Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 967 –984 (2025)
www.ejabf.journals.ekb.eg

Comparative Analysis of Protein Profiles in Albumin Concentrates from Various Freshwater Fish Species: Implications for Nutraceuticals and Functional Foods

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ARTICLE INFO

Article History:
Received: Jan. 3, 2025
Accepted: March 4, 2025
Online: March 20, 2025

Keywords:
Albumin,
Molecular weight,
Protein,
SDS-PAGE

ABSTRACT

This study investigated the protein band profile of albumin concentrates from various freshwater fish species, including *Trichopodus trichopterus*, *Monopterus albus*, *Clarias gariepinus*, *Oreochromis niloticus*, *Pangasius pangasius*, *Cypinus carpio*, and *Channa striata*. The protein analysis was conducted using SDS-PAGE, revealing consistent protein bands across all species, with molecular weights ranging from 10 to 250kD. Major proteins identified include myosin, β-galactosidase, bovine serum albumin, glutamate dehydrogenase, ovalbumin, carbonic anhydrase, myoglobin, trypsin inhibitor, lysozyme, and aprotinin. Despite the commonality of these proteins, differences in their expression levels were noted, attributed to genetic factors, environmental conditions, and physiological roles specific to each species. The study highlights the potential industrial applications of these proteins, especially in pharmaceuticals, food processing, and biotechnology, emphasizing the importance of species selection in optimizing protein yield and functionality.

INTRODUCTION

Protein is an essential component that plays a crucial role in the biological structure and function of organisms **(Morris *et al*., 2022; Austin *et al*., 2024; James & Jowza, 2024)**. Among various types of proteins, albumin is particularly important, primarily for maintaining osmotic pressure and transporting various substances in the blood **(Van De Wouw & Joles, 2022; Zheng *et al*., 2022)**. Freshwater fish albumin, specifically, has gained attention in numerous studies due to its wide range of potential benefits across various fields, including the food industry, healthcare, and fishery technology **(Duran-Güell *et al*., 2021; Alves *et al*., 2023; Elmira *et al*., 2023; Fitriani & Nugraha, 2023; Asrorov *et al*., 2024)**.

As demand for protein-based products in the food and healthcare industries continues to grow **(Prasad *et al*., 2023; Austin *et al*., 2024)**, it is essential to explore protein sources that offer added value. Freshwater fish, an abundant natural resource,

remains underutilized, especially in terms of product diversification. Freshwater fish albumin possesses unique characteristics that could serve as an alternative high-quality protein source, yet comprehensive studies on the albumin protein profiles of various freshwater fish species are still limited **(Nurfaidah *et al*., 2021)**. Therefore, this research is vital to fill this knowledge gap.

The SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) method has been widely used to analyze protein profiles based on molecular weight. This method enables precise identification and characterization of proteins, which is crucial for further application development **(Mazur *et al*., 2023; Gore *et al*., 2024)**. The albumin protein profiles from various freshwater fish species analyzed using SDS-PAGE not only provide basic information about protein composition but also open opportunities for further utilization.

The novelty of this study lies in its comprehensive approach to identifying and comparing albumin protein profiles from different freshwater fish species. By using the SDS-PAGE method, this study provides a detailed overview of albumin protein composition and creates opportunities for broader applications in various industries and healthcare. This research is expected to serve as a foundation for the development of innovative products based on freshwater fish protein, as well as contributing to an enhanced economic value of fishery resources in a more sustainable manner.

In the food industry, freshwater fish albumin has the potential to be used as a high-quality protein additive **(Fitriani & Nugraha, 2023; Simanjuntak *et al*., 2023)**. Its properties, such as solubility, stability, and emulsification ability, make albumin a promising candidate for food formulations like nutritional supplements and functional products **(Das *et al*., 2021; Alves *et al*., 2023)**. In healthcare, fish albumin can serve as an alternative source of albumin for medical use, such as wound treatment and drug-binding agents **(Elmira *et al*., 2023; Silaban & Nurjanah, 2024)**. Furthermore, in fishery technology, an in-depth understanding of freshwater fish protein profiles can support the development of innovative, high-value products **(Hutapea *et al*., 2022; Alfonso *et al*., 2024)**.

