Protein extraction efficiency depends critically on solvent selection, which influences the disruption of tissue matrices, protein solubilization, and preservation of bioactive properties. Previous studies have explored various extraction approaches, yet comparative analyses examining different solvent systems under standardized conditions are scarce. Acidic solvents, saline solutions, and aqueous extraction represent three fundamentally different chemical approaches, each potentially affecting protein yield, structural integrity, and biological activity differently.

Furthermore, the translation of fish protein extracts into stable, convenient dosage forms suitable for pharmaceutical or supplement applications requires careful formulation development. Capsule formulations offer advantages including precise dosing, improved stability, and enhanced patient compliance compared to liquid or semi-solid preparations. However, formulation parameters such as extract concentration, excipient selection, and storage stability require systematic evaluation to ensure product quality and therapeutic consistency.

The present study addresses these gaps through two primary objectives. First, we compare the protein extraction efficiency of three solvent systems: 1% hydrochloric acid (HCl), 0.9% sodium chloride (NaCl), and distilled water (aquadest). These solvents represent acidic, isotonic saline, and neutral aqueous extraction approaches, respectively, providing mechanistically distinct conditions for protein solubilization. Second, we develop and evaluate capsule formulations containing varying concentrations of the optimal extract, assessing physical stability, dissolution characteristics, and storage stability. This integrated approach, combining extraction optimization with formulation development, provides practical guidance for developing *C. striata*-based protein supplements suitable for commercial production and clinical application.

Methods

Sample preparation and extraction

Fresh *Channa striata* specimens (1 kg total weight) were obtained from Way Sekampung River, Pekon Sidoharjo, Pringsewu District, Lampung Province, Indonesia. Taxonomic identification was performed at the Zoology Museum, Bandung Institute of Technology, confirming the species as *C. striata* (identification record No. 5747/IT1.C11.2/TU/2023).

Fish specimens were manually processed to separate meat and skin from bones and viscera. The edible portions were washed thoroughly with distilled water and cut into small pieces to facilitate homogenization. Three extraction solvents were prepared using analytical-grade reagents: 1% (v/v) hydrochloric acid (HCl), 0.9% (w/v) sodium chloride (NaCl), and distilled water. Each solvent represented distinct extraction mechanisms: acidic hydrolysis, ionic interaction, and neutral aqueous extraction, respectively.

For extaction, fish tissue was homogenized with each solvent at a material-to-solvent ratio of 1:4 (w/v, based on fresh tissue weight) using a high-speed blender until a uniform paste was obtained. The homogenate was transferred to centrifuge tubes and centrifuged at 3,000 rpm for 15 minutes at room temperature (25 ± 2°C). The supernatant was carefully separated and subjected to freeze-drying at -50°C under vacuum (0.1 mbar) for 48 hours to obtain dry extract powder [7]. Extraction yield was calculated as:

Extraction yield (%) = (Weight of dried extract / Weight of fresh tissue) × 100%

All extractions were performed in triplicate for each solvent system.

Protein content determination

Protein quantification was performed using the Lowry method with UV-Vis spectrophotometric detection. Lowry reagent A was prepared by diluting 5 mL Folin-Ciocalteu reagent with 50 mL distilled water. Lowry reagent B was prepared fresh daily by mixing 15 mL alkaline carbonate solution (2% Na_2CO_3 in 0.1 M NaOH), 0.75 mL of 1% copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution, and 0.75 mL of 2% potassium sodium tartrate tetrahydrate ($C_4H_4O_6KNa \cdot 4H_2O$) solution.

Extract samples were dissolved in distilled water to prepare test solutions. To 1 mL of sample solution, 1 mL of Lowry reagent B was added, mixed thoroughly using

Table 1. Formulation of snakehead fish extract capsules [10]

No	Ingredient	Formula				Function
		F0 (-)	F1	F2	F3	
1	Snakehead fish extract powder	-	300 mg	400 mg	500 mg	Active ingredient
2	Vivapur 101	1.5 mg	1.5 mg	1.5 mg	1.5 mg	Adsorben
3	Calcium sorbate	0,1%	0,1%	0,1%	0,1%	Preservative
4	Starch	5%	5%	5%	5%	Disintegrant
5	Talcum powder	2%	2%	2%	2%	Lubricant
6	Magnesium stearate	1%	1%	1%	1%	Lubricant
7	Lactose	Ad 100%	Ad 100%	Ad 100%	Ad 100%	Filler

a vortex mixer, and allowed to stand for 15 minutes at room temperature. Subsequently, 3 mL of Lowry reagent A was added, mixed gently, and incubated for 45 minutes in the dark. The development of blue color indicated positive protein reaction. Absorbance was measured using a UV-Vis spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific) at 595 nm against a reagent blank. Protein concentration was determined using a calibration curve prepared from bovine serum albumin (BSA) standard solutions. All measurements were performed in triplicate.

