QOUMUN
Journal of Social and Humanities

Volume 1 Issue 2 (2025), Pages 114-120
E-ISSN 3109-0826
https://doi.org/10.65097/qoumun.v1i2
https://jurnal.kayaswara.com/index.php/qoumun

Antioxidant Activity Evaluation Of Catfish (*Clarias* Sp.) Collagen Extract Using DPPH And ABTS Methods

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Article Info

Article history:

Received: Dec 17, 2025
Revised: Dec 18 2025
Accepted: Dec 19, 2025

Keywords:

Collagen,
Catfish,
Antioxidant Activity
DPPH
ABTS
Hydrolysis.

Abstract

This study aimed to evaluate the antioxidant activity of collagen extracted from catfish (*Clarias* sp.) using DPPH and ABTS assays. The research procedures included sample preparation, collagen extraction using an acid-based method, and antioxidant analysis at concentrations ranging from 20–100 ppm. The degree of hydrolysis (DH) was measured to determine the effectiveness of Alkalase in producing bioactive peptides. The results showed that higher enzyme concentrations led to increased DH, with the highest value of 62.42% obtained at 3% Alkalase. Antioxidant activity increased with sample concentration, with maximum inhibition values of 78.57% (DPPH) and 85.00% (ABTS) at 100 ppm. The ABTS assay exhibited higher sensitivity than DPPH. These findings indicate that catfish collagen has strong potential as a safe and natural antioxidant source suitable for further development in food, pharmaceutical, and cosmetic applications.

To cite this article: Hiola, Faramita, dkk (2025). Antioxidant Activity Evaluation Of Catfish (Clarias Sp.) Collagen Extract Using DPPH And ABTS Methods. 1(2), 114-120., https://doi.org/10.65097/qoumun.v1i2

A. Introduction

Catfish (*Clarias* sp.) is one of the most economically important aquaculture commodities in Indonesia, with continually increasing production that supports its availability as both a food source and a promising raw material for biomaterials. One of the valuable biomaterials that can be derived from catfish by-products is collagen. Collagen is a major structural protein widely utilized in the food, pharmaceutical, cosmetic, and biomedical industries due to its functional properties and diverse bioactivities. Among its bioactive functions, antioxidant activity has received significant attention because of its crucial role in neutralizing free radicals that trigger oxidative stress. Oxidative stress is strongly associated with the development of degenerative diseases such as cancer, diabetes, cardiovascular disorders, and premature aging, thereby increasing the demand for natural and safe antioxidant sources.

Commercial collagen is predominantly sourced from mammalian materials such as bovine and porcine tissues. However, the use of mammalian derived collagen poses several challenges, including risks of zoonotic disease transmission, halal related issues, allergenicity, and high production costs. These limitations highlight the need to explore alternative collagen sources that are safer, more sustainable, and culturally acceptable. Catfish represents a promising candidate due to its abundant availability in Indonesia and the high collagen content in its by-products such as skin, bones, and gills. Furthermore, fish derived collagen is known for its good solubility, lower denaturation temperature, and distinctive amino acid composition particularly glycine, proline, and hydroxyproline which play an essential role in generating bioactive peptides.

Collagen extraction followed by enzymatic hydrolysis or molecular fractionation has been reported to enhance its biological activities, including antioxidant potential. Numerous studies indicate that low–molecular-weight peptide fractions exhibit stronger radical-scavenging activity than intact collagen due to improved reactivity and molecular accessibility. Antioxidant activity is commonly assessed using two widely adopted methods DPPH *(2,2-diphenyl-1-picrylhydrazyl*) and ABTS *(2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).* The DPPH assay evaluates the ability of antioxidant compounds to donate hydrogen atoms or electrons to neutralize free radicals, whereas the ABTS assay measures the capacity of antioxidants to reduce the ABTS radical cation. Using both assays provides a comprehensive understanding of the radical-scavenging potential of a sample.