This study aimed to analyze albumin protein profiles from various freshwater fish species using the SDS-PAGE method and to assess their potential applications in various fields, especially for nutraceuticals or functional food. The results of this research are expected to contribute to the broader development of fishery technology applications and to increase the economic value of fishery products.

MATERIALS AND METHODS

Materials and equipment

The fish used in this study include the common carp (*Cyprinus carpio*), catfish (*Clarias gariepinus*), eel (*Monopterus albus*), three-spot gourami (*Trichopodus*

trichopterus), pangas catfish (*Pangasius pangasius*), tilapia (*Oreochromis niloticus*), and snakehead fish (*Channa striata*), obtained from waters around the Makassar area. The equipment and materials used for the analysis include vertical electrophoresis (Mini-PROTEAN® Tetra Cell), PowerPac™ power supply, water bath, micropipette set with tips, 1.5ml microtubes, gloves, shaker, 10% APS, ddH2O/aquadest, Laemmli sample buffer, β-mercaptoethanol, acrylamide/Bis, Tris-Glycine-SDS running buffer, SDS, Tris-HCl, Coomassie brilliant blue staining solution, Coomassie destaining solution, TEMED, protein samples, protein marker, and PVDF membrane.

Sample preparation and fish albumin extraction

In this study, fresh fish were sourced from markets in Makassar. The fish were collected in varying sizes: common carp ranged from 340 to 824 grams, tilapia from 263 to 361 grams, eel from 140 to 172 grams, three-spot gourami from 116 to 162 grams, catfish from 271 to 374 grams, pangas catfish from 276 to 497 grams, and snakehead fish from 243 to 640 grams. Upon arrival at the laboratory, the fish were stored at approximately ±10°C in a bucket containing water and ice blocks. The fish samples were then frozen at temperatures below -20°C in HDPE zip-lock plastic bags until analysis.

The fish were cleaned by removing the gills and internal organs, then rinsed with running water and allowed to drain. The fish were filleted, and the flesh was separated from the bones and skin. The fish meat was minced, homogenized using a blender, placed in HDPE zip-lock plastic bags, and stored at -20°C until analysis. The albumin extraction process was adapted from **Nurfaidah *et al.* (2021)**. Albumin was extracted from fish meat by weighing 50g of the meat into a 250ml beaker, diluted with aquadest at a ratio of 1:4, and homogenized for 1 minute using a homogenizer. After homogenization, the sample was incubated at 50°C for 1 hour in a water bath with continuous stirring. Following incubation, the sample was filtered using a vacuum pump, and the filtrate volume was recorded. The filtrate was transferred into dark glass bottles, sealed tightly, and stored at -20°C until analysis.

Analysis of fish albumin protein molecular weight

The sample buffer was prepared by mixing 50µl of β-mercaptoethanol with 950µl of Laemmli sample buffer. A total of 500µl of the buffer mixture was combined with 500µl of the sample (1:1 ratio). The protein mixed with the buffer was heated in a water bath at 85°C for 5 minutes. Equipment such as spacer plates, short plates, and the casting gel system was assembled to prevent leakage during gel preparation. A 12% separating gel was prepared by mixing 6ml of 30% acrylamide/bis stock, 3.75ml of 1.5M Tris pH 8.8, 5.03ml aquabides, 150µl of 10% SDS, 75µl of 10% APS, and 7.5µl TEMED. The mixture was poured into the mold and was left for approximately 30 minutes.

A 4% stacking gel was prepared by mixing 0.99ml of 30% acrylamide/bis, 1.89ml of 0.5M Tris pH 6.8, 4.5ml aquabides, 75µl of 10% SDS, 40µl of 10% APS, and 7.5µl TEMED. The mixture was poured over the separating gel and was allowed to solidify.

The gel was placed in the mini-protean tetra cell chamber and was filled with running buffer. After loading the protein marker and samples into the wells, the chamber was sealed, and gel running was performed at 100-120V for 60-90 minutes. The SDS-PAGE results were verified by Coomassie blue staining, followed by destaining. Visualization was carried out by identifying the protein bands based on the positions of known molecular weight marker bands. The marker served as a standard for comparing the molecular weight of sample proteins.

