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Antioxidant Potential of Bioactive Peptides Derived from Fish Waste: A Focus on *Catla catla* Liver and Intestinal Tissue

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Abstract: Fish waste, if not managed properly, poses a significant environmental threat. Scientists worldwide have been exploring innovative ways to utilize this resource, finding applications in pharmaceuticals and nutraceuticals. One promising avenue is isolating bioactive peptides from *Catla catla* fish waste and assessing their antioxidant potential. Using Papain digestion, fractions were obtained from *Catla catla* liver waste, showing significant antioxidant activity, especially those with molecular weights of 10-100 kDa. These fractions, derived from 2% Papain digestion for 180 minutes, displayed the highest DPPH and ABTS scavenging activity. They hold promise for further investigation as potential anticarcinogenic agents. This study highlights the potential of fish waste, particularly from *Catla catla* liver, as a source of bioactive peptides with antioxidant properties. Further research into these fractions could lead to the development of nutraceuticals with such antioxidant peptides.

Introduction

Fish is a primary source of protein globally, leading to the generation of significant fishery waste in various forms, such as frames, scales, fins, viscera, gills and heads. While some of this waste is utilized for low-value market products like fish oil, fish feed and organic manure, a considerable amount is disposed of, contributing to environmental pollution (Saha et al., 2017; Coppola et al., 2021; Mondal et al., 2022). Scientists worldwide have been exploring novel methods to utilize this waste in recent years effectively. Through processes like hydrolysis using acid, alkali, or enzymes, fish protein hydrolysates (FPH) are produced, from which bioactive peptides are isolated and investigated for their potential roles in various health applications, including anticancer, antimicrobial, antidiabetic, antihypertensive, antioxidant and immunomodulatory activities (Ese-roghene and Ikechukwu, 2018; Behera et al., 2022; Idowu et al., 2021; Moreira et al., 2023; Tambunan et al., 2024). This innovative approach has led to the integration of fish waste into pharmaceuticals and nutraceuticals, benefiting humanity.

Fish protein hydrolysates (FPHs) are rich sources of bioactive peptides (BAPs), comprising small peptides containing 2-20 amino acids obtained through controlled hydrolysis (Ortizo et al., 2023). These peptides exhibit diverse bioactivities, making them valuable for developing nutraceuticals and supplements for human and animal consumption (Awuchi et al., 2022). Notably, research has unveiled the anticarcinogenic and antioxidant properties of peptides derived from fish waste, particularly non-edible parts like frames, bones, scales, and viscera. Studies indicate that the antioxidant activity of BAPs is influenced by amino acid

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composition, sequence, and hydrophobicity, with amino acids like tyrosine and histidine playing key roles. Carp, among other fish species, stands out as a significant source of BAPs, contributing to the exploration of their health-promoting properties (Jafar et al., 2024).

In our recent study, we aimed to optimize the enzyme hydrolysis process to extract fish hydrolysates from *Catla catla* intestine and liver tissues, typically discarded as visceral waste. Subsequently, we isolated smaller peptides from these hydrolysates using molecular weight cut-off centrifuge tubes, resulting in peptides ranging between 10 - 100 kDa and those <10 kDa. We evaluated the antioxidant properties of the fractions obtained from the intestinal and liver tissues of *Catla catla* using various antioxidant assays. This research contributes to the ongoing efforts to unlock the potential of fish waste, transforming it into valuable bioactive compounds with promising health benefits.

Materials and Methods

Raw Materials

Intact *Catla catla* specimens were obtained from the local market of Baripada, Odisha. Upon collection, they were promptly placed in an icebox and transported to the laboratory within 30 minutes. Subsequently, the fish underwent washing with both tap water and distilled water before being stored in sealed plastic bags at -20°C.

Collection of Visceral Waste

The fish were longitudinally dissected using a knife and scissors to extract the entire viscera, and the liver was also separated. After dissection, the intestine and liver tissues were meticulously washed under running tap water, followed by a rinse with distilled water. The cleaned tissues were then stored at -20°C.

Preparation of Fish Waste Mince (FWM)

To prepare the mince, the fish viscera were thawed and left at room temperature for 30 to 60 minutes, followed by washing with distilled water. After washing, the intestine and liver waste were minced without the addition of water, using a Hariom meat mincer (Hariom - 1200), resulting in a homogenate. This homogenate was stored at -20°C until further use.

Chemicals Used

Papain, Folin-Ciocalteau, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trichloroacetic Acid, and Bovine Serum Albumin (BSA) were procured from Hi-Media Laboratories, Mumbai, India. Sodium Hydroxide, Sodium Carbonate, Copper Sulphate, Ethanol and Ferrous Sulphate were procured from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India. All reagents used were of analytical grade.