Method verification

Method verification was conducted to ensure analytical reliability and included evaluation of linearity, accuracy, and precision. BSA was used as the reference standard for all verification parameters.

Linearity: A series of BSA standard solutions at concentrations of 0, 2, 3, 4, 5, and 6 mg/mL were prepared in distilled water. Each standard was reacted with Lowry reagents following the procedure described above. Absorbance values were plotted against concentration to establish a calibration curve. The linear regression equation ($Y = bX + a$) and correlation coefficient (R) were calculated. Linearity was considered acceptable if $R \geqslant 0.999$ [8].

Accuracy: Three concentration levels of BSA standards within the calibration range were prepared and analyzed in triplicate. Recovery percentage was calculated using the formula:

Recovery (%) = (Measured concentration / Theoretical concentration) × 100%

Acceptable recovery was defined as 98-102% with relative deviation <10% [11].

Precision: Repeatability was assessed by measuring six replicate samples of BSA standard solution on the same day under identical conditions. Reproducibility was evaluated by repeating the measurement on three consecutive days. Relative standard deviation (RSD) was calculated as:

RSD (%) = (Standard deviation / Mean) × 100%

Precision was considered acceptable if RSD <2% for both repeatability and reproducibility [9].

Capsule formulation

Based on protein content analysis results, the extract with the highest protein concentration was selected for capsule formulation. Four formulations were prepared with varying extract concentrations: F0 (0 mg, negative control), F1 (300 mg), F2 (400 mg), and F3 (500 mg) (Table 1). Pharmaceutical-grade excipients were used as follows: microcrystalline cellulose (Vivapur 101, 1.5 mg) as adsorbent, calcium sorbate (0.1% w/w) as antimicrobial preservative, starch (5% w/w) as disintegrant, talcum powder (2% w/w) as glidant, magnesium stearate (1% w/w) as lubricant, and lactose as filler to achieve 100% total weight. All ingredients were accurately weighed using an analytical balance and mixed geometrically in a mortar to ensure uniform distribution. The powder blend was manually filled into size 0 hard gelatin capsules using a manual capsule filling apparatus.

Quality control evaluation

Organoleptic properties: Physical characteristics including color, odor, texture, and taste of capsule contents were evaluated by trained observers under standardized conditions. Observations were recorded systematically using a standardized evaluation form [10].

Weight uniformity: Twenty capsules from each formulation were individually weighed using an analytical balance. Average weight and deviation from the mean were calculated. Formulations met acceptance criteria if not more than two capsules deviated by more than ±25% from the average weight [11].

Moisture content: Approximately 1 g of powder from each formulation was placed in pre-weighed aluminum foil and dried using a moisture analyzer at 105°C for 15 minutes. Moisture content was calculated as percentage weight loss. Acceptable moisture content range was 1-5% (w/w) [12].

Dissolution testing: Dissolution studies were performed to evaluate protein release characteristics. Capsules were placed in dissolution vessels containing 900 mL phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C with paddle rotation at 50 rpm. Samples (5 mL) were withdrawn at predetermined time intervals (0, 5, 10, 15, 20, 30, 45, and 60 minutes) and replaced with fresh medium to maintain sink conditions. Protein concentration in each sample was determined using the Lowry method. Dissolution profiles were plotted as cumulative protein released versus time.

Stability testing: Capsules from each formulation were stored at three different temperatures: -4°C (refrigerated), 24°C (room temperature), and 40°C (accelerated conditions) for 28 days. Samples were withdrawn at the end of the storage period and analyzed for protein content using the established Lowry method. Stability was considered acceptable if protein content remained ⩾95% of the initial value [13].

Statistical analysis

All experimental data are expressed as mean ± standard deviation from triplicate determinations. Data were analyzed using SPSS software version 26. Normal distribution was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. For normally distributed and homogeneous data, one-way analysis of variance (ANOVA) was performed to determine significant differences among groups. When significant differences were detected ($p < 0.05$), Duncan's multiple range test was applied as a post-hoc analysis to identify specific group differences. For non-normally distributed data, the non-parametric Kruskal-Wallis test was employed. Statistical significance was set at $\alpha = 0.05$ for all analyses [14–17].

Results

Method verification

Method verification was performed to establish the reliability of protein quantification using the Lowry method. Linearity was evaluated using BSA standard solutions ranging from 0 to 6 mg/mL. The relationship between absorbance and concentration yielded a linear regression equation of $y = 0.0901x + 0.2246$ with a correlation coefficient (R) of 0.998 (Figure 1A), demonstrating excellent linearity across the tested concentration range.