Data analysis

Data analysis was conducted descriptively using the results of SDS-PAGE to identify and compare the protein band profiles of albumin from different freshwater fish. Each protein band formed on the SDS-PAGE gel was analyzed based on its intensity and presence in various fish species. The protein profile data obtained are presented in tables and figures to facilitate comparison between species.

The data processing involved the following steps: 1) Protein band identification: the protein profiles of each fish species were identified by comparing the band positions on the gel with the known molecular weight marker. 2) Protein band classification: the bands were categorized as major or minor based on thickness and intensity. 3) Inter-species comparison: the protein profiles of each species were compared to identify differences in the composition and intensity of protein bands. These results were analyzed to evaluate the influence of genetic, environmental, and dietary factors on protein variation. 4) Result interpretation: the analysis results were used to evaluate the potential industrial and medical applications of the identified proteins.

RESULTS

The SDS-PAGE electrophoresis analysis of various freshwater fish species revealed distinct protein band profiles. In each sample, the bands formed represent proteins of varying molecular weights. These bands were compared to a standard protein marker to identify the types of proteins present in the samples.

In Fig. (1), it is evident that all tested fish species exhibit the presence of protein bands within a certain molecular weight range, identified as albumin. The albumin band profiles for each fish species vary in both intensity and the number of bands, indicating differences in the composition and structure of albumin proteins among the species.