Preparation of Fish Protein Hydrolysates with Papain (FPH)

Enzymatic Hydrolysis

For enzymatic hydrolysis, the intestine and liver of *Catla catla* were mixed separately with distilled water (2:100 w/v) and were adjusted to pH and temperature (as suggested by the manufacturer) for Papain (pH 7; 55°C). The 1% enzymatic reaction ratio of enzyme to sample was 1:100 (w/w) and 2 % enzymatic reaction ratio of enzyme to sample was 2:100 (w/w). The incubation time intervals were 30, 60, 120, and 180 minutes. The enzymatic hydrolysis was stopped by heating at 90°C for 10 min. The hydrolysate was centrifuged at 10000 rpm for 20 min, and the supernatant was kept at 2-8°C for analysis. (Figure 1).

Isolation of Peptides (10-100kDa and < 10kDa)

Molecular weight cut-off centrifugation was performed using a Vivaspin Turbo 15 (VS 15T01, Sartorious) (Centrifugal Concentrator) with a 10kDa cut-off filter. Each hydrolysates was centrifuged at 4000 rpm for 10 minutes at 4°C. The fractions of 10 – 100kDa and fractions of <10kDa were collected from the centrifuge tubes in different vials and stored at 2° - 8°C for further use. Different fractions of both intestine and liver were labelled properly with specific code before storing (1: 1% papain; 2: 2% papain; A: Liver; B: Intestine; C: 30min; D: 60min; E: 120min and F: 180min) (Figure 1).

Determination of Degree of Hydrolysis

The degree of hydrolyzed (DH) protein was assessed following the method outlined by Hoyle & Merritt (1994), as detailed by Safari et al., (2012). Subsequent to the specified hydrolysis, 20% trichloroacetic acid (TCA) was introduced into each treatment to halt the reaction. Following centrifugation, the 20% TCA-soluble material was collected as the supernatant. The DH was then calculated using the formula:

$$DH = \frac{\text{Supernatant protein content}}{\text{Total protein content}} \times 100\%$$

Protein Estimation of Crude Protein, 10-100kDa, & < 10kDa Proteins

The Lowry method determined the protein concentration according to the procedure described by Gerhardt et al. (1994).

The antioxidant properties of the fractions obtained from visceral hydrolysates

DPPH Assay

The DPPH assay, a widely used method for evaluating antioxidant activity, was conducted following the

protocol of Baliyan et al. (2022). Initially, a stock solution of 0.075 mM DPPH was prepared by dissolving DPPH powder in 95% ethanol. For the reaction setup, 50µL of the peptide (whose antioxidant properties are to be evaluated) was added to 1950µL of the DPPH solution, and the mixture was thoroughly mixed. Following a 30-minute incubation in darkness, the absorbance of the solution was measured at 517 nm using a UV/Visible Spectrophotometer, with 95% ethanol serving as a blank for baseline correction.

The DPPH scavenging activity was calculated using the formula

Scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$

A_0 is the absorbance of the control and A1 is the absorbance of the sample.

Blank correction involved subtracting the blank absorbance from both sample and control readings. A lower absorbance indicated higher DPPH scavenging activity, providing insights into the antioxidant potential of the tested compounds. This DPPH assay protocol offers a straightforward and effective approach for assessing free radical-scavenging capabilities.

ABTS Assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) antioxidant assay is a widely employed method for assessing the antioxidant activity of compounds and in this context, peptides obtained from papain digestion of intestine and liver tissue of *Catla catla* serve as the test samples (Re et al., 1999). In this assay, a 7 mM ABTS stock solution is initially prepared and activated by mixing it with 2.45 mM potassium persulfate. The resulting reaction mixture is left to stand in the dark for 12-16 hours at room temperature. After activation, the solution is diluted with an appropriate buffer to achieve an absorbance of approximately 0.70 at 734 nm. Subsequently, the activated ABTS solution is combined with the test samples, and the reduction in absorbance is monitored spectrophotometrically at the designated wavelength using a UV-Visible Spectrophotometer. The percentage inhibition of ABTS radicals by the peptides derived from papain digestion is then calculated, providing a quantitative measure of their antioxidant activity. This method allows for a precise evaluation of the free radical-scavenging potential of the peptides from *Catla catla* intestine and liver tissue under investigation.

Scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$

A_0 is the absorbance of the control and A1 is the absorbance of the sample.

Statistical Analysis

Data were analyzed using one-way ANOVA to compare the FPH obtained from 1 % and 2% of enzyme digestion at different time intervals in both intestine and liver tissue. ANOVA was again used to compare the mean of scavenging activity of both 1% and 2% digested fractions obtained for different time period of enzyme incubation in both the tissues. A correlation test was used to observe the pattern of scavenging activity of different tissue fractions using SPSS 20.0 software. The level of statistical significance was defined as $P < 0.05$, and all data were given as mean ± SD.

