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Development of Fish (*Oreochromis niloticus*) Protein Hydrolysate-based Enteral Formula

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ABSTRACT: The study aimed to explore the usage of fish protein to produce fish protein hydrolysate (FPH) that may find application in clinical aspects, especially in critical care. The findings might increase the number of value-added products produced for the pharmaceutical industry while improving environmental sustainability. The study focused on the development and characterization of a fish protein hydrolysate (FPH)-based enteral formula derived from enzymatically hydrolyzed tilapia fish (*Oreochromis niloticus*) fillets. The freeze-dried fish protein hydrolysate was mixed with selected ingredients such as cocoa powder, xanthan gum, ascorbic acid, soy lecithin, potato starch, maltodextrin, milk powder, powdered sugar, salt, and pure medium-chain triglycerides (MCT) to develop an enteral formulation. The product was standardized by incorporating different proportions of FPH and was selected based on the sensory score.

Keywords: *Oreochromis niloticus*, fish protein hydrolysate, flavourzyme, enteral nutrition, enteral formula.

INTRODUCTION

Enteral nutrition is a crucial treatment method used to supply balanced nourishment to patients who cannot satisfy their nutritional needs via standard oral consumption. It is particularly crucial for people in medical environments dealing with chronic diseases, trauma, or digestive issues that hinder their capacity to eat or process whole foods (Wischmeyer, 2017). An essential aspect of every enteral formula is its protein component, which needs to be easily digestible, hypoallergenic, and readily bioavailable. In this context, tilapia-derived fish protein hydrolysates (FPHs) have become a notable protein source for developing fish protein hydrolysate-based enteral formula.

Tilapia (*Oreochromis niloticus*) is one of the most widely farmed freshwater fish species globally, contributing substantially to aquaculture output and food security. The fish is not only financially sustainable but also abundant in high-quality proteins and vital amino acids (Canton, 2021). By the process of enzymatic hydrolysis, tilapia proteins can be decomposed into small peptides and free amino acids, yielding fish protein hydrolysates that provide enhanced digestibility, quick absorption, and lower allergenic potential (Chalamaiah *et al.*, 2012; He *et al.*, 2013). These qualities are particularly advantageous for individuals with compromised gastrointestinal function, as FPHs reduce the digestive burden and enhance nutrient uptake (Minkiewicz *et al*., 2019).

Additionally, FPHs contain biologically active peptides that demonstrate antioxidative, anti-inflammatory, immunomodulatory, and antihypertensive effects, rendering them functionally more advantageous than conventional intact proteins (Ngo *et al.*, 2014). These biological activities are highly significant in enteral nutrition, which frequently serves patients in catabolic states, with inflammatory conditions, or under oxidative stress. Such properties make FPHs particularly suitable as protein sources for semi-elemental or peptide-based enteral formulas designed for individuals with impaired digestion, malabsorption, or increased metabolic demands.

Regardless of these advantages, the effective integration of FPH into a clinically appropriate format necessitates meticulous focus on formulation science and product design. A significant advancement in this area is the creation of a ready-to-reconstitute formula, which is a powdered, shelf-stable version that can quickly be rehydrated before use. This format tackles significant logistical issues like storage, transportation, and shelf life, while preserving the nutritional and functional integrity of the formula (Schaafsma, 2009).

According to recent research, hydrolysates generated from tilapia can produce bioactive peptides with particular health-promoting properties. Developing a fish protein hydrolysate-based enteral formula using

tilapia FPH requires several important factors to consider, such as optimizing hydrolysis conditions, nutritional analysis, functional attributes like solubility and dispersibility, and long-term stability. In addition, choosing suitable carbohydrates, fats, micronutrients, and stabilizers must guarantee that the formula adheres to dietary guidelines for at-risk groups like the elderly, critically ill, or malnourished individuals.

Currently, a substantial gap remains in utilizing fish resources, such as tilapia, for a valuable medical nutrition supplement. Using by-products or entire tilapia enhances nutrient recovery and sustainability while also promoting circular bioeconomy initiatives within aquaculture and food processing sectors (Rustad *et al.*, 2011). Consequently, developing a fish protein hydrolysate-based enteral formula that utilizes tilapia FPH addresses clinical nutritional requirements and advances sustainable protein innovation. But even with encouraging in vitro and animal results, the majority of peptide-based enteral nutrition products still on the market use whey or casein hydrolysates instead of protein sources obtained from fish.