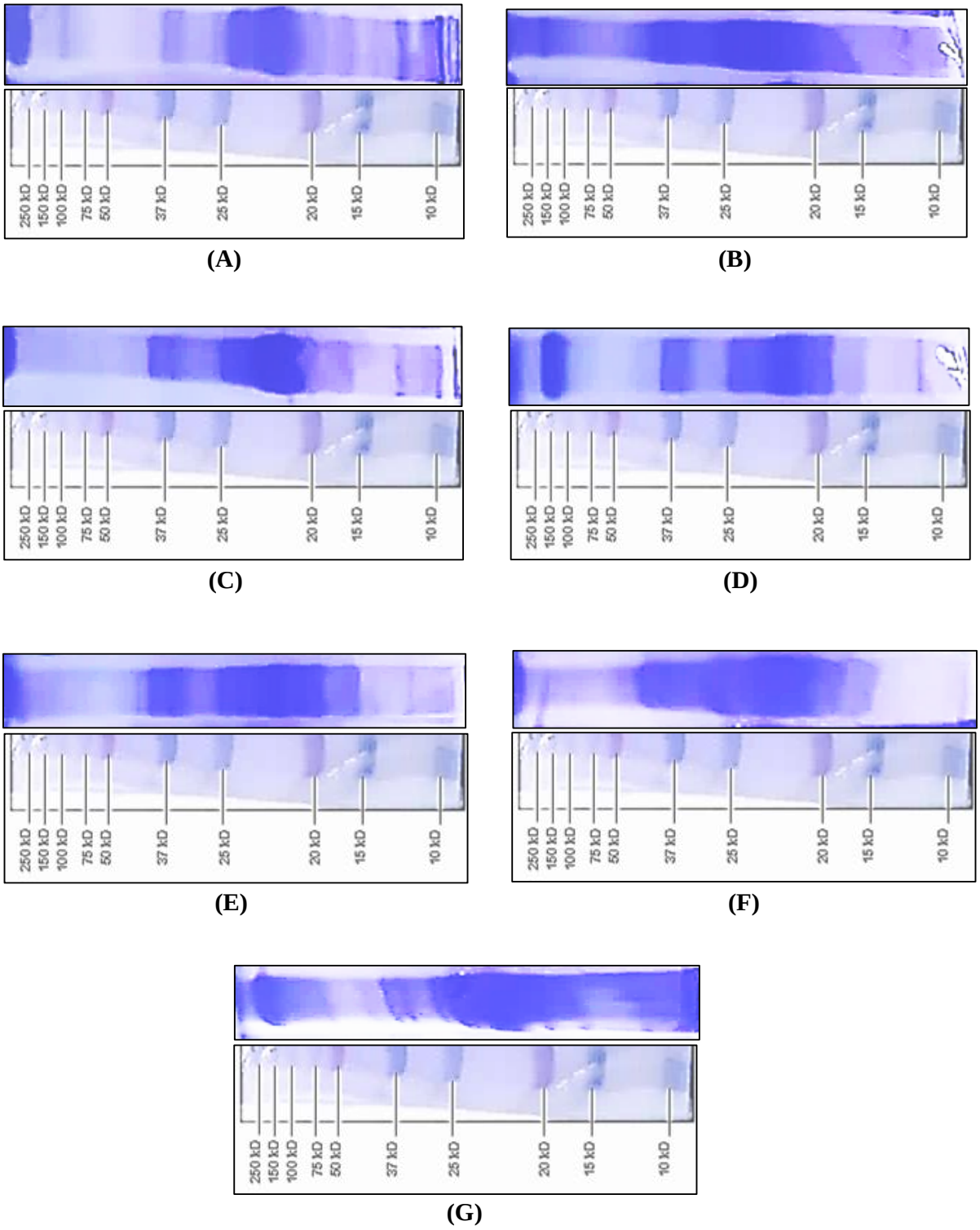

Fig. 1. Molecular weight profiles of albumin proteins from various freshwater fish species analyzed using SDS-PAGE, where **(A)**: *Trichopodus trichopterus*; **(B)**: *Monopterus albus*; **(C)**: *Clarias gariepinus*; **(D)**: *Oreochromis niloticus*; **(E)**: *Pangasius pangasius*; **(F)**: *Cyprinus carpio*; **(G)**: *Channa striata*

Table (1) below presents the molecular weight profiles of albumin proteins detected in several fish species. Each species exhibited various protein molecular weights, ranging from 10 to 250kD. The identified proteins include Myosin, β-Galactosidase, Bovine Serum Albumin, Glutamate Dehydrogenase, Ovalbumin, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Lysozyme, and Aprotinin. These data illustrate the diversity of proteins in fish bodies and can aid in understanding the metabolic adaptations and biological functionalities of each species.

All fish species examined displayed a wide range of proteins with varying molecular weights, from 10 to 250kD. Three-spot gourami and eel exhibited a broad protein spectrum, indicating similar diversity in proteins and complex metabolic functions. Catfish, tilapia, pangas catfish, common carp, and snakehead fish also showed similar protein diversity, suggesting strong adaptive capacities to environmental conditions and a wide range of biological functions. This protein diversity reflects similar metabolic and functional adaptations among the studied fish species. The protein profiles provide insight into their biological functions and the adaptations these species have developed to thrive in their respective environments.

Table 1. Molecular weight profiles of albumin proteins in various fish species

Fish species	Molecular weight (kD)	Protein profile*
Trichopodus trichopterus	250, 150, 75, 37, 25, 20, 15, 10	Myosin, β-galactosidase, Glutamate Dehydrogenase, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Lysozyme, Aprotinin
Monopterus albus	100, 25, 20, 10	Bovine Serum Albumin, Myoglobin, Trypsin Inhibitor, Aprotinin
Clarias gariepinus	250, 100, 37, 25, 20, 10	Myosin, Bovine Serum Albumin, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Aprotinin
Oreochromis niloticus	100, 50, 37, 25, 20, 10	Bovine Serum Albumin, Ovalbumin, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Aprotinin
Pangasius pangasius	100, 50, 37, 25, 20, 10	Bovine Serum Albumin, Ovalbumin, Carbonic

		Anhydrase, Myoglobin, Trypsin Inhibitor, Aprotinin
Cypinus carpio	250, 50, 37, 25, 20, 10	Myosin, Ovalbumin, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Aprotinin
Channa striata	250, 75, 37, 25, 20, 15, 10	Myosin, Glutamate Dehydrogenase, Carbonic Anhydrase, Myoglobin, Trypsin Inhibitor, Lysozyme, Aprotinin

*(Nacalai, n.d.)

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DISCUSSION

Protein band analysis of fish albumin concentrate and its potential applications

Protein bands can be categorized into major and minor bands. Major bands contain higher protein concentrations than the others, indicated by their thickness and stronger color intensity. In contrast, minor bands have lower protein concentrations with thinner bands and lighter intensity **(Chong *et al.*, 2022)**. Each fish species studied contained various types of proteins with specific molecular weights. While the types of proteins present in each fish were similar, the specific characteristics and potential applications of each species may vary depending on factors such as availability, market demand, and the fish's physical or chemical properties.