Results

Degree of Hydrolysis

The figure 2 provides insight into the impact of time invested in hydrolysis and enzyme concentration on the degree of hydrolysis and peptide content of *Catla catla* visceral protein hydrolysate obtained from intestine and liver samples. Samples from both (intestine and liver) were treated with 1% and 2% Papain for varying durations (30, 60, 120, and 180 minutes). The degree of hydrolysis, expressed as a percentage, increased with longer times of hydrolysis and higher enzyme concentrations. For instance, in the intestine samples, the degree of hydrolysis ranged from 9.12% to 41.25% with 2% Papain over the 180-minute duration, indicating greater protein breakdown compared to 1% Papain. Similarly, peptide content increased with increasing hydrolysis time and enzyme concentration. This data highlights the importance of enzyme concentration and hydrolysis time in optimizing the protein hydrolysis process for peptide production from the intestine and liver of *Catla catla*.

Table 1. Correlation Matrix of Enzyme Concentration, Time Taken for Hydrolysis, and Percentage of Hydrolysis in Intestine Samples *Catla catla*.

Intestine	Enzyme Concentration in percentage	Time Taken for Hydrolysis	Percentage of Hydrolysis
Enzyme Concentration in percentage	1		
Time Taken for Hydrolysis	0	1	
Percentage of Hydrolysis	0.289900314	0.929693028	1

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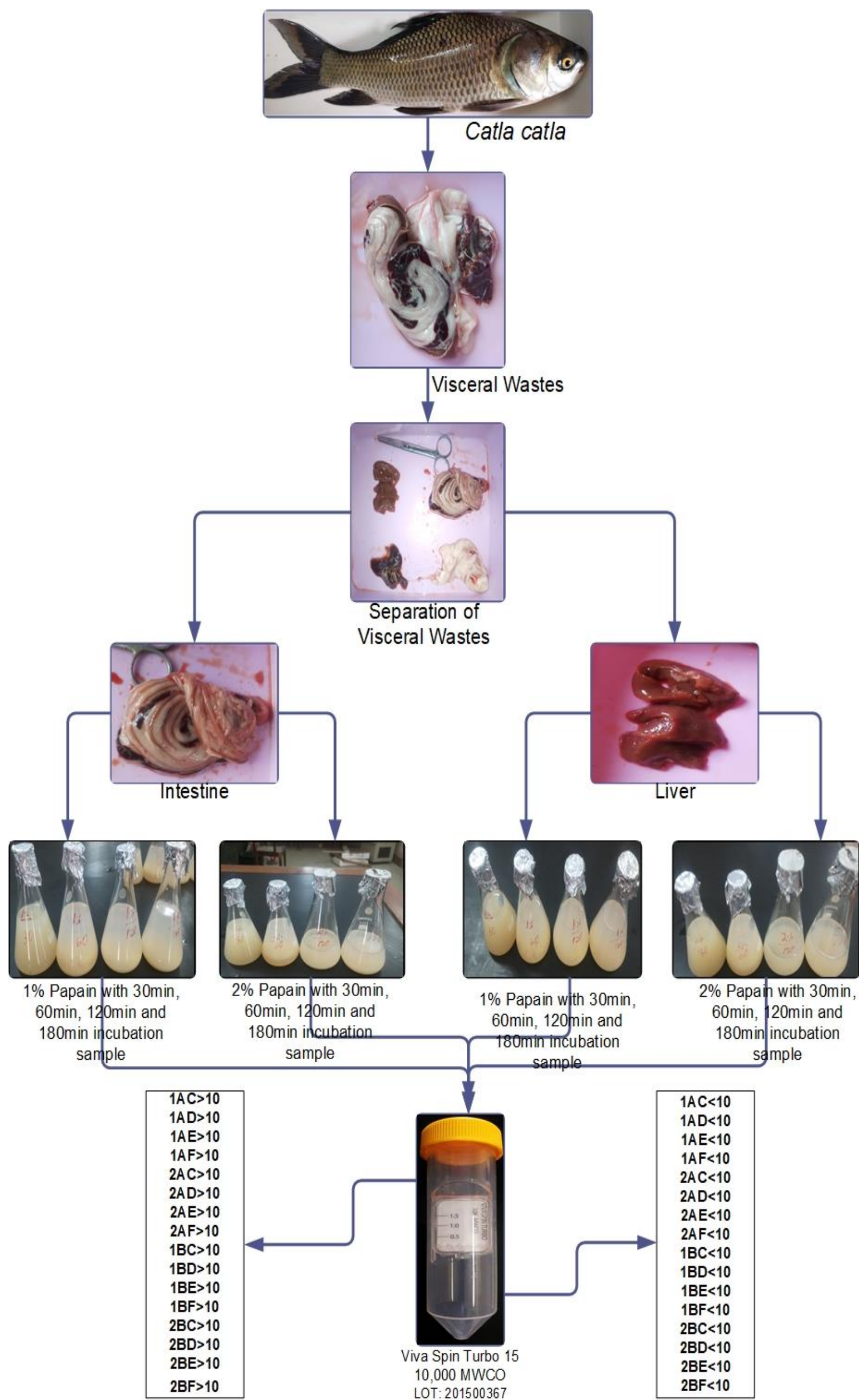

Figure 1. Steps of Enzyme hydrolysis followed by Molecular weight cut-off centrifugation.