Accuracy assessment was conducted at three concentration levels (low, medium, and high) within the calibration range. Mean recovery values were 98.35 ± 1.21%, 101.66 ± 0.89%, and 100.60 ± 1.15% for the three concentration levels, respectively (Figure 1B). All recovery values fell within the acceptable range of 98-102%, indicating satisfactory accuracy of the analytical method

Precision evaluation showed high reproducibility and repeatability. The repeatability test yielded an RSD value of 0.499% based on six replicate measurements performed on a single day, while the reproducibility test across three consecutive days produced an RSD of 0.688% (Figure 1C). Both values were well below the acceptance criterion of 2%, confirming adequate precision for protein quantification.

Extraction yield and protein content

Extraction yield varied significantly among the three solvent systems. The 1% HCl solvent produced the highest yield at 21.4 ± 0.000% (w/w), followed by 0.9% NaCl at 16.7 ± 0.000% (w/w), and distilled water at 15.0 ± 0.001% (w/w). Correspondingly, protein content analysis revealed that 1% HCl extraction yielded the highest protein concentration of 4.9 ± 0.071 mg/mL, significantly exceeding that obtained with 0.9% NaCl (4.0 ± 0.024 mg/mL) and distilled water (3.4 ± 0.080 mg/mL) (Figure 2). Statistical analysis using one-way ANOVA indicated significant differences among the three extraction methods ($p < 0.05$). Duncan's post-