As of 2024, there were still significant research gaps. Several controlled clinical trials, standardized ingredient characterization, and safety and allergenicity data in human populations have not directly assessed the efficacy of O. niloticus FPHs as primary protein sources in enteral nutrition, despite their good techno-functional and biological qualities (Honrado *et al*., 2024). The shift from laboratory-scale innovation to clinical-grade enteral medications has also been hampered by regulatory approval procedures, formulation stability, and sensory attributes (fishy odor, bitterness).

By addressing these gaps through systematic clinical testing, formulation enhancement, and regulatory alignment, tilapia-derived FPH must be recognized as a sustainable and practical protein source in medical nutrition. This research aims to develop and assess a fish protein hydrolysate-based enteral formula that comprises tilapia fish protein hydrolysate, emphasizing its nutritional makeup, functional characteristics, reconstitution performance, and possible clinical use. The results are anticipated to enhance the growing field of functional medical nutrition while encouraging sustainable use of aquatic resources.

MATERIALS AND METHODS

A. Procurement of Sample

Fresh tilapia fillets were purchased from the markets in Ernakulam and transported to the laboratory in containers with an ice pack to maintain a temperature of 4°C. Fresh tilapia fillets were minced and stored at -18°C to -20°C. The tilapia fillet mince was used immediately within 24 hours after freezing at -18°C before processing to maintain its quality. Before use, the fresh minced tilapia was thawed (kept in a refrigerator at 4°C for approximately 24 hours) and washed by rinsing with tap water.

B. Enzymatic preparation of fish protein hydrolysate (FPH) from Tilapia fish fillet

Fish protein hydrolysate was prepared from tilapia using flavourzyme according to the procedure developed with slight modification (Elavarasan *et al*., 2014). The frozen mince was thawed at 4 ± 2°C and used for the preparation of the hydrolysate. The fish mince was mixed with water in a 1:4 ratio [fish mince: water] (Jamshidi *et al*., 2018), and it was blended for 3 minutes using a mechanical homogenizer to obtain a slurry.

The mixture was subjected to 85°C for 30 min to achieve complete inactivation of endogenous enzymes present in the substrate. Further, the temperature was lowered, and the following conditions were maintained for hydrolysis, *i.e.*, temperature at 50°C, pH 6.5 ± 0.2, and time of hydrolysis for 90 minutes, and the addition of 1% enzyme initiated the reaction. After incubation, the slurry was heated at 90°C for 15 min, then centrifuged at 7,500 g for 15 min at 4°C. The supernatant was subjected to a freeze-drying process to get FPH. The obtained freeze-dried fish protein hydrolysate was stored at a temperature of -21°C (Elavarasan *et al*., 2014).

C. Standardisation and development of fish protein hydrolysate-based enteral formula

(i) Development of fish protein hydrolysate-based enteral formula. The freeze-dried fish protein hydrolysate is mixed homogenously with the selected ingredients: cocoa powder, xanthan gum, ascorbic acid, soy lecithin, potato starch, maltodextrin, milk powder, powdered sugar, salt, and pure medium-chain triglycerides (MCT) to obtain the fish protein hydrolysate-based enteral formula.

(ii) Standardization of fish protein hydrolysate-based enteral formula. To develop the fish protein hydrolysate-based enteral formula, the freeze-dried fish protein hydrolysate is combined with specific components such as pure medium-chain triglycerides (MCT), milk powder, powdered sugar, ascorbic acid, soy lecithin, potato starch, maltodextrin, xanthan gum, cocoa powder, and salt. The product was standardized by incorporating different proportions of fish protein hydrolysate into the above ingredients and was selected based on the sensory score.

D. Sensory analysis

The fish protein hydrolysate-based enteral formula was evaluated using 9-hedonic scales, with 20 panelists as judges (Viriyajaree, 1992). The acceptability of ready-to-reconstitute fish-based enteral formula at various concentrations of tilapia fish protein hydrolysate (5%, 10%, 15%, 20%, 25%, and 30%). They record their preference for color, fishy odor, fishy flavor, sweetness, bitterness, and overall liking.

The 9-point Hedonic Scale is a subjective sensory evaluation tool that panelists or consumers use to gauge how much they like or dislike a product. In addition to measuring individual sensory qualities like color, scent, taste, texture, and appearance, it also gauges general acceptability. By using this scale, researchers may quantitatively evaluate customer preferences and determine which sensory characteristics have the biggest effects on product adoption.