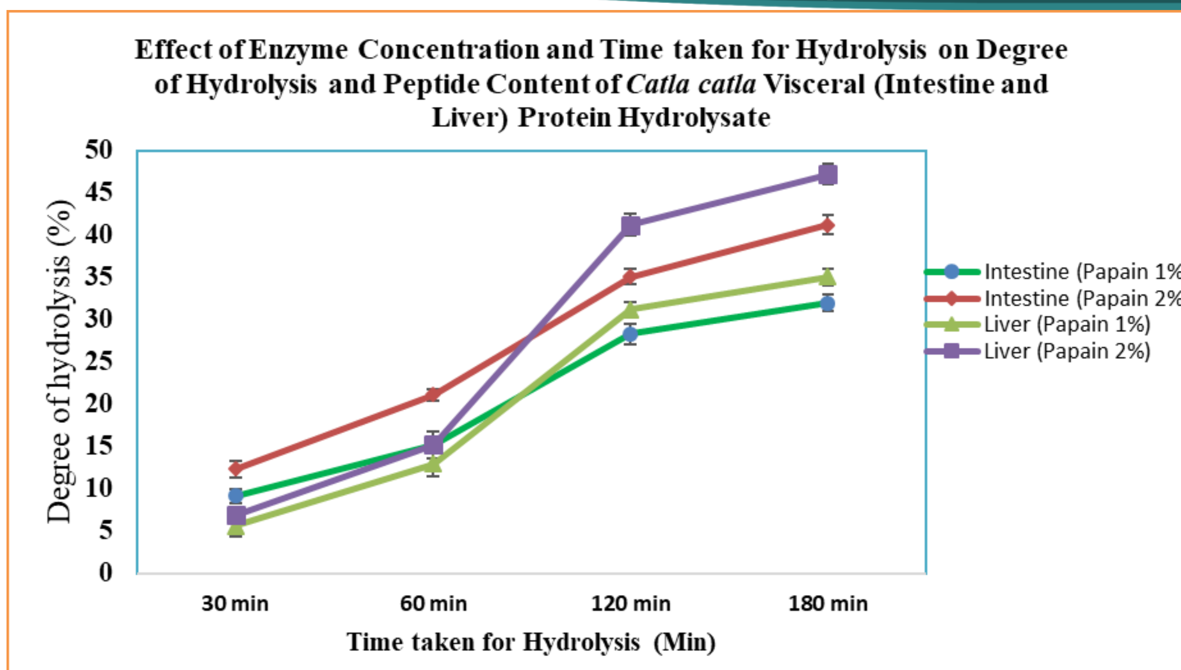

Figure 2. Effect of Time taken for Hydrolysis and Enzyme Concentration on Degree of Hydrolysis of *Catla catla* obtained from intestine and liver Protein Hydrolysate.

Table 2. Correlation Matrix of Enzyme Concentration, Time Taken for Hydrolysis, and Percentage of Hydrolysis in Liver Samples of *Catla catla*.

Liver	Enzyme Concentration in percentage	Time Taken for Hydrolysis	Percentage of Hydrolysis
Enzyme Concentration in percentage	1		
Time Taken for Hydrolysis	0	1	
Percentage of Hydrolysis	0.212268666	0.932859015	1

The correlation matrices Tables 1 and 2 highlight the results of "Intestine Samples" and "Liver Samples," unveiling key insights into the relationships among enzyme concentration, time taken for hydrolysis, and percentage of hydrolysis in respective contexts. In "Intestine Samples," a correlation coefficient of 0 suggests no significant linear relationship between enzyme concentration and hydrolysis duration, while a correlation of approximately 0.29 indicates a weak positive association between enzyme concentration and percentage of hydrolysis. However, the latter correlation is relatively feeble, implying the influence of other factors on hydrolysis extent. Conversely, a strong positive correlation coefficient of roughly 0.93 highlights a robust link between hydrolysis duration and percentage of substrate breakdown. In "Liver Samples," a correlation coefficient of 0 between enzyme concentration and hydrolysis duration implies minimal influence, while a weak positive correlation of about 0.21 suggests a slight association between enzyme concentration and percentage of hydrolysis. This correlation, though weak, may suggest potential effects of enzyme concentration on hydrolysis outcomes. Notably, both datasets demonstrate a strong positive correlation between hydrolysis duration and percentage of hydrolysis, emphasizing the significant impact of time on substrate breakdown. These findings underscore the distinct dynamics between enzyme concentration, hydrolysis duration, and substrate breakdown in intestinal and liver environments.

Protein Yield

The protein yield from *Catla catla* visceral waste, specifically from the intestine and liver, during Papain hydrolysis at different time intervals (30, 60, 120, and 180 minutes) and two enzyme concentrations (1% and 2%). Protein concentrations, presented in mg/ml, are shown as mean values with standard deviations (SD) for each condition.

The figure 3 provide insight into how the protein yield varies based on the duration of hydrolysis and the concentration of the enzyme Papain. For each time interval and enzyme concentration combination, the mean protein concentration is depicted along with its corresponding standard deviation, indicating the variability of the data around the mean value.