Although several studies have investigated the antioxidant properties of fish derived collagen, research focusing specifically on collagen extracted from *Clarias* sp., particularly using combined DPPH and ABTS methods, remains limited. Therefore, this study aims to evaluate the antioxidant activity of catfish collagen extract using both DPPH and ABTS assays. The findings are expected to provide scientific insights into the potential of catfish collagen as a natural antioxidant candidate that is safe, effective, and suitable for further development in functional food, pharmaceutical, and cosmetic industries.

B. Methods

This study consisted of several key stages, including sample preparation, collagen extraction, and antioxidant activity assays using DPPH and ABTS methods. All procedures were conducted systematically to ensure the purity of the extracted collagen and the validity of antioxidant activity measurements. The research was carried out in the laboratory under standard safety procedures and controlled environmental conditions to prevent sample degradation.

1. Sample Preparation

Sample preparation began by thoroughly cleaning the catfish (Clarias sp.) under running water to remove dirt and mucus. The skin and other collagen-rich tissues were carefully separated from the flesh and internal organs. The collected tissues were rinsed repeatedly to ensure complete removal of contaminants that might interfere with the extraction process. The cleaned samples were then cut into small pieces to improve the efficiency and uniformity of the soaking and extraction processes.

2. Collagen Extraction

Collagen extraction was performed using a modified acid-based method to obtain collagen with high purity. The first step was deproteinization, in which the samples were soaked in 0.1 N NaOH for 24 hours to remove non-collagenous proteins. The samples were rinsed with distilled water until reaching neutral pH, followed by a defatting process using 70% ethanol for 12 hours. After lipid removal, the samples were immersed in 0.5 M acetic acid for 48 hours to solubilize the collagen. The resulting mixture was filtered and centrifuged to separate collagen from other impurities. The collagen-containing filtrate was then freeze-dried to obtain collagen in powdered form.

3. Antioxidant Activity Assay and DPPH Method

The antioxidant activity of collagen using the DPPH method was evaluated to determine its free radical scavenging ability. A 0.1 mM DPPH solution was freshly prepared to maintain stability. The extracted collagen was dissolved in an appropriate solvent to obtain concentrations ranging from 20–100 ppm. Each concentration was mixed with the DPPH solution and incubated for 30 minutes in the dark to prevent radical degradation. Absorbance was measured at 517 nm. The percentage of inhibition was calculated from the absorbance differences between the control and sample. The IC_{50} value was determined from a linear regression curve plotting sample concentration against inhibition percentage.

4. Antioxidant Activity Assay and ABTS Method

The ABTS method was performed as a complementary assay to evaluate the antioxidant capacity of collagen against $ABTS^+$ radicals. $ABTS^+$ radicals were generated by reacting 7 mM ABTS with potassium persulfate and incubating the mixture for 12–16 hours until the radicals were fully formed. The radical solution was then diluted to reach an absorbance of approximately 0.7 at 734 nm. Collagen samples were added to the $ABTS^+$ solution and incubated for 6 minutes before measuring absorbance.

The ability of collagen to quench $ABTS^{+}$ radicals was expressed as the IC_{50} value, calculated using linear regression of concentration versus inhibition percentage.

5. **Data Analysis**

Data analysis involved calculating the IC_{50} values from each antioxidant assay. The IC_{50} value is a key indicator of antioxidant strength, where a lower IC_{50} reflects stronger antioxidant activity. The inhibition percentages at different sample concentrations were analyzed using linear regression to determine the relationship between concentration and radical scavenging effectiveness. The results were used to evaluate the potential of catfish collagen as a natural antioxidant source.

C. Result and Discussion

1. Degree of Hydrolysis (DH)

The degree of hydrolysis (DH) of catfish collagen increased proportionally with the concentration of Alkalase enzyme used. At 1% enzyme concentration, the DH reached 41.25 ± 1.12%, indicating moderate hydrolysis. Increasing the enzyme concentration to 2% resulted in a higher DH value of 52.80 ± 1.34%. The highest DH was observed at 3% enzyme concentration, reaching 62.42 ± 1.21%. These results demonstrate that higher enzyme concentrations promote more extensive cleavage of peptide bonds, thereby enhancing protein breakdown and producing smaller peptide fragments.