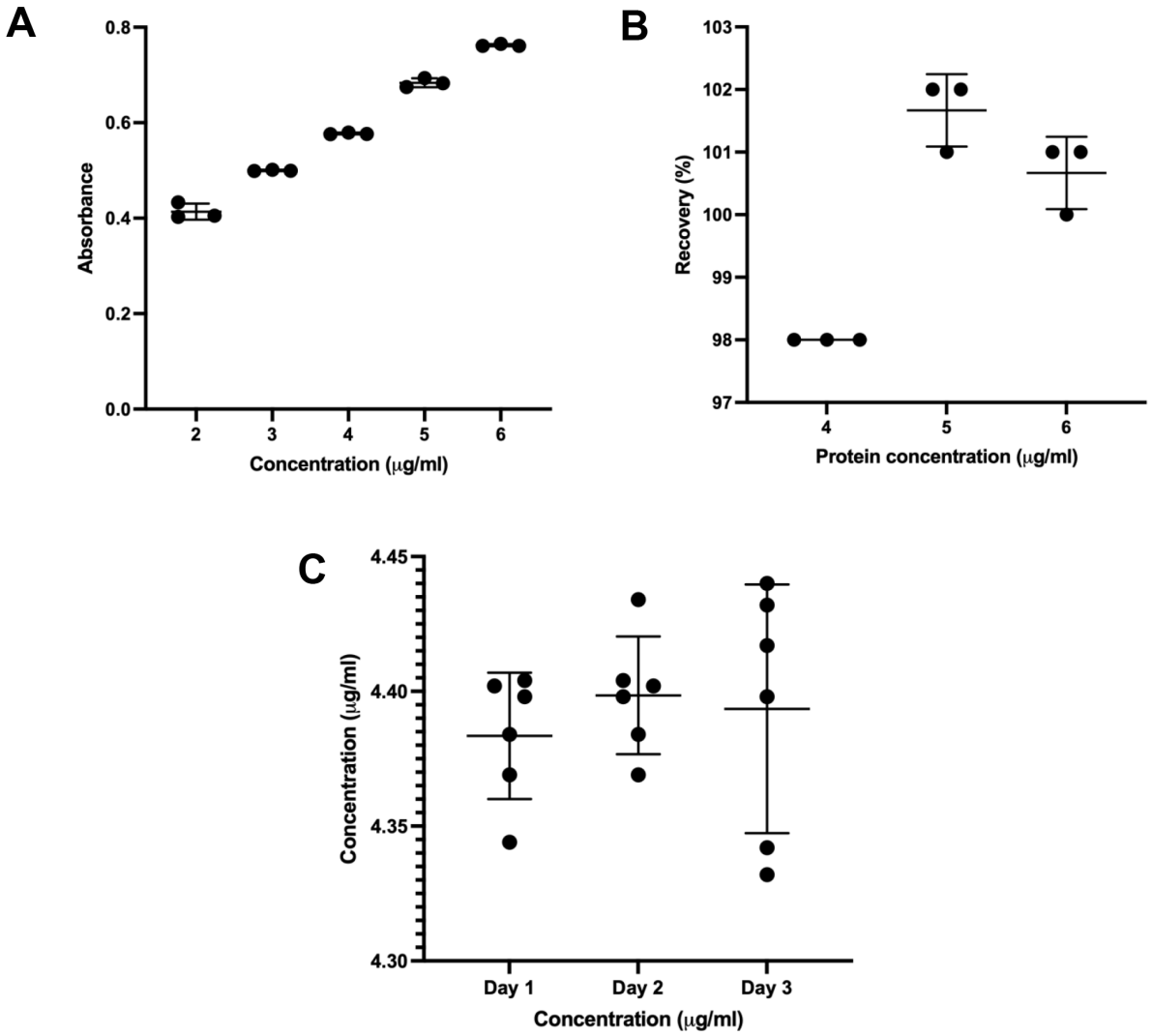

Figure 1. Method verification. (A) Linearity curve of bovine serum albumin (BSA) standard solutions. The linear regression equation (y = 0.0901x + 0.2246) with correlation coefficient R = 0.998 indicates excellent linearity within the tested concentration range. (B) Accuracy test results at three concentration levels. All values fall within the acceptable range of 98-102%, demonstrating satisfactory method accuracy. (C) Precision test results showing repeatability and reproducibility. Repeatability (intra-day precision) was assessed through six replicate measurements on day 1, yielding RSD of 0.499%. Reproducibility (inter-day precision) was evaluated across three consecutive days, producing RSD of 0.688%. Both values are below the 2% acceptance criterion. Data are expressed as mean ± SD (n=3). For precision test result, data represent mean ± SD (n=6 for repeatability; n=18 for reproducibility).

hoc test confirmed that each solvent system produced statistically distinct protein yields, with 1% HCl demonstrating superior extraction efficiency. Based on these results, the 1% HCl extract was selected for subsequent capsule formulation development.

Organoleptic evaluation

All capsule formulations (F0, F1, F2, and F3) exhibited consistent organoleptic properties. The powder contents displayed characteristic white coloration, fine texture, and mild sweet aroma attributable to the lactose excipient. Visual inspection revealed no color variations, clumping, or phase separation across different formulations. The uniformity of sensory characteristics indicated that variations in extract concentration (0-500 mg) did not substantially affect the physical appearance or odor profile of the final product.

Weight uniformity

Weight uniformity testing demonstrated acceptable consistency across all formulations. Mean capsule

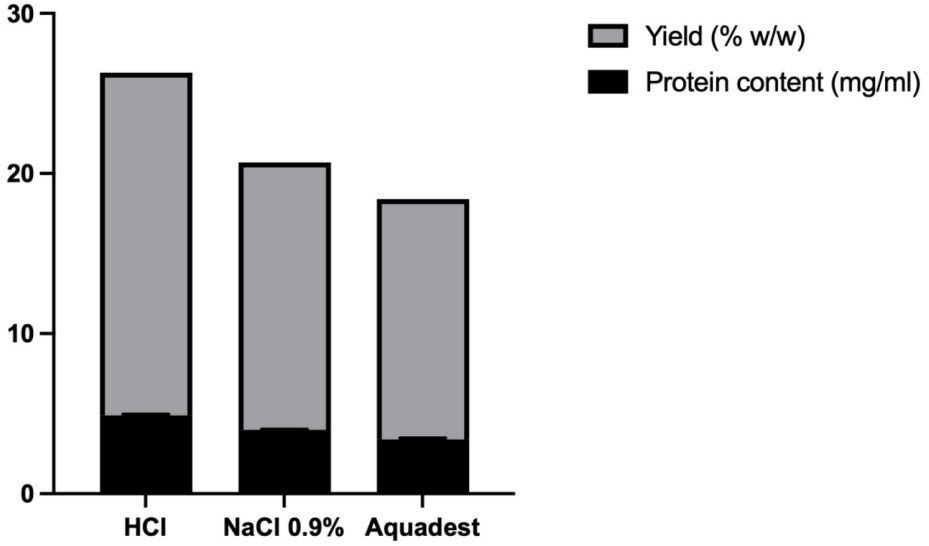

Figure 2. Extraction yield and protein content using different solvent systems. Comparison of extraction yield (% w/w) and protein content (mg/ml) obtained using 1% HCl, 0.9% NaCl, and distilled water as extraction solvents. The 1% HCl solvent produced significantly higher yield (21.4 ± 0.000%) and protein content (4.9 ± 0.071 mg/mL) compared to other solvents. Different letters indicate significant differences based on Duncan's post-hoc test ($p < 0.05$). Data are expressed as mean ± SD (n=3).

weights were 708.530 ± 6.667 mg for F0, 708.530 ± 6.208 mg for F1, 710.236 ± 7.607 mg for F2, and 709.090 ± 7.791 mg for F3. All formulations met pharmacopeial requirements, with no individual capsule deviating by more than 25% from the average weight. The calculated RSD values were 0.94%, 0.88%, 1.07%, and 1.10% for F0, F1, F2, and F3, respectively, all falling well below the 2% acceptance limit. These results confirm adequate manufacturing consistency and dose uniformity.