E. Proximate analysis of fish protein hydrolysate-based enteral formula

The methods of the Association of Official Analytical Chemists (AOAC, 2005) were used for the determination of moisture, ash, protein, and fat content of the fish protein hydrolysate-based enteral formula. All the determinations were done in duplicate. 5 g, each in duplicate, was used for the determination of moisture content by weighing in a crucible and drying in the oven at 105℃ until a constant weight was obtained. Determination of ash content was done by ashing at 550℃ for about 3 hours. The Kjeldahl method (AOAC, 2005) was used to determine the protein content by multiplying the nitrogen value by a conversion factor of 6.25. The lipid was extracted by the Soxhlet extraction method (AOAC, 2005).

F. Amino acid composition of fish protein hydrolysate-based enteral formula

Amino acid profiling of the fish protein hydrolysate-based enteral formula was performed using LC-MS/MS Waters Acquity UPLC H class combined with TQD MS/MS (USA) according to the procedure of Nimbalkar *et al*. (2012).

G. Physical parameters of fish protein hydrolysate-based enteral formula

The color was measured with a Hunter Lab CT-1100 Colour QUEST reflectance colorimeter (USA). An Aqua Lab water activity meter was used to assess the water activity level. The viscosity was determined using a Brookfield viscometer with appropriate spindles based on the slurry consistency and formulation procedures. The pH was measured using a pH meter that had previously been calibrated to three points.

H. Functional parameters of fish protein hydrolysate-based enteral formula

The oil-holding capacity and water-holding capacity of the fish protein hydrolysate-based enteral formula were determined according to Foh *et al*. (2010). The foaming capacity and foaming stability of the fish protein hydrolysate-based enteral formula were determined according to the method of Parvathy *et al*. (2018). The bulk density and tapped density were determined by the method described by Jangam and Thorat (2010). The nitrogen solubility of the fish protein hydrolysate-based enteral formula's determination was conducted based on the method described by Moon & Cho (2023).

I. Biochemical parameters of fish protein hydrolysate-based enteral formula

PV and FFA were estimated by the American Oil Chemists' Society method (Connell, 1975). The Conway microdiffusion method (Conway, 1947) was implemented for estimating TVBN and TMA, and the thiobarbituric acid reactive substance of the fish protein hydrolysate-based enteral formula was estimated according to the method developed by Tarladgis *et al*. (1960).

J. Microbiological parameters of fish protein hydrolysate-based enteral formula

Microbiological parameters of the fish protein hydrolysate-based enteral formula were determined by following the procedure of the FDA (2011).

K. Statistical Analysis

The mean value and standard deviation were calculated from the data obtained from proximate parameters, physical parameters, functional parameters, and biochemical parameters.

RESULTS AND DISCUSSION

A. Development and standardisation of fish protein hydrolysate-based enteral formula

The development of a fish protein hydrolysate-based enteral formula involved eleven ingredients, including freeze-dried tilapia protein hydrolysate as the main protein source. The dry ingredients included maltodextrin, whole milk powder, powdered sugar, potato starch, salt, medium-chain triglyceride, cocoa, xanthan gum, soy lecithin, and ascorbic acid. These ingredients were combined to create a homogeneous powdered mixture and transferred into metallized low-density polyethylene packaging. A control was also prepared without freeze-dried tilapia protein hydrolysate and stored for supplement development.

Using the 9-point Hedonic Scale sensory evaluation method, which was done by a panel of 20 judges, the above ingredients were standardized with tilapia fish protein hydrolysate in various ratios, namely 5%, 10%, 15%, 20%, 25%, and 30%, to develop the ready-to-reconstitute fish-based enteral formula. The ratio of ingredients for the ready-to-reconstitute fish-based enteral formula that was chosen based on organoleptic assessment is shown in Table 1, and the selected ready-to-reconstitute fish-based enteral formula ingredient ratio was highlighted in Table 1 and was finalized based on organoleptic evaluation.

Table 1: Standardised ingredients for the development of a fish protein hydrolysate-based enteral formula, and the selected ratio are highlighted.