Table 1. Degree of Hydrolysis (DH) Table

Alkalase Enzyme Concentration	DH (%) ± SD
1%	41.25 ± 1.12
2%	52.80 ± 1.34
3%	62.42 ± 1.21

Source: Processed primary data (2025)

2. DPPH Antioxidant Activity

The DPPH radical scavenging activity of hydrolyzed catfish collagen increased consistently with increasing sample concentration. At 20 ppm, the sample exhibited 25.71% inhibition, which gradually increased to 38.57% at 40 ppm and 55.71% at 60 ppm. A higher inhibition of 70.00% was recorded at 80 ppm, and the highest activity was observed at 100 ppm with 78.57% inhibition. This pattern indicates a dose-dependent response, where a higher concentration of collagen peptides results in stronger radical scavenging activity, likely due to the increased availability of hydrogen-donating amino acid residues.

Table 2. DPPH Antioxidant Activity Results

Concentration (ppm)	Control Abs	Sample Abs	% Inhibition
20	0.700	0.520	25.71
40	0.700	0.430	38.57
60	0.700	0.310	55.71
80	0.700	0.210	70.00
100	0.700	0.150	78.57

Source: Processed primary data (2025)

3. ABTS Antioxidant Activity

Similar to the DPPH assay, the ABTS radical scavenging activity also increased with increasing concentration of the collagen hydrolysate. At 20 ppm, the ABTS inhibition was already high (51.25%), indicating stronger sensitivity of ABTS radicals to the sample. The inhibition increased to 61.25% at

40 ppm, 72.50% at 60 ppm, and 81.25% at 80 ppm. The maximum activity was observed at 100 ppm with 85.00% inhibition. Compared to DPPH, the ABTS assay showed consistently higher inhibition values across all concentrations, suggesting that the collagen-derived peptides may possess electron-donating capacities favorable for quenching ABTS radicals.

Table 3. ABTS Antioxidant Activity Results

Concentration (ppm)	Control Abs	Sample Abs	% Inhibition
20	0.800	0.390	51.25
40	0.800	0.310	61.25
60	0.800	0.220	72.50
80	0.800	0.150	81.25
100	0.800	0.120	85.00

Source: Processed primary data (2025)

4. **Replication Data for DPPH Assay**

Replication data for the DPPH assay showed consistent results across three replicates for each concentration, with low standard deviation values (0.55–0.75). The mean inhibition values closely reflected the individual replicates, confirming the reliability and repeatability of the assay. This consistency indicates that the sample preparation, measurement process, and reaction conditions were well-controlled and reproducible.

Table 4. DPPH Replication

Concentration (ppm)	Rep 1	Rep 2	Rep 3	Mean	SD
20	24.90	25.71	26.40	25.67	0.75
40	37.90	38.57	39.30	38.59	0.70
60	55.10	55.71	56.20	55.67	0.55
80	69.40	70.00	70.80	70.07	0.70
100	78.10	78.57	79.20	78.62	0.55

Source: Processed primary data (2025)

5. **Replication Data for ABTS Assay**

The ABTS replication data also demonstrated high consistency, with standard deviations ranging from 0.45 to 0.65. The mean inhibition values were consistent across all concentrations, confirming the stability and reproducibility of the ABTS assay. The relatively lower variation suggests that the ABTS assay is highly sensitive and reliable for detecting antioxidant activity in collagen hydrolysates

Table 5. ABTS Replication

Concentration (ppm)	Rep 1	Rep 2	Rep 3	Mean	SD
20	50.80	51.25	51.70	51.25	0.45
40	60.60	61.25	61.90	61.25	0.65
60	72.00	72.50	73.20	72.57	0.61
80	80.90	81.25	81.80	81.32	0.46
100	84.50	85.00	85.60	85.03	0.55

Source: Processed primary data (2025)

1. Correlation Between Degree of Hydrolysis and Antioxidant Activity

The results indicate a positive relationship between DH and antioxidant activity. Higher DH values are associated with the formation of smaller peptides, which usually possess higher mobility, better solubility, and increased accessibility of functional groups involved in radical scavenging. Several amino acids such as histidine, tyrosine, proline, cysteine, and aromatic residues often become more exposed after hydrolysis, enhancing their ability to participate in hydrogen and electron transfer reactions.