Snakehead fish *(Channa striata)*

The albumin concentrate from the snakehead fish shows the presence of three major protein bands at approximately 10, 25, and 250kD, and four minor bands at around 15, 20, 37, and 75kD. The major proteins in this concentrate include aprotinin, myoglobin, and myosin, which play significant roles in both medical and food industries. The

snakehead fish albumin concentrate can be utilized in the production of myoglobin-based health supplements, as myoglobin is an oxygen-binding protein that facilitates oxygen transport in muscles, particularly useful in health supplements to enhance tissue oxygenation. It also has potential as a supplement to boost immunity and to improve tissue oxygenation in patients with cardiovascular diseases **(Ordway & Garry, 2004; Vanek & Kohli, 2023)**. In the food industry, the snakehead fish albumin extract can be used in surimi production to improve texture due to its myosin content, which plays a role in muscle contraction and is essential for gel formation and coagulation **(Yongsawatdigul *et al*., 2005)**. Additionally, aprotinin in this albumin can be used in pharmaceuticals as a protease inhibitor for reducing blood loss during surgeries and as an anti-inflammatory agent **(De Hert *et al*., 2022; Eloïse Gallo *et al*., 2024)**.

Numerous studies have explored the benefits of the snakehead fish albumin. One study found that 6% snakehead fish albumin gel significantly accelerated wound closure in rats, achieving 80% closure by day seven **(Puspitasari & Suprayitno, 2020)**. The high protein content and amino acid profile of the snakehead fish albumin are crucial in stimulating tissue regeneration, making it a viable alternative to expensive human serum albumin **(Mardiyah *et al*., 2022)**. In patients with hypoalbuminemia, the snakehead fish extract significantly increased albumin levels from 2.95 to 3.17g/ dL, along with enhanced protein intake **(Fauzan *et al*., 2020)**.

Three-spot gourami *(Trichopodus trichopterus)*

In three-spot gourami, two major protein bands were observed at approximately 10 and 25kD, with six minor bands at around 20, 37, 50, 75, 150, and 250kD. The major proteins include aprotinin and myoglobin, while the minor proteins consist of ovalbumin, trypsin inhibitor, carbonic anhydrase, and β-galactosidase. Aprotinin serves as a protease inhibitor, similar to its role in the snakehead fish. The three-spot gourami also has a high concentration of aprotinin and myoglobin, but it additionally contains ovalbumin and β-galactosidase. Ovalbumin is commonly used in allergy testing and has potential medical applications for metal poisoning detoxification **(Olsen, 2008)**. Meanwhile, β-galactosidase, an important enzyme in the food industry, hydrolyzes lactose, making products more digestible for individuals with lactose intolerance, highlighting the potential of three-spot gourami in lactose-free dairy products **(Patel *et al*., 2016)**.

Consumption of three-spot gourami offers various health benefits for humans and the food industry. Although there has been no specific research on the benefits of its albumin, other studies have shown that it is not only a source of high-quality protein but also contains essential omega-3 fatty acids and various micronutrients, contributing to overall health and disease prevention. Fish like three-spot gourami provide all essential amino acids important for metabolism and muscle maintenance, as well as omega-3 fatty acids, which reduce the risk of cardiovascular disease and improve brain function **(Chen *et al*., 2022; Phogat *et al*., 2022)**. Additionally, three-spot gourami is rich in vitamins and minerals that support immune function and overall health **(Phogat *et al*., 2022)**.

Eel *(Monopterus albus)*

The albumin concentrate from eel exhibits one major protein band at 25kD and three minor bands at around 10, 20, and 100kD. Myoglobin is the dominant major protein, with aprotinin and trypsin inhibitor present in smaller amounts. The dominance of myoglobin in the eel albumin concentrate indicates its potential as a health product that can enhance oxygenation in muscle tissues. Although aprotinin is present in smaller quantities, it still adds value as a protease inhibitor for medical applications.