Sr. No.	TPH	Malt Dextrin	Whole Milk Powder	Sugar Powder	Cocoa Powder	Xanthan Gum	Ascorbic Acid	Salt	Soy Lecithin	MCT	Potato Starch
1.	0	30	25	17	5	0.1	1	0.5	0.05	10	1.35
2.	**5**	**25**	**25**	**17**	**5**	**0.1**	**1**	**0.5**	**0.05**	**10**	**1.35**
3.	10	20	25	17	5	0.1	1	0.5	0.05	10	1.35
4.	15	15	25	17	5	0.1	1	0.5	0.05	10	1.35
5.	20	10	25	17	5	0.1	1	0.5	0.05	10	1.35
6.	25	5	25	17	5	0.1	1	0.5	0.05	10	1.35
7.	30	0	25	17	5	0.1	1	0.5	0.05	10	1.35

B. Sensory analysis of fish protein hydrolysate-based enteral formula

Twenty panelists judged the organoleptic evaluation of Fish Protein Hydrolysate-Based Enteral Formula at various concentrations of tilapia fish protein hydrolysate (5%, 10%, 15%, 20%, 25%, and 30%), shown in Fig. 1 & 2. The fish protein hydrolysate-based enteral formula developed with a 5% tilapia fish protein hydrolysate incorporation level was found to have a higher acceptability level than others. Based on the requirement, the FPH was incorporated at different ratios, and the final product was chosen based on organoleptic evaluation.

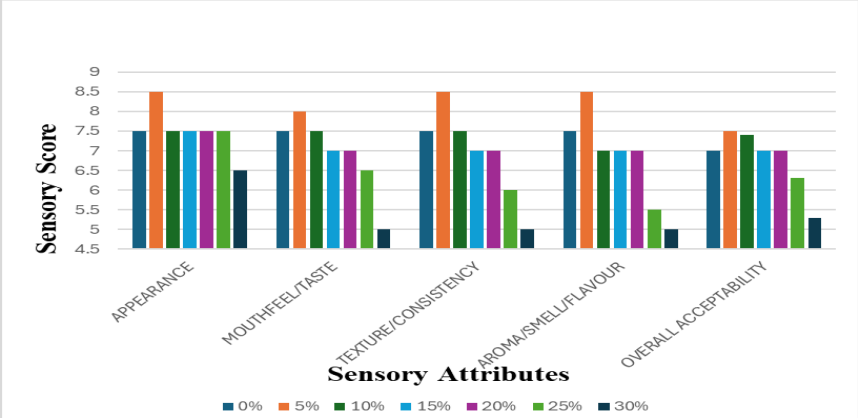

Fig. 1. Sensory scores obtained for fish protein hydrolysate-based enteral formula with different ratios of tilapia protein hydrolysate.

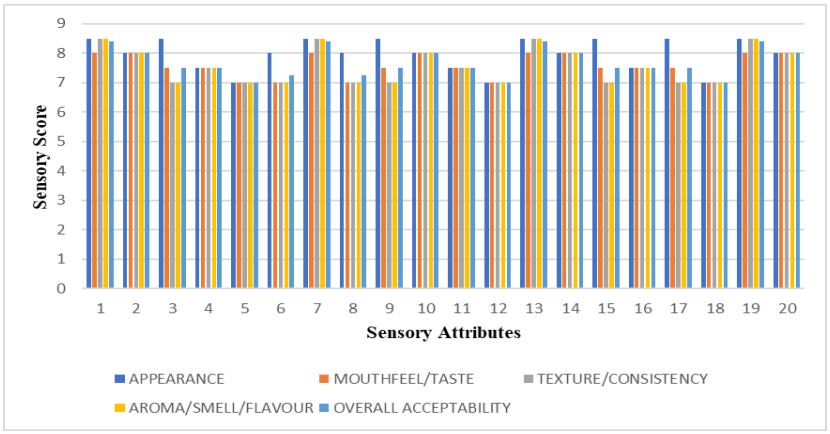

Fig. 2. Sensory evaluation with 20 panellists was conducted for the fish protein hydrolysate-based enteral formula with 5% tilapia protein hydrolysate.

C. Proximate analysis of fish protein hydrolysate-based enteral formula

The proximate composition of the fish protein hydrolysate-based enteral formula without fish protein hydrolysate and with fish protein hydrolysate, *i.e.*, 5% tilapia protein hydrolysate, was evaluated, and the results are presented in Table 2. The observation showed that all the proximate constituents were almost the same in both samples, except in the case of protein and lipid. There was a higher protein content in the fish protein hydrolysate-based enteral formula added with 5% tilapia protein hydrolysate (15.48±0.03%) than without tilapia protein hydrolysate (5.35±0.03%). In the case of lipids, there is only a mild difference; the formula with fish protein hydrolysate is 7.1±1.09%, and the formula without fish protein hydrolysate is 4.94±0.12%.