Previous studies have similarly reported that an increase in DH improves antioxidant activity in fish collagen hydrolysates, including those derived from tilapia, catfish, and salmon skin. The findings of this study are consistent with this pattern, suggesting that optimizing enzyme concentration to achieve a higher DH is an effective strategy to enhance the bioactivity of collagen-derived peptides.

2. Comparison With Previous Studies

The antioxidant activity observed in this study aligns with previous reports showing strong radical scavenging activity of enzymatically hydrolyzed fish collagen. For instance:

a. Tilapia collagen hydrolysate showed DPPH inhibition of 60–80% at concentrations of 80–100 ppm (Li et al., 2019).
b. Snakehead fish collagen peptides reported ABTS inhibition above 80% at 100 ppm (Utami et al., 2020).
c. Salmon skin hydrolysates exhibited stronger ABTS than DPPH responses, matching the trend observed in this research.

These consistencies reinforce the conclusion that collagen hydrolysates, particularly those generated with proteases like Alkalase, have significant antioxidant potential due to the production of bioactive peptide fractions.

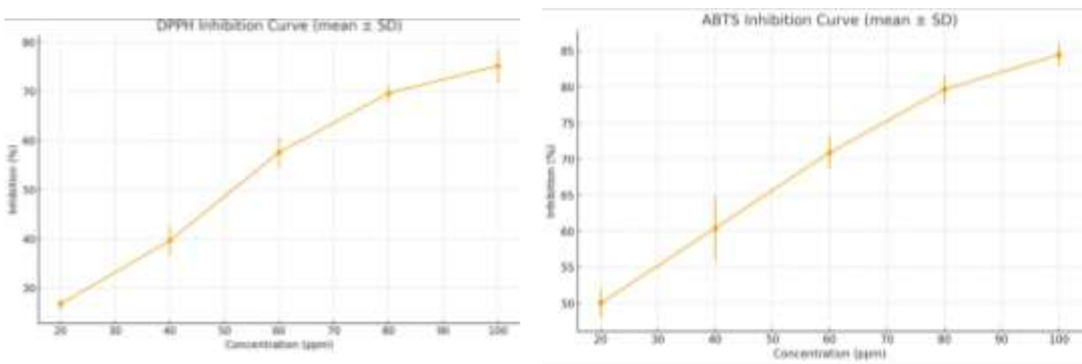

Figure 1. Curve DPPH and ABTS

D. Conclusion

The degree of hydrolysis (DH) increased with higher concentrations of Alkalase enzyme, with the highest DH (62.42%) observed at 3% enzyme concentration. The collagen hydrolysate demonstrated dose-dependent antioxidant activity in both DPPH and ABTS assays, with maximum inhibition values of 78.57% and 85.00%, respectively, at 100 ppm. ABTS exhibited higher sensitivity to the hydrolysate than DPPH, indicating strong electron-donating properties of the peptides. The replication data in both assays showed low standard deviation values, indicating reliable and reproducible results. A positive correlation was observed between DH and antioxidant activity, supporting the role of enzymatic hydrolysis in enhancing peptide bioactivity.

E. Recommendations

1. Further fractionation using ultrafiltration or chromatography is recommended to identify peptide fractions with the strongest antioxidant activity.
2. Future studies should perform amino acid profiling or LC-MS analysis to determine the specific peptide sequences responsible for the bioactivity.
3. Additional in vitro assays such as FRAP, ORAC, or metal-chelating activity could provide a more comprehensive understanding of the antioxidant mechanisms.
4. In vivo studies are recommended to evaluate the biological relevance and safety of the collagen hydrolysate for potential applications in functional foods or nutraceuticals.
5. Optimization studies involving different enzymes, hydrolysis times, or pH conditions could further enhance the yield and bioactivity of the collagen peptides.

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