Moisture content

Moisture content analysis revealed progressive increases corresponding to extract concentration. Values obtained were 0.580 ± 0.000% (w/w) for F0, 1.677 ± 0.002% (w/w) for F1, 1.883 ± 0.019% (w/w) for F2, and 2.193 ± 0.001% (w/w) for F3 (Figure 3). Despite the concentration-dependent trend, all formulations remained within the acceptable moisture content range of 1-5% for capsule powders, indicating appropriate drying and acceptable storage stability characteristics.

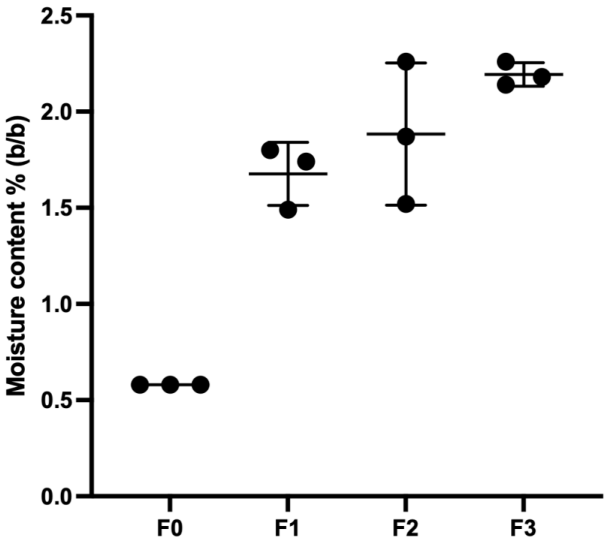

Figure 3. Moisture content of capsule formulations. Moisture content (% w/w) increased progressively with extract concentration, ranging from 0.58% (F0) to 2.19% (F3). All formulations remained within the acceptable range of 1-5% for capsule powders. Data are expressed as mean ± SD (n=3).

Dissolution profile

Dissolution testing revealed concentration-dependent protein release patterns. Figure 4 illustrates the cumulative protein release profiles for all formulations over 60 minutes. Formulation F3 demonstrated the highest maximum concentration, followed by F2, F1, and F0 in descending order. The release profiles showed rapid initial release within the first 15 minutes, followed by gradual approach to equilibrium. Statistical analysis using one-way ANOVA indicated significant differences among formulations ($p < 0.05$). Duncan's post-hoc comparison revealed that F2 and F3 did not differ significantly from each other ($p > 0.05$) but both showed significantly higher release

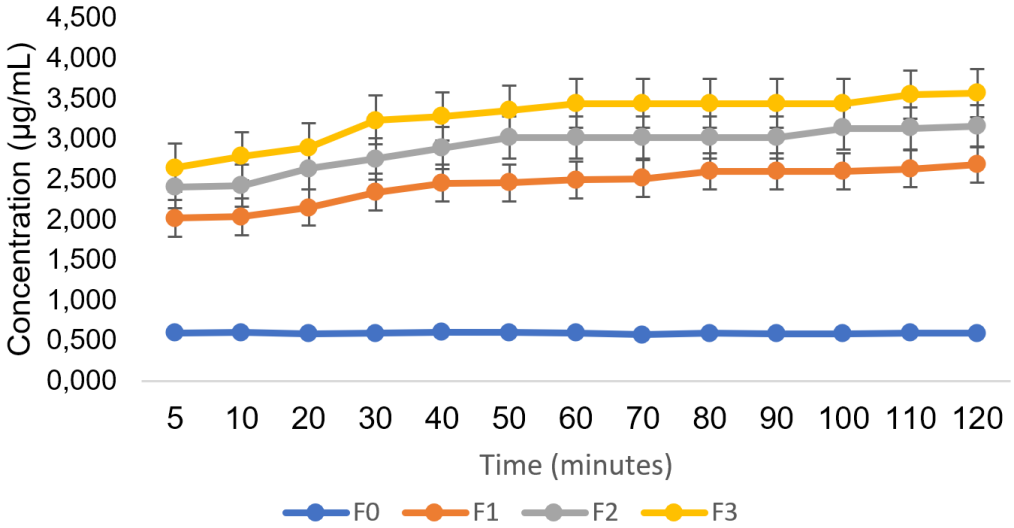

Figure 4. Dissolution profiles of *C. striata* extract capsule formulations. Cumulative protein release over 60 minutes for formulations F0 (0 mg), F1 (300 mg), F2 (400 mg), and F3 (500 mg). All formulations showed rapid initial release within 15 minutes followed by gradual equilibrium. F3 exhibited the highest dissolution rate and maximum protein release. Duncan's post-hoc test indicated no significant difference between F2 and F3 ($p > 0.05$), but both differed significantly from F0 and F1 ($p < 0.05$). Data are expressed as mean ± SD (n=3).

compared to F0 and F1 ($p < 0.05$). The dissolution efficiency, calculated as the area under the curve, increased proportionally with extract concentration, with F3 exhibiting the most favorable release characteristics.