Table 2: Comparison of proximate constituents of the fish protein hydrolysate-based enteral formula with and without fish protein hydrolysate.

Proximate Constituents	Formula with fish protein hydrolysate	Formula without fish protein hydrolysate
Protein (%)	15.48±0.03	5.35±0.03
Fat (%)	7.1±1.09	4.94±0.12
Ash (%)	3.83±0	3.637±0.03
Moisture (%)	2.44±0.02	2±0

The protein content of around 15.5% indicates that the formula provides a good source of high-quality protein, which is especially significant in clinical and nutritional applications such as enteral feeding. Fish protein hydrolysates have a balanced amino acid profile and are highly digestible, which is important for individuals with limited digestion or elevated protein requirements

(Kristinsson & Rasco 2000). This protein content promotes muscle maintenance and general nutritional health, making it appropriate for nutritional supplements and recovery assistance in therapeutic settings.

The 7.1% fat content greatly increases the caloric density of the formula, ensuring that energy requirements are met even with a moderate volume of ingestion. Fat not only increases energy value, but it also aids in the absorption of fat-soluble vitamins and supplies important fatty acids required for various metabolic activities. The observed variability (±1.09%) may be due to modest changes in the formulation or processing; however, it stays within acceptable limits for a specialized nutritional product.

The term "ash" refers to the formula's overall mineral content. A 3.83% rating indicates that the formula contains a significant amount of minerals, which are required for electrolyte balance, bone health, and overall metabolic function. The lack of change in ash content suggests a consistent formulation process.

A low moisture level of 2.44% improves the formula's shelf durability and microbiological safety. Reduced moisture reduces the risk of microbiological development and chemical deterioration, extending the product's shelf life while maintaining nutritional quality. This low moisture content is especially significant in powdered formulations, where water activity must be managed to avoid spoiling (Karel and Heidelbaugh 1973).

D. Amino acid composition of fish protein hydrolysate-based enteral formula

The amino acid composition of the fish protein hydrolysate-based enteral formula reveals essential information about its nutritional value, functioning, and possible bioactivity. The profile in Table 3 shows a balance of essential and non-essential amino acids, which is critical for protein synthesis, tissue repair, and metabolic function.

Table 3: Amino acid profiling of the fish protein hydrolysate-based enteral formula.

Amino Acids (mg/100 gm)	Values
Glycine	0.03
Alanine	12.79
Serine	19.41
Proline	39.13
Valine	9.46
Threonine	5.65
Cysteine	0.71
Leucine	31.92
Asparagine	1.62
Aspartic acid	14.33
Lysine	15.56
Glutamic acid	52.98
Methionine	15.64
Histidine	5.94
Methionine	0.03
Phenylalanine	12.03
Arginine	10.44
Citrulline	1.09
Tyrosine	8.82
Beta 3-4 dihydroxyphenylalanine	0.03
Tryptophan	0.07

Glutamic acid is commonly found in fish proteins, contributing not only to nutritional value but also to umami flavor, which can improve palatability (Wu, 2009). Proline (39.13 mg/100 g) and alanine (12.79 mg/100 g) are necessary for collagen production and energy metabolism. Their presence contributes to the structural and functional integrity of tissues. The formula contains a reasonable amount of essential amino acids, including leucine (31.92 mg/100 g) and lysine (15.56 mg/100 g).

Hydrolyzed fish proteins generally exhibit high digestibility and absorption rates, which makes them an excellent protein source for clinical nutrition, sports supplements, and functional foods. The presence of a broad range of amino acids enhances the nutritional completeness of the formula (Kristinsson & Rasco 2000). Amino acids like glutamic acid contribute to flavor enhancement, while others, such as proline and alanine, can affect the texture and solubility of protein formulations. These properties are essential in determining the acceptability and performance of protein powders in various applications (Damodaran, 1997). The dual listing of phenylalanine (12.03 vs. 0.03 mg/100 g) and very low levels of glycine and tryptophan warrant further investigation.