The potential uses of albumin from eel are primarily in the health and nutrition sectors. Research on eel albumin is mostly limited to extraction and yield measurements, though some studies have highlighted its benefits. **Rufniarti (2021)** reported that eel albumin extract increased albumin levels in patients with hypoalbuminemia and post-operative conditions and was effective in accelerating wound healing. This is supported by **Yang *et al.* (2024)**, who noted that eel albumin contains high protein content and has strong antioxidant properties, aiding in wound healing and immune support **(Yang *et al.*, 2024)**. Furthermore, eel albumin is used in the cosmetics and pharmaceutical industries due to its properties that improve skin structure and tissue repair **(Yang *et al.*, 2024)**.

Several studies have also shown that eel albumin holds potential as a therapeutic agent for treating various medical conditions. In the industrial sector, this albumin extract can be used in supplements and nutraceuticals, as well as raw materials in pharmaceutical formulations **(Tay *et al.*, 2003)**. Apart from its high protein content, swamp eel has free-radical scavenging abilities **(Rahman *et al.*, 2021)** and is rich in omega-3 fats **(Putri *et al.*, 2024)**.

Catfish *(Clarias gariepinus)*

In the catfish, two major protein bands were observed at approximately 10 and 37kD, along with four minor bands at approximately 20, 25, 100, and 250kD. The major proteins in catfish are aprotinin and carbonic anhydrase. Aprotinin functions as a protease inhibitor, playing a role in controlling bleeding during surgical procedures **(Padín *et al.*, 2024)**. Carbonic anhydrase plays a key role in maintaining acid-base balance and has applications in medical therapy for respiratory disorders **(Occhipinti & Boron, 2019)**. The presence of these two major proteins makes the catfish an ideal candidate for use in medical applications requiring protease inhibitors and acid-base stabilizers. Carbonic anhydrase suggests that the catfish could also serve as a raw material in medical products targeting patients with respiratory disorders or conditions related to body acidity.

Although no specific studies have been found discussing the role of the catfish albumin extract for respiratory disorders, the potential of the catfish albumin as a raw material for medical or health products is supported by several previous studies. Catfish albumin is recognized for its role in wound healing and boosting the immune system, as demonstrated in ointment formulations that enhance postoperative healing **(Risnawan & Nugraha, 2023)**. Further, the catfish albumin plays a role in brain tissue development and helps improve immune function effectiveness **(Fitriani & Nugraha, 2023)**.

Tilapia *(Oreochromis niloticus)* and pangas catfish *(Pangasius pangasius)*

Tilapia and pangas catfish share a similar protein banding pattern, with one major protein band at approximately 10kD and five minor protein bands at approximately 20, 25, 37, 50, and 100kD. Aprotinin is the major protein in both fish species. As with other fish species, aprotinin in tilapia and pangas catfish also has significant medical applications. The similarity in protein content suggests that these two fish species can be used interchangeably in certain industrial applications, depending on availability and cost. The albumin in tilapia and pangasius plays a crucial role in human health, particularly through its nutritional benefits and potential health applications. This protein is essential for maintaining colloid oncotic pressure and has various physiological functions that can positively impact human health.

Previous studies have shown that tilapia and pangas catfish are high-quality protein sources, including albumin, which is essential for muscle repair and growth in humans **(Throop *et al*., 2004)**. An example of product utilization from tilapia and the pangas catfish as high-protein food sources is the production of the pangas catfish biscuits by **Anugrahati *et al*. (2012)** and tilapia fish flakes by **Safitri *et al*. (2023)**. Consuming foods containing albumin, such as those from tilapia and pangasius, can enhance immune responses, potentially increasing resistance to infections **(Chakrabarti & Rao, 2012)**.

Common carp *(Cyprinus Carpio)*

The albumin concentrate from common carp exhibited a major protein band around 10kDa and five minor bands at approximately 20, 25, 37, 50, and 250kDa. Aprotinin emerged as the major protein in carp, with ovalbumin and carbonic anhydrase being significant minor proteins. Aprotinin, known for its role as a protease inhibitor with medical applications in controlling bleeding, stands out, while the presence of ovalbumin highlights common carp's potential in medical applications related to allergy testing and toxin neutralization. Common carp, with aprotinin as its dominant protein and a diverse range of minor proteins such as carbonic anhydrase and ovalbumin, is particularly suited for use in medical products requiring a combination of anti-inflammatory, acid-base stabilizing, and allergy therapy functions. This unique combination positions common carp as a potential source for the development of multifunctional supplements or pharmaceuticals.