Cumulative protein release over 60 minutes for formulations F0 (0 mg), F1 (300 mg), F2 (400 mg), and F3 (500 mg). All formulations showed rapid initial release within 15 minutes followed by gradual equilibrium. F3 exhibited the highest dissolution rate and maximum protein release. Duncan's post-hoc test indicated no significant difference between F2 and F3 ($p > 0.05$), but both differed significantly from F0 and F1 ($p < 0.05$). Data are expressed as mean ± SD (n=3).

Stability assessment

Stability testing over 28 days at three storage temperatures (-4°C, 24°C, and 40°C) revealed concentration-dependent protein retention across formulations. Formulations F0 (Figure 5A) and F1 (Figure 5B) exhibited substantial protein degradation, with retention rates falling below 90% at all storage temperatures, thereby failing to meet the 95% acceptance criterion. F2 demonstrated improved stability compared to F0 and F1, yet still exhibited protein retention below the required threshold, with values ranging from 87.0% to 89.0% across different storage conditions (Figure 5C).

In contrast, F3 maintained acceptable stability at all tested temperatures, with protein retention exceeding 97% in all conditions (Figure 5D). The highest stability was observed under refrigerated conditions (-4°C, 99.3% retention), followed by accelerated storage (40°C, 98.4% retention) and room temperature (24°C, 97.1% retention). Statistical analysis confirmed that F3 exhibited significantly superior protein stability compared to other formulations ($p < 0.05$). Notably, temperature variations within the tested range (-4°C to 40°C) did not substantially affect F3 stability, suggesting robust formulation characteristics and adequate protection of protein content during storage.

Discussion

This study demonstrated that solvent selection critically influences protein extraction efficiency from *Channa striata*, with 1% HCl yielding significantly higher protein content (4.947 ± 0.071 mg/mL) and extraction yield (21.390%) compared to 0.9% NaCl and distilled water. Subsequently, capsule formulations containing varying extract concentrations (0-500 mg) were developed and evaluated for pharmaceutical quality parameters. All formulations met acceptable standards for organoleptic properties, weight uniformity, and moisture content. Dissolution studies revealed concentration-dependent protein release, with formulations F2 (400 mg) and F3 (500 mg) exhibiting