E. Physical parameters of fish protein hydrolysate-based enteral formula

The physical properties of food products are key indicators of quality, consistency, and consumer acceptance (Pinela *et al*., 2022). Table 4 shows the results for the fish protein hydrolysate-based enteral formula, including color, pH, water activity, and viscosity, which provide information on its quality and shelf life.

Table 4: Physical parameters of the fish protein hydrolysate-based enteral formula.

Physical Parameters	Values
Colour: -	
L*	59.84±0.01
a*	8.84±0.02
b*	12.84±0.12
pH (%)	6.55±0.01
Water activity (a_w)	0.37±0.01
Viscosity (CP)	6±0

The color measurement of the fish protein hydrolysate-based enteral formula results in L* 59.84±0.01, a* 8.84±0.02, and b* 12.84±0.12. A lightness value of roughly 60 implies that the product is reasonably light. In many culinary products, this score indicates adequate brightness and consistency. Consistent L* readings indicate that ingredients are processed and mixed uniformly (Pathare *et al*., 2013).

The a* value is positive, indicating a faint red hue. This could be useful in products that require a warm or natural tint. Small variations in a* are frequently linked to the raw material used and the presence of natural pigments (Mortazavi *et al*., 2023). The b* value represents the yellow component and is moderate. The combined a* and b* ratings indicate that the product has a balanced color that customers may find natural

and appealing. The color profile (L*, a*, b*) confirms that the product meets its expected visual characteristics, which is critical for consumer acceptance (Pathare *et al*., 2013).

The pH measurement of the fish protein hydrolysate-based enteral formula results in 6.55±0.01%. The pH close to neutral (6.55) indicates that the product is neither too acidic nor too alkaline. This pH range is typical for many food products, especially those based on protein powders or dairy, and can affect both flavor and microbial stability. A near-neutral pH is also beneficial for maintaining the stability of bioactive compounds and ensuring compatibility with packaging materials (Fellows, 2022).

The water activity (a_w) is an important factor regulating microbial development and shelf life. The water activity of the fish protein hydrolysate-based enteral formula was 0.37±0.01. An a_w value of 0.37 is low, which is beneficial because it inhibits the growth of most bacteria, yeasts, and molds. Low water activity is especially significant in dried products and powders, which contribute to longer shelf life and stability (Barbosa-Cánovas *et al*., 2020). This figure also indicates that the product has been thoroughly dried and/or contains humectants, which bind water.

The viscosity of the fish protein hydrolysate-based enteral formula was 6±0. Low viscosity is desirable in reconstituted powders or liquid formulations because it makes handling, mixing, and administration easier. Low viscosity also implies that the product can be easily swallowed or pumped through feeding systems without clogging or requiring excessive energy during processing (Seville *et al*., 2007).

F. Functional parameters of fish protein hydrolysate-based enteral formula

The functional properties of the fish protein hydrolysate-based enteral formula are key determinants (Rana *et al*., 2023; Saidi *et al*., 2018) of their performance in food formulations and end-use applications. The following parameters—water holding capacity, oil holding capacity, foam capacity, foam stability, bulk density, tapped density, and protein solubility—result in the values shown in Table 5.

Table 5: Functional parameters of the fish protein hydrolysate-based enteral formula.

Functional Properties	Values
Water Holding Capacity (WHC) (ml water/gm sample)	3.54±0.12
Oil Holding Capacity (OHC) (ml oil/gm sample)	1.14±0.05
Foam Capacity (FC) (%)	63.76±0.02
Foam Stability (FS) (%)	68.12±0.02
Bulk Density (BD) (gm/dl)	0.44±0.05
Tapped Density (TD) (gm/dl)	0.54±0.05
Protein Solubility (NS) (%)	83.96±0.05

The fish protein hydrolysate-based enteral formula has a water holding capacity (WHC) of 3.54 ± 0.12 ml/g sample, indicating moderate water absorption and retention. High WHC is beneficial in food systems where moisture retention influences texture, juiciness, and overall mouthfeel (Kinsella & Melachouris 1976; Damodaran, 1997). In many formulations, a WHC in this range indicates that the powder can keep hydration during processing and storage, which is advantageous for products such as baked goods, meat analogues, or reconstituted beverages.

The OHC of 1.14 ± 0.05 ml oil/g sample indicates the fish protein hydrolysate-based enteral formula's capacity to bind lipids. This capacity is essential for flavor preservation and improving the texture of emulsified products (Zamora-Sillero *et al*., 2018). A modest OHC value might be useful in formulations like dressings and spreads, where fat retention is critical for sensory qualities. The observed result suggests that the protein matrix has a balanced hydrophobic-hydrophilic profile, allowing fat incorporation without significant oil separation.