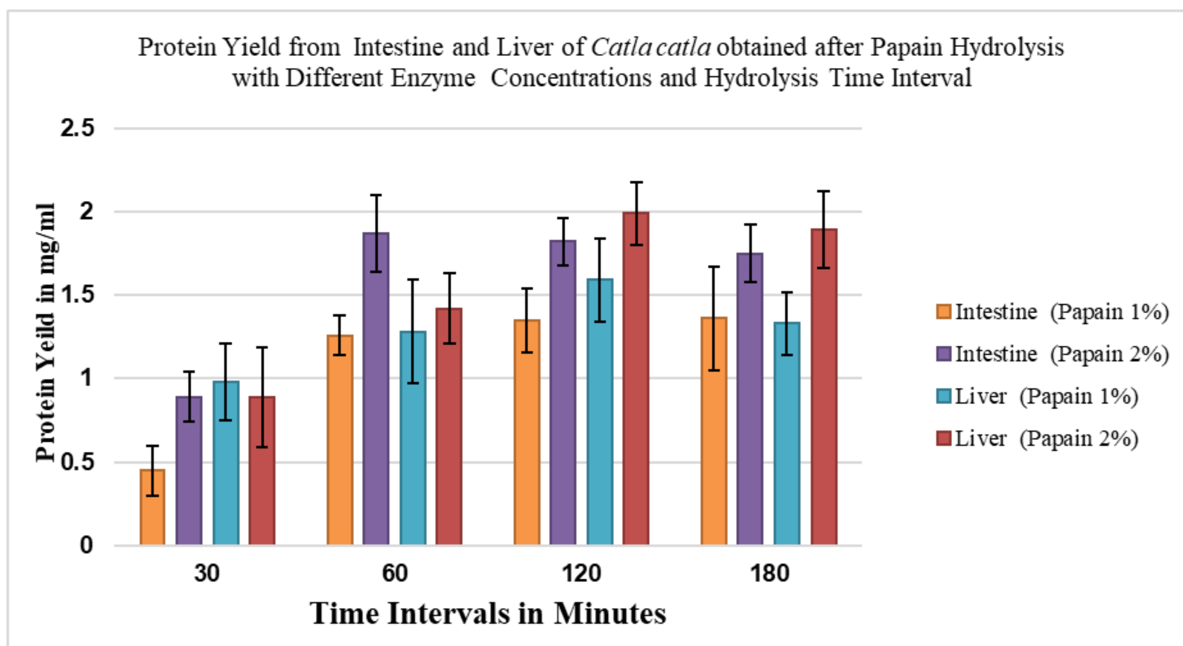

Figure 3. Figure illustrates the protein yield after enzyme hydrolysis at different concentrations of Papain exposed to various time intervals for the intestine and liver tissue of *Catla catla*.

Observations can be made regarding the effect of time and enzyme concentration on protein yield from both the intestine and liver of *Catla catla*. Generally, longer hydrolysis times and higher enzyme concentrations lead to increased protein yields, as evidenced by the higher mean protein concentrations observed at 60, 120, and 180 minutes compared to 30 minutes, and at 2% enzyme concentration compared to 1%. However, variations in protein yield are also observed between the intestine and liver samples, suggesting potential differences in the substrate composition and enzymatic activity.

The figure 4 reveals several significant findings regarding the enzymatic digestion process. Firstly, there is a noticeable increase in the protein quantity of peptides with longer incubation times, indicating a time-dependent digestion process. Additionally, higher enzyme concentrations (2%) yield higher protein quantities than lower concentrations (1%), suggesting a concentration-