Nurfaidah *et al*. (2024) formulated a nutrient-dense complementary food by adding common carp meat flour and albumin, which proved rich in essential amino acids and fatty acids vital for growth and development. This aligns with previous research by **Ljubojević *et al*. (2017)**, which confirmed that common carp meat is high in protein, crucial for tissue growth and repair due to its high-quality amino acid profile. Common carp can be utilized in the production of health supplements and traditional medicines because of its rich nutritional content. Additionally, extracts from common carp, such as collagen, are used in cosmetics and skincare products due to their benefits in improving skin health and anti-aging properties **(Ljubojević *et al*., 2017)**.

Based on the analysis of protein band patterns from the albumin concentrate of various freshwater fish species, three types of proteins-aprotinin, trypsin inhibitor, and carbonic anhydrase were found in all fish species studied. These proteins could serve as markers for fish albumin. The protein band analysis of each fish species revealed variations in major and minor proteins, which can be exploited in various medical, health, and food industry applications. Aprotinin, identified in all observed fish species, plays a crucial role in medical applications, particularly in surgical procedures to reduce bleeding. Myoglobin, myosin, and carbonic anhydrase also present significant opportunities in health and the food industry, aligning with their biological and functional roles. This combination of proteins highlights the great potential of fish albumin in both medical and commercial fields.

Comparison of albumin protein components across species

At the molecular level, the protein profile of albumin reveals striking similarities across various fish species, with key proteins such as myosin, myoglobin, and several enzymes playing similar functional roles. This uniformity suggests that these proteins are crucial for fish physiology, including muscle contraction and oxygen storage. Although the protein patterns are generally consistent, variations in the types and quantities of proteins occur between species, influenced by genetic factors, environmental conditions, diet, developmental stage, and physiological states.

Genetic differences among fish species can lead to variations in the structure and function of proteins such as myosin and myoglobin, even though their molecular weights remain similar **(Koch *et al.*, 2016; Usman, 2016; Chen *et al.*, 2020)**. Environmental conditions also significantly impact the protein composition of fish **(Ariyanto *et al.*, 2019; Kandyliari *et al.*, 2020)**. In addition to genetic and environmental factors, diet plays a crucial role in protein variation between fish species **(Smith *et al.*, 2013; Kondratiuk & Otchenashko, 2021)**. For example, the snakehead fish, a predator, may have more active proteins like trypsin inhibitors to cope with a protein-rich diet, while common carp, which are more herbivorous, exhibit less dominant activity of these proteins. This variation reflects specific dietary adaptations in each species, influencing the need and function of proteins in fish bodies.

Developmental stage or age also contributes to differences in protein expression **(Payuta & Flerova, 2021)**. Proteins such as bovine serum albumin (BSA) and ovalbumin may show differences in expression between adult and juvenile fish, as observed in the catfish compared to eel. Furthermore, physiological conditions and stress **(Raposo De Magalhães *et al.*, 2020; Sánchez-Velázquez *et al.*, 2024)**, such as those experienced by eels in low-oxygen muddy environments, can lead to variations in proteins like carbonic anhydrase, which regulates pH and CO_2 expulsion. These factors demonstrate that while proteins may have similar structures, their specific roles and functions in fish physiology can vary according to environmental adaptations and the individual conditions of each

species. Further research is needed to understand the specific impacts of each protein across different fish species.

CONCLUSION

This study reveals that albumin concentrates from seven species of freshwater fish exhibit relatively similar protein band profiles, though with certain significant variations between species. Genetic factors, environmental adaptations, and the dietary patterns of each fish species contribute to these variations. The main proteins identified, such as myosin, bovine serum albumin, and myoglobin, hold considerable potential for broad applications in the food, health, and biotechnology industries. This study underscores the importance of a deep understanding of fish protein composition to optimize its utilization in various commercial applications. Further research is needed to explore the correlation between specific environmental conditions and the expression of certain proteins, as well as investigating additional potential applications of these albumin proteins.

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