The fish protein hydrolysate-based enteral formula has a foam capacity (FC) of 63.76 ± 0.02% and foam stability (FS) of 68.12 ± 0.02%. A high FC indicates that proteins can quickly adsorb at the air-water interface, generating a large foam (Kinsella & Melachouris 1976).

The FS value measures the foam's capacity to resist collapse over time, which is critical for retaining texture and consistency during processing and storage (Damodaran, 1997). These results imply that the protein powder is good at creating and maintaining foams, making it appropriate for formulations that require lightness and aeration.

The bulk density of the fish protein hydrolysate-based enteral formula was 0.44 ± 0.05 g/dl. This statistic represents the mass of the powder per unit volume when loosely packed. A lower bulk density is frequently associated with powders that are more aerated or have a wider particle size distribution. Tapped density of the fish protein hydrolysate-based enteral formula was 0.54 ± 0.05 g/dL. It is determined after mechanically compacting the powder, represents the potential for volume reduction, and is critical for packaging and storage efficiency (Saw *et al.*, 2013). The difference in bulk and tapped density measures powder flowability and compressibility. In this situation, the moderate values indicate that the protein powder is rather free-flowing, which is ideal for consistent mixing and handling in industrial applications.

The fish protein hydrolysate-based enteral formula's high protein solubility (83.96 ± 0.05%) makes it ideal for protein supplements and beverage mixes. Proteins with high solubility are easily disseminated and digested, allowing for greater nutritional and physiological benefits (Damodaran, 1997). This amount of solubility also indicates that the protein has been properly processed, such as by spray drying or

enzymatic hydrolysis, to produce a product that dissolves well in aqueous systems.

G. Biochemical parameters of fish protein hydrolysate-based enteral formula

To determine the quality of a fish protein hydrolysate-based enteral formula, the study evaluates key oxidative and protein stability parameters such as peroxide value (PV), free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS), total volatile bases nitrogen (TVB-N), and trimethylamine (TMA). Table 6 shows the key parameters of fish protein hydrolysate-based enteral formulations, which are important indications of protein powder freshness and consistency.

Table 6: Biochemical parameters of the fish protein hydrolysate-based enteral formula.

Biochemical Parameters	Values
Peroxide Value (%)	0.45 0.02
Free Fatty Acid Value (FFA) (%)	1.02±0.02
Thiobarbituric acid reactive substance (TBARS) (mg%)	0.07±0
Total Volatile Bases (TVB-N) (mg N/100 gm)	7.34±0.15
Trimethylamine (TMA) (mg N/100 gm)	1.24±0.12

Lipid oxidation, protein degradation, and microbial activity all have an impact on their quality, stability, and shelf life (Shahidi & Zhong 2010). Lipid oxidation, protein breakdown, and microbiological safety all contribute to the stability and quality of these formulations. Lipid oxidation causes rancidity, off-flavors, and decreased nutritional value, whereas protein degradation can impair digestibility and nitrogen retention (Khalid *et al*., 2023). These formulations' stability and quality are determined by lipid oxidation, protein degradation, and microbiological safety (Sharma *et al*., 2021).

The peroxide value represents the amount of primary oxidation products (*i.e.*, hydroperoxides) produced in lipids. A PV of 0.45% indicates that there is minimal oxidative degradation. This is especially important for products that contain polyunsaturated fatty acids (PUFAs), which are easily oxidized. Research suggests that keeping PV values below 5 meq O_2/kg is ideal for product freshness and stability (Shahidi & Zhong 2010). In this case, the low PV indicates effective processing conditions, which are most likely aided by the use of antioxidants or oxygen-reducing packaging strategies.

The FFA value is a measure of hydrolytic rancidity, which is the degradation of triglycerides into free fatty acids. An FFA value of roughly 1.0% is acceptable for many food and nutraceutical products (Frankel, 1980). Elevated FFA levels can have an impact on flavor, stability, and nutritional quality. The low FFA reported here indicates that lipolytic enzyme activity and moisture-induced hydrolysis were adequately regulated during processing and storage, preserving product quality (O'Keefe & Pike 2010).