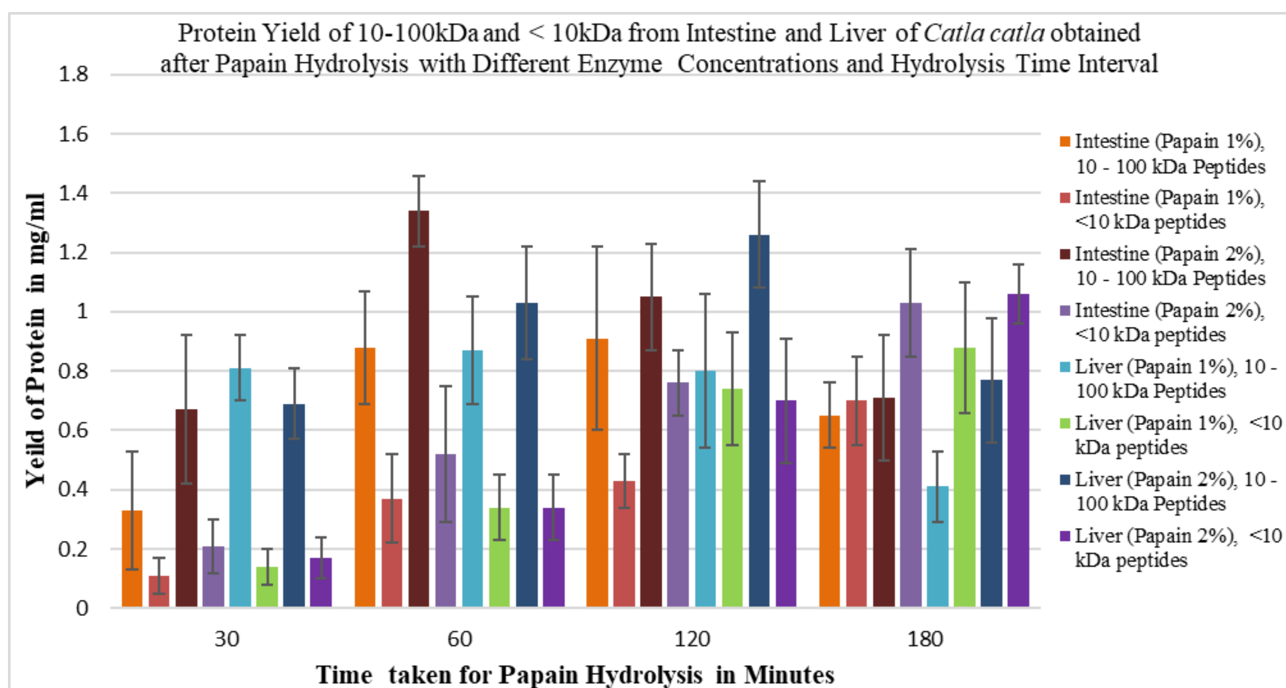

Figure 4. Protein Yield of Different Categories of Peptides Obtained from the Crude Hydrolysates of intestine and liver Tissue of *Catla catla*.

dependent efficiency in peptide breakdown. Furthermore, variations in protein quantity between different tissue types suggest potential differences in substrate accessibility or enzyme activity. Moreover, differences in protein quantity are observed between peptides of different sizes (10-100 kDa and <10 kDa), indicating potential preferences or efficiencies in peptide digestion based on size. Finally, interaction effects between time, enzyme concentration, and tissue type may influence protein quantity, highlighting the complexity of the enzymatic digestion process. These findings provide valuable insights into the factors influencing peptide digestion and underscore the intricate interplay between time, enzyme concentration, tissue type, and peptide size in determining protein quantity.

Table 3. Scavenging Activity of DPPH Radicals by Papain-Digested Peptides in Intestine and Liver Tissues at Different Time Intervals and Enzyme Concentrations by Different Sizes of Peptides.

Time Interval in Minutes	Type of Tissue	Concentration of Enzyme (Papain)	% of Scavenging Activity (%RSA) of 10-100kDa Mean ± SD	% of Scavenging Activity (%RSA) of <10kDa Mean ± SD
30	Intestine	1%	54.69 ± 1.78^{a1}	35 ± 2.5^{a1}
		2%	$38 \pm 2.64\ 2^{b2}$	43.5 ± 3.04^{a2}
	Liver	1%	22 ± 2.65^{c3}	37.90 ± 3.34^{a3}
		2%	29 ± 5^{d4}	44.46 ± 3.97^{a4}
60	Intestine	1%	58.89 ± 3.44^{a1}	53.7 ± 3.06^{a1}
		2%	51.6 ± 3.83^{b2}	27.7 ± 3.51^{a2}
	Liver	1%	30.9 ± 2.85^{c3}	55.53 ± 2.60^{a3}
		2%	25 ± 5.29^{d4}	35 ± 3^{a4}
120	Intestine	1%	45 ± 2^{b2}	41 ± 4.58^{a1}
		2%	41 ± 2.64^{b2}	30 ± 3.4^{b1}
	Liver	1%	30.36 ± 2.51^{c3}	56.4 ± 3.93^{c1}
		2%	39.2 ± 1.70^{d4}	39.3 ± 3^{d1}
180	Intestine	1%	54.83 ± 4.25^{a1}	39 ± 3^{a1}
		2%	20 ± 2^{d4}	56 ± 3.6^{a2}
	Liver	1%	30.76 ± 1.36^{c3}	41 ± 3.6^{a3}
		2%	59.23 ± 3.32^{a}	26 ± 3.60^{d4}

*The different alphabet showing the significant difference between tissue type and different numerical are showing significant differences between different enzyme concentrations, $p < 0.05$

Antioxidant Activity by DPPH

The analysis of table 3 presents data on the scavenging activity of intestine and liver tissues treated with different concentrations of the enzyme papain over varying time intervals (30, 60, 120, and 180 minutes). Scavenging activity is measured by the percentage of scavenging activity (%RSA) of two peptide size fractions: 10-100 kDa and <10 kDa.

