

Effect of Physicochemical Parameters on Antioxidant Activity of Hydrolysed Tilapia Protein

ABDULSALAM, I.¹, ISHOLA, N. O.², ODEDERE, A. O.³, DICKSON MUSA, A.⁴, ABADI, N.⁵,
ABDULAZEEZ, M.⁶, HASHIMU, A.⁷, YUSUF BIN, M.⁸

^{1, 2, 3, 5} Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos State, Nigeria

⁴ Ibrahim Badamasi Babangida University Lapai (IBBUL), Niger State, Nigeria

^{6, 7, 8} National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria

*Corresponding Author: Odedere, A. O.

Abstract- Marine-derived proteins and their hydrolysed peptide fractions exhibit high bioactivity due to their unique amino acid composition distinct from terrestrial protein sources. This study examined the effect of enzyme concentration, temperature, and hydrolysis time on the antioxidant activity of papain-hydrolysed tilapia protein. Protein hydrolysates were prepared by enzymatic hydrolysis using papain and purified by gel filtration chromatography, following standard analytical protocols. Response surface methodology (RSM) based on a Box-Behnken design was used to model the influence of enzyme concentration, temperature, and hydrolysis time on antioxidant activity (DPPH radical scavenging), across 15 experimental runs. DPPH scavenging activity ranged from 51.86% to 92.17% across the design space. Maximum antioxidant activity occurred at an enzyme concentration of 4%, temperature of 60 °C, and hydrolysis time of 5 h. The temperature × hydrolysis time interaction (AC) was non-significant ($p > 0.05$), indicating that the two variables exert independent main effects rather than acting synergistically. The model followed an additive response topology; whereby peak antioxidant activity is predicted when temperature and hydrolysis time are set concurrently at their individually optimal values. These findings demonstrate that enzyme concentration, temperature, and hydrolysis time independently govern the antioxidant activity of papain-hydrolysed tilapia protein, providing a basis for optimising hydrolysis conditions to maximise bioactive peptide yield.

Keywords: Tilapia protein, physicochemical parameters, antioxidant

I. INTRODUCTION

Oceans cover over 70% of the earth's surface, hosting many chemicals and biological and ecological biodiversity. The interest in marine organisms as alternative and novel natural product sources has recently increased (Khan *et al.*, 2021). Several

compounds with distinctive physical properties have been identified in the marine ecosystem, such as phlorotannin, ziconotide, arenastatin A, and others (Srinivasan 2021; Ameen. 2021). The high bioactivity of marine-derived proteins and peptides is due to several factors, including their unique amino acid composition, 3-dimensional structure, and molecular size (Hu *et al.*, 2022; Suo *et al.*, 2022).

These peptides have been implicated with several bioactivities, hence categorized as angiotensin-converting enzyme (ACE)-inhibitory, antioxidant, antimicrobial, anticancer, and anti-inflammatory peptides (Prataama *et al.*, 2022). Studies have vastly obtained these bioactive peptides from tuna, salmon, herring, and cod (Valimaa *et al.*, 2019). Various bioactive peptide groups found in fish contribute to various physiological benefits. Among these are ACE inhibitory peptides, known for their ability to lower blood pressure by impeding the activity of ACE—an enzyme responsible for blood vessel constriction. Angiotensin-converting enzyme inhibition and antihypertensive activities have been popular with fish peptides and hydrolysate (Hu *et al.*, 2023; Suo *et al.*, 2022). Examples of ACE inhibitory peptides from fish are tuna hydrolysate, Val-Leu Pro from salmon muscle, and Ile-Asn-Pro identified in tilapia muscle. Additionally, antioxidant peptides in fish play a crucial role in safeguarding the body against oxidative stress, a significant contributor to numerous chronic diseases (Wu *et al.*, 2023).

These unique proteins and peptides often have specialized functions that require high stability, such as enzymatic activity or structural support. Collagen is known for its high stability and resistance to degradation, making it valuable for various

applications, including wound healing and tissue engineering. The stability of marine-derived peptides can make them effective therapeutic agents, as they are less likely to degrade in vivo and can remain active for extended periods. Additionally, the stability of marine derived proteins and peptides can make them valuable for use in food and beverage applications, where they can provide functional benefits such as improved texture and stability (Coppola *et al.*, 2022; Giordano *et al.*, 2018). The aim of this research work is to determine the effect of physicochemical parameters on enzyme activity.

II. MATERIALS AND METHOD

Sample Collection and Preparation

Purchase 10 kg each of tilapia (*Oreochromis* spp.) and catfish (*Clarias* spp.) from fishermen in Badagry/Lagos. The fish species were identified using FAO morphological keys. The fish were placed on ice in insulated containers and transported to the laboratory for analysis within 6 hours.

Preparation

Dissection was done using sterilized dissection tools, the skin and gills were removed, rinse tissues thoroughly with cold distilled water to remove blood and debris. Homogenize ~100 g tissue in 500 mL of

extraction buffer (0.1 M acetic acid, pH 4.0) using a blender. The samples were kept in an ice throughout to minimize proteolysis. Aliquot homogenates were kept in sterile 50 mL tubes and stored at -20 °C until extraction.

Extraction

The extraction was done using enzymatic hydrolysis. Homogenate in 0.1 M phosphate buffer, pH 7.4 was prepared, pepsin enzyme (1% w/w relative to protein content) was added and incubated at 37 °C with continuous stirring for 6 hours. The enzyme was inactivated by heating to 80 °C for 10 minutes and then centrifuged at 5,000 rpm for 20 minutes. The supernatant (peptide hydrolysate) was then collected.