TBARS is a measurement of secondary lipid oxidation products, specifically malondialdehyde (MDA), which contribute to off-flavors and rancidity. A TBARS value of 0.07 mg% is extremely low, implying that secondary oxidation is insignificant. High-quality products often have acceptable TBARS readings of less than 1.0 mg MDA/kg (Piranavatharsan *et al*., 2023). The low TBARS level supports the conclusion that the formulation and storage conditions effectively slowed the advancement of lipid oxidation beyond its initial stages.

TVB-N assesses volatile nitrogenous chemicals produced by protein degradation, such as ammonia and amines. Many protein-rich goods have TVB-N values < 20 mg N/100 g, indicating freshness. The reported value of 7.34 mg N/100 g falls well within safe limits. implying negligible microbial spoilage or enzymatic protein degradation. This low TVB-N measurement demonstrates that the product's protein fraction is effectively preserved (Sarkar *et al*., 2020).

TMA is one of the volatile amines produced by the microbial decomposition of trimethylamine oxide (TMAO), particularly in marine-derived goods. A TMA value of 1.24 mg N/100 g is within acceptable limits for assuring product freshness. Values below 5 mg N/100 g are generally considered acceptable (Hultmann & Rustad 2004). The low TMA level contributes to the product's overall microbiological and chemical stability.

H. Microbiological parameters of fish protein hydrolysate-based enteral formula

The Total Plate Count (TPC) and Fungal Count (Yeast & Mold) serve as key indicators of microbial contamination, hygiene control, and product stability. The microbiological parameters of fish protein hydrolysate-based enteral formula, shown in Table 7, have a low microbial load, which assures that they are safe to be consumed by critically ill patients, reducing infection risks.

Table 7: Microbiological parameters of the fish protein hydrolysate-based enteral formula.

Microbiological Parameters	Values
Total plate count	0.3×10^3 cfu/g
Fungal count: Yeast & Mould	0.1×10^1 cfu/g

The Total Plate Count (TPC) of 0.3×10^3 CFU/g (300 CFU/g) suggests a low bacterial burden in the sample. This level is generally deemed appropriate for a wide range of food products, particularly those that are little processed or ready-to-eat. Food safety guidelines suggest that a TPC of less than 10^3 CFU/g indicates acceptable hygiene and effective processing controls (Canton, 2021). The low TPC indicates that the raw materials and processing environments were properly handled, lowering the risk of spoiling and foodborne illness.

The fungal value of 0.1×10^1 CFU/g (about 1 CFU/g) indicates minimal fungal contamination. Yeast and mold count in food products are crucial for determining spoilage and safety, especially in products where moisture and nutrient availability may promote fungal development. According to regulatory rules and research, fungus counts of less than 10^2 CFU/g are safe for many food products (Jacxsens *et al*., 2009). In this

scenario, the extremely low count indicates that the product was processed and stored in settings that minimize fungal multiplication.

CONCLUSIONS

Fish protein hydrolysate is an abundant source of protein that may be used to make powdered fish protein supplements with enhanced value. The hydrolyzed protein may be successfully extracted from fish flesh using the flavorzyme extraction process. With good ratings for consumer approval, the resulting fish protein hydrolysate can be utilized as a useful component in protein supplements for critical care. The pharmaceutical business may employ FPH from fish fillet flesh for more research and also to expand the utilization of extremely valuable fish protein hydrolysates. Comparing the protein supplement powder made from tilapia fillet flesh with other commercial protein supplements on the market should be the subject of future research.

FUTURE SCOPE

The study's future scope highlights a number of crucial avenues to improve the suitability of enteral formulations based on fish protein hydrolysate (FPH). Assessing digestibility, bioavailability, safety, and therapeutic effects in patients requires clinical evaluation through in vivo and clinical trials. Furthermore, to assure microbiological safety, nutritional retention, and sensory consistency throughout storage, thorough shelf life and stability studies—both expedited and real-time—are necessary. The functional bioactivity of FPH, in particular its immunomodulatory, antihypertensive, and antioxidant qualities, should also be further studied.

Clinical applicability could be increased by diversifying formulations for particular patient groups, such as elderly, diabetic, renal, and oncology patients, using enteral formulas and tube-feeding solutions. More broadly, commercialization and integration into the medical nutrition industry depend on industrial-scale manufacturing. cost-benefit analysis, and regulatory validation. Finally, to support circular economy principles in the food and healthcare sectors, future research should concentrate on sustainability by valuing fish by-products and underutilized marine resources.

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