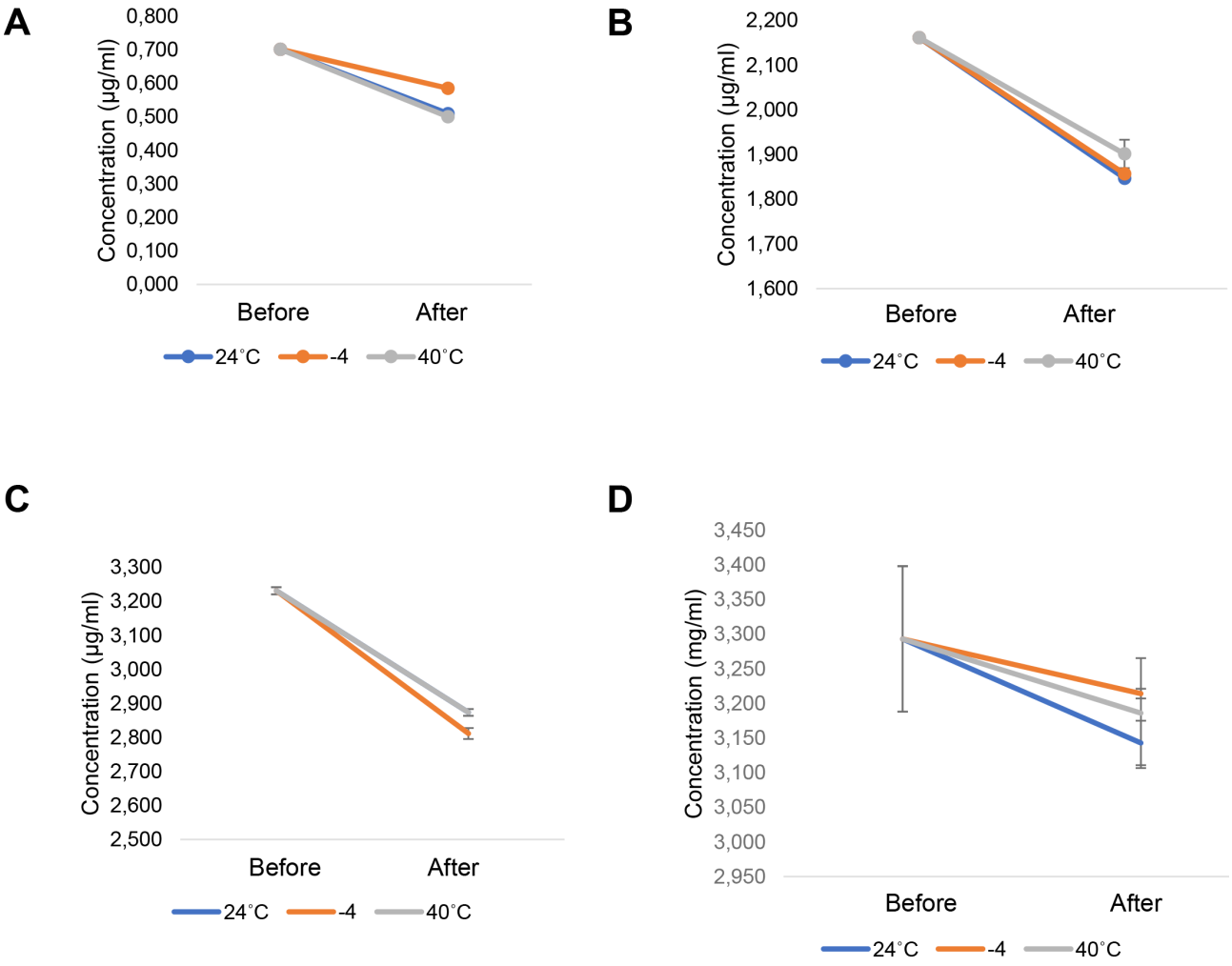

Figure 5. Stability profiles of *C. striata* extract capsule formulations after 28-day storage. Protein retention (%) at -4°C, 24°C, and 40°C for (A) F0, (B) F1, (C) F2, and (D) F3 formulations. Dashed lines indicate 95% acceptance criterion. Only F3 achieved satisfactory stability (>97% retention) across all storage conditions. Data are expressed as mean ± SD (n=3).

statistically comparable release profiles that were superior to lower-concentration formulations. Critically, stability assessment identified F3 as the optimal formulation, maintaining protein content above 97% across all tested storage conditions (-4°C, 24°C, and 40°C) over 28 days, while formulations F0, F1, and F2 failed to meet the 95% retention criterion. These findings establish 1% HCl as the preferred extraction solvent and identify F3 as a stable, pharmaceutically acceptable formulation for *C. striata*-based protein supplementation.

The superior extraction efficiency of 1% HCl can be attributed to its strong acidic properties, which facilitate disruption of tissue matrices and protein solubilization through multiple mechanisms. Hydrochloric acid, as a strong mineral acid, undergoes complete dissociation in aqueous solution, generating high concentrations of hydrogen ions (H^+) that effectively protonate peptide bonds and disrupt electrostatic interactions within protein structures [18]. This protonation induces protein unfolding and enhances solubility by increasing net positive charge, thereby promoting protein extraction from muscle and dermal tissues. The classic method of fish protein hydrolysis using HCl (6 M) for 20-24 hours at 110°C has been well-documented for achieving quantitative protein release [18]. In the present study, the use of 1% HCl at room temperature represents a milder extraction condition that still achieves superior yield compared to alternative solvents, suggesting that even dilute acid concentrations are sufficient for effective protein extraction when combined with appropriate mechanical disruption. Additionally, acidic

conditions facilitate hydrolysis of cross-links between collagen molecules present in connective tissues, further improving extraction yield [7]. The complete dissociation characteristic of HCl enables achievement of optimal extraction conditions at relatively low concentrations (1%), minimizing reagent consumption while maximizing efficiency.