Purification

The collected peptide hydrolysate was purified by Gel Filtration Chromatography. Glass column (50 cm × 2 cm) with Sephadex G-25 resin was packed and equilibrated with distilled water. Dialyzed extract and eluted with distilled water at 1 mL/min was loaded and 5 mL fractions were collected. Absorbance at 280 nm was measured to identify peptide-containing fractions.

III. RESULTS AND DISCUSSION

Factor Coding: Actual

Antioxidant activity (%)

● Design Points
--- -95% CI Bands

Actual Factors

A = 4

B = 60

C = 10

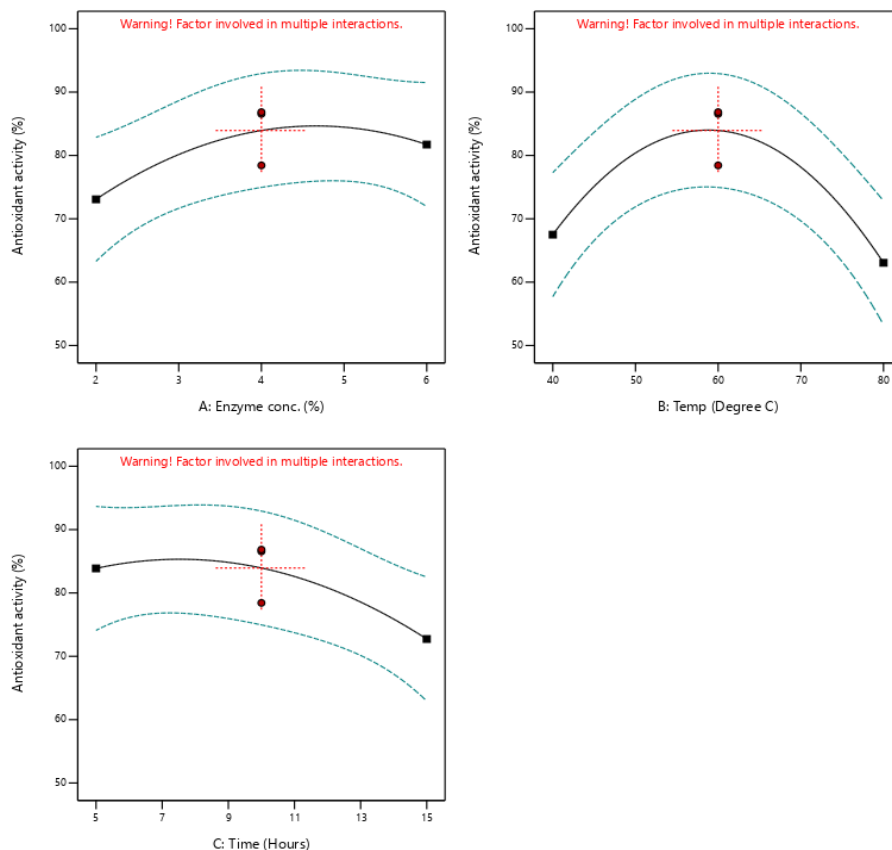


Figure 1: Effect of Enzyme Concentration (A), Temperature (B), and Hydrolysis Time (C) on Antioxidant Activity of Hydrolyzed Tilapia Protein

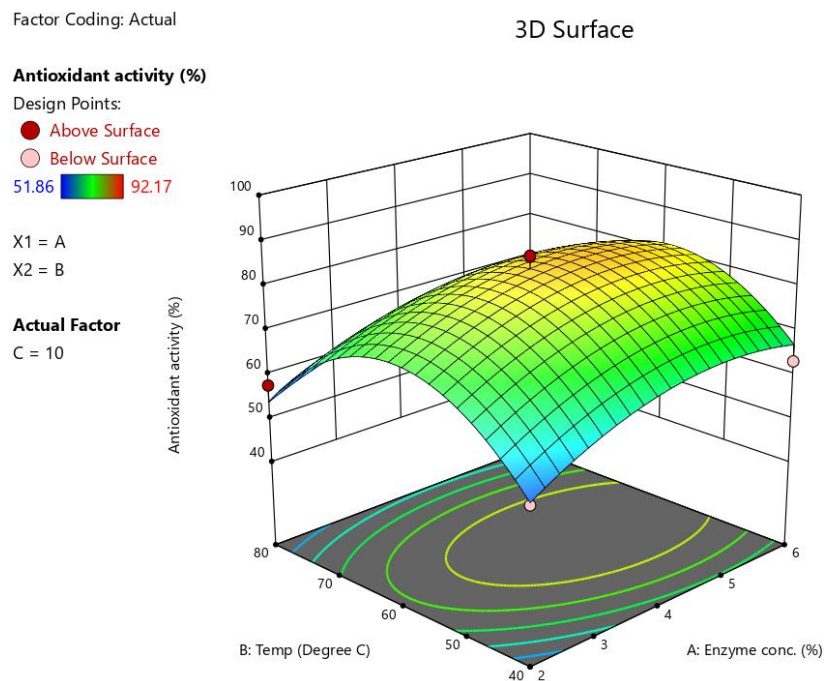


Figure 2: Interaction Effect Enzyme Concentration and Temperature on Antioxidant Activity of Hydrolyzed Tilapia Protein