One notable finding is the significant variation in scavenging activity observed with different concentrations of papain across both tissue types. Generally, a higher concentration of papain (2%) exhibits greater scavenging activity compared to a lower concentration (1%). Additionally, differences in scavenging activity are observed between intestine and liver tissues, with the intestine often showing higher scavenging activity, particularly at specific time intervals and enzyme concentrations. Moreover, scavenging activity fluctuates over time, with distinct trends noted for different peptide size fractions. For instance, in intestine tissue, the scavenging activity of the 10-100 kDa fraction tends to increase over time, while the <10 kDa fraction shows fluctuations. Overall, these findings underscore the influence of tissue type, enzyme concentration, and treatment duration on the scavenging activity of papain, providing valuable insights into its potential antioxidant properties and therapeutic applications.

Antioxidant Activity by ABTS

The table 4 present the % of Inhibition of ABTS radicals by papain-digested peptides in intestine and liver tissues at various time intervals (30, 60, 120, and 180 minutes) and enzyme concentrations (1% and 2%). Notably, in the liver tissue, at 180 minutes, the 10-100kDa digested peptides exhibit the highest scavenging activity, with a mean % of Inhibition of 75 ± 4.35 for 2% enzyme concentration and a mean % of Inhibition of 64 ± 3 for 1% enzyme concentration.

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Table 4. Scavenging Activity of ABTS Radicals by Papain-Digested Peptides in Intestine and Liver Tissues at Different Time Intervals and Enzyme Concentrations by Different Sizes of Peptides.

Time Interval in Minutes	Type of Tissue	Concentration of Enzyme (Papain)	% of Inhibition of 10-100kDa Mean ± SD	% of Inhibition of <10kDa Mean ± SD
30	Intestine	1%	54.69 ± 1.78[a1]	27.67 ± 5.5[a1]
		2%	18 ± 4.35[a2]	35.33 ± 6.50[a1]
	Liver	1%	22 ± 2.65[a3]	32 ± 3.46[a1]
		2%	25 ± 1[a4]	18 ± 5.56 [a1]
60	Intestine	1%	46.00 ± 3.6[a1]	63 ± 5[a1]
		2%	54 ±5.56[b2]	47± 4[a2]
	Liver	1%	56 ± 2.64[c3]	62 ± 5.29[a3]
		2%	26 ± 3[d4]	51 ± 3.6[a4]
120	Intestine	1%	42 ± 3[a1]	56 ± 2.64[a1]
		2%	38 ± 4[b1]	69 ± 2[a2]
	Liver	1%	29 ± 2.64[c1]	58 ± 5.29[a3]
		2%	30 ± 5[d1]	48 ± 2[a4]
180	Intestine	1%	35 ± 4.35[a1]	61 ± 1.73[a1]
		2%	55 ± 2.64[b2]	61± 3.6[b2]
	Liver	1%	64± 3[c3]	55 ± 4[c3]
		2%	75 ± 4.35[d4]	37 ± 3.46[d4]

*The different alphabet showing the significant difference between tissue type and different numerical values are showing significant differences between different enzyme concentrations, $p < 0.05$.

Conversely, in intestine tissue, at 60 minutes, the <10kDa digested peptides demonstrate the highest scavenging activity, with a mean % of Inhibition of 63 ± 5 for 1% enzyme concentration and a mean % of Inhibition of 47 ± 4 for 2% enzyme concentration. These results indicate the variation in scavenging activity based on tissue type, time interval, and peptide fragment size in ABTS radical scavenging assay.

Discussion

Proteins and peptides possess antioxidant properties, forming the basis for extensive research in this domain. Notably, studies have concentrated on identifying antioxidant peptides derived from diverse food sources, including milk, eggs, fish, and plant-based proteins, as demonstrated by Samaranayaka and Li-Chan (2011). These investigations have resulted in the isolation and characterization of specific peptide sequences with potent antioxidant activity, showcasing the potential of food-derived peptides as natural antioxidants (Coppola et al., 2021).

Additionally, research has delved into the antioxidant properties of peptides sourced from marine proteins such as fish, shellfish, and algae, revealing their significant free radical-scavenging abilities (Lopez-garcia et al., 2022; You et al., 2010). Furthermore, enzymatic hydrolysis of proteins has emerged as a common method for producing bioactive peptides with antioxidant activity, as demonstrated by Wu et al. (2012). They investigated the enzymatic hydrolysis of proteins from various sources to obtain peptides with antioxidant properties.

Evaluating the in vivo antioxidant effects of peptides using animal models has also been a focus, with studies demonstrating the protective effects of peptides against oxidative damage (Cipolari et al., 2020; Zhang et al., 2024). Moreover, research has explored the bioavailability and mechanisms of action of antioxidant peptides, providing insights into their absorption, distribution, metabolism, and excretion, thereby enhancing our understanding of their physiological effects (Losada-Barreiro et al., 2022). Together, these pioneering works have advanced our knowledge of proteins and peptides with antioxidant properties, paving the way for their potential applications in promoting health and preventing diseases.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assays serve as commonly used methods to measure the antioxidant activity of compounds, including peptides, owing to their simplicity, reliability, and sensitivity. Both assays rely on the principle of electron transfer or hydrogen atom transfer to assess the ability of an antioxidant to neutralize free radicals or oxidizing agents (Ambigaipalan and Shahidi, 2017; Baliyan et al., 2022).