In contrast, the 0.9% NaCl solution, while capable of extracting salt-soluble myofibrillar proteins through ionic interactions, demonstrated lower overall efficiency. Sodium chloride functions by disrupting electrostatic interactions between protein molecules and enhancing solubility of myofibrillar proteins that are insoluble in pure water. At low ionic concentrations, protein solubility typically increases as salt ions shield charged protein surfaces, preventing aggregation. However, at higher ionic strengths, the "salting-out" phenomenon occurs, wherein salt ions compete with proteins for water molecules, leading to protein dehydration and precipitation [19]. This dual effect—initial solubilization followed by potential precipitation—may limit the net extraction efficiency of saline solvents. The phenomenon follows the Hofmeister series, where different ions exhibit varying capacities to promote protein precipitation or solubilization [19]. Furthermore, NaCl extraction primarily targets water-soluble and salt-soluble protein fractions, potentially leaving collagen and other structural proteins less efficiently extracted compared to acidic hydrolysis [20]. Saline extraction methods typically yield lower collagen recovery rates because neutral salts cannot effectively break down the cross-linked collagen structure present in fish connective tissues [20]. Distilled water extraction yielded the lowest protein content, consistent with its inability to disrupt strong intermolecular interactions or solubilize hydrophobic protein components without additional chemical or physical treatments.

The formulation development phase revealed important insights into the relationship between extract concentration and product performance. The progressive increase in moisture content with higher extract concentrations (from 0.580% in F0 to 2.193% in F3) likely reflects the hygroscopic nature of protein and amino acid components in the extract [4]. Despite this trend, all formulations remained within acceptable moisture limits (1-5%), indicating adequate stability against microbial growth and chemical degradation [12]. The dissolution profiles demonstrated that F2 and F3 achieved similar maximum protein release, suggesting that concentrations above 400 mg may approach a saturation point for release kinetics under the tested conditions. However, the stability data clearly differentiated these formulations, with only F3 maintaining protein integrity above the 95% threshold across all storage conditions [13].

The superior stability of F3 merits particular consideration. Higher extract concentrations may provide protective effects through several mechanisms, including formation of a more cohesive protein matrix that resists oxidative and hydrolytic degradation, or through the presence of endogenous antioxidants and protective compounds that increase proportionally with extract concentration. Previous studies on fish protein extracts have demonstrated that albumin-rich formulations exhibit enhanced stability due to the protein's inherent structural properties and resistance to denaturation [6]. The observation that F3 maintained excellent stability even under accelerated conditions (40°C) suggests robust formulation characteristics suitable for tropical climates where ambient temperatures frequently exceed standard room temperature. This is particularly relevant for Indonesia, where tropical conditions pose challenges for pharmaceutical product stability. Interestingly, refrigerated storage (-4°C) provided only marginally better protection compared to room temperature for F3, indicating that the formulation possesses inherent stability that does not critically depend on cold chain maintenance, an important practical advantage for distribution and patient compliance.

The findings of this study have practical implications for development of *C. striata*-based nutritional supplements. The identification of 1% HCl as an optimal extraction solvent provides a scalable, cost-effective approach for protein isolation that can be readily implemented in pharmaceutical manufacturing. The F3 capsule formulation offers a convenient oral dosage form with demonstrated stability, potentially suitable for therapeutic applications including wound healing support, postoperative nutritional supplementation, and management of protein-energy malnutrition [5,6]. However, several limitations warrant acknowledgment. The stability study was conducted over 28 days, representing an abbreviated timeframe compared to typical pharmaceutical shelf-life requirements of 24-36 months [13]. Extended stability studies under ICH-compliant conditions would be necessary for commercial development. Additionally,

this study focused exclusively on protein content as the stability indicator; comprehensive stability assessment should include evaluation of amino acid profiles, microbiological quality, and functional bioactivity of the protein components.

Future research directions should address these gaps while expanding the scope of investigation. In vivo bioavailability studies are essential to determine whether the formulated capsules deliver biologically active protein that can be absorbed and utilized by the body. Comparative clinical trials evaluating the therapeutic efficacy of F3 against standard protein supplements would provide evidence for clinical applications. Furthermore, investigation of alternative stabilizing excipients or encapsulation technologies (such as enteric coating or lipid-based delivery systems) might further enhance protein protection and bioavailability. Finally, comprehensive safety assessment including acute and chronic toxicity studies would be necessary to support regulatory approval for human consumption.

Conclusion

This study established that 1% HCl is the most efficient solvent for protein extraction from *Channa striata*, yielding significantly higher protein content (4.947 ± 0.071 mg/mL) and extraction efficiency (21.390%) compared to 0.9% NaCl and distilled water. Among the developed capsule formulations, F3 containing 500 mg extract demonstrated optimal pharmaceutical characteristics, maintaining protein retention above 97% across all storage conditions (-4°C, 24°C, and 40°C) over 28 days. These findings provide a foundation for scalable production of *C. striata*-based protein supplements. However, extended stability studies, bioavailability assessment, and clinical efficacy trials remain necessary for commercial development and therapeutic applications.

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Author contributions

PDPS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. DDAK: Methodology, Resources, Supervision, Validation, Writing – review & editing. SA: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. All authors have read and approved the final manuscript.

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