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In the DPPH assay, DPPH radicals react with antioxidants, reducing DPPH radicals to form the non-radical form DPPH-H. The decrease in the purple colour, measured spectrophotometrically at a specific wavelength (usually 515 nm), is directly proportional to the scavenging ability of the antioxidant present in the sample. Antioxidants with higher scavenging activity exhibit stronger colour reduction, indicating greater free radical scavenging capacity (Baliyan et al., 2022).

On the other hand, the ABTS assay measures the ability of antioxidants to scavenge ABTS radicals, generated by the oxidation of ABTS with a strong oxidizing agent such as potassium persulfate. The decrease in absorbance at 734 nm, measured spectrophotometrically, correlates with the antioxidant capacity of the sample, with stronger antioxidants producing greater reductions in absorbance (Ambigaipalan and Shahidi, 2017; Christodoulou et al., 2022; Re et al., 1999).

Several peptides derived from both freshwater and marine water species have been identified for their potent antioxidant properties, offering potential health benefits. For example, peptides obtained from fish protein hydrolysates have garnered considerable attention due to their diverse bioactive properties. Research on peptides from freshwater fish species, such as *Catla catla* and *Labeo rohita,* has revealed their antioxidant potential (Elavarasan and Shamasundar, 2022; Mohanty et al., 2021).

Studies have identified antioxidant peptides from *Catla catla* visceral waste protein hydrolysates, highlighting their ability to scavenge free radicals and mitigate oxidative stress (Murthy et al., 2018). Similarly, peptides derived from protein hydrolysates of *Labeo rohita* have demonstrated significant antioxidant activity, attributed to their amino acid composition and sequence (Mohanty et al., 2021).

In addition to freshwater species, peptides derived from marine water fish have also shown promising antioxidant properties. Peptides from marine fish protein hydrolysates have been reported to exhibit potent radical-scavenging activity and metal-chelating ability, contributing to their antioxidative effects (Cheung et al., 2015). For example, peptides derived from marine fish species like tuna, salmon, and cod have demonstrated antioxidant activity due to their high content of essential amino acids and specific peptide sequences (Nikoo and Benjakul, 2015).

The results of our experiments unveil the complex interplay of protein hydrolysis, peptide production, and antioxidant activity in *Catla catla* visceral protein hydrolysate derived from intestine and liver samples. Notably, the findings highlight the crucial influence of enzyme concentration and hydrolysis time on optimizing the production of bioactive peptides with antioxidant properties(Bhaskar and Mahendrakar, 2008). Initially, we observed that the degree of hydrolysis and peptide content increased with longer hydrolysis times and higher enzyme concentrations, indicating enhanced protein breakdown and peptide liberation. These results underscore the significance of enzyme concentration and hydrolysis duration in maximizing the yield of bioactive peptides from visceral protein sources. Correlation matrices further revealed distinct relationships between enzyme concentration, hydrolysis duration, and the percentage of hydrolysis, emphasizing the influence of these factors on substrate breakdown efficiency (Bhaskar et al., 2008).

Subsequent investigations evaluated the protein yield from intestine and liver samples, with longer hydrolysis times and higher enzyme concentrations consistently leading to increased protein yields, indicative of efficient enzymatic digestion. However, variations in protein yield between tissue types suggest potential differences in substrate composition and enzymatic activity, emphasizing the importance of tissue-specific considerations in peptide production (Abeyrathne et al., 2016; Shahi et al., 2020).

Further studies on the hydrolysates' scavenging activity (%RSA) revealed significant differences between tissue types and enzyme concentrations across various time intervals, with the intestine consistently exhibiting higher %RSA compared to the liver, indicating its superior antioxidative capacity. Higher enzyme concentrations generally resulted in enhanced scavenging activity, particularly in the intestine, suggesting a concentration-dependent effect on antioxidative properties.

Overall, these findings underscore the potential of visceral protein hydrolysates from both intestine and liver sources as valuable sources of bioactive peptides with antioxidant properties, with implications for various applications in health and disease management and go with the previous findings of many pioneer fish scientists (Cipolari et al., 2020; Wang et al., 2022).

Conclusion

In conclusion, our study sheds light on the promising antioxidant properties of the 2AF>10 (2% Enzyme in Liver tissue with 180 min incubation) peptide derived from *Catla catla* fish liver. Furthermore, our investigation into the antioxidant properties of fish-

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derived peptides underscores their potential as natural antioxidants, with implications for health promotion and disease prevention. Peptides from fish sources, including *Catla catla*, have demonstrated significant antioxidant activity, offering promising avenues for therapeutic development. The influence of enzyme concentration and hydrolysis time on peptide production and antioxidative effects highlights the importance of optimizing peptide extraction processes to maximize their bioactivity. Overall, these findings contribute to advancing our understanding of peptide-based therapies and their potential applications in combating cancer and oxidative stress-related disorders, offering hope for improved treatment strategies and health outcomes in the future.

Conflict of Interest

There is no conflict of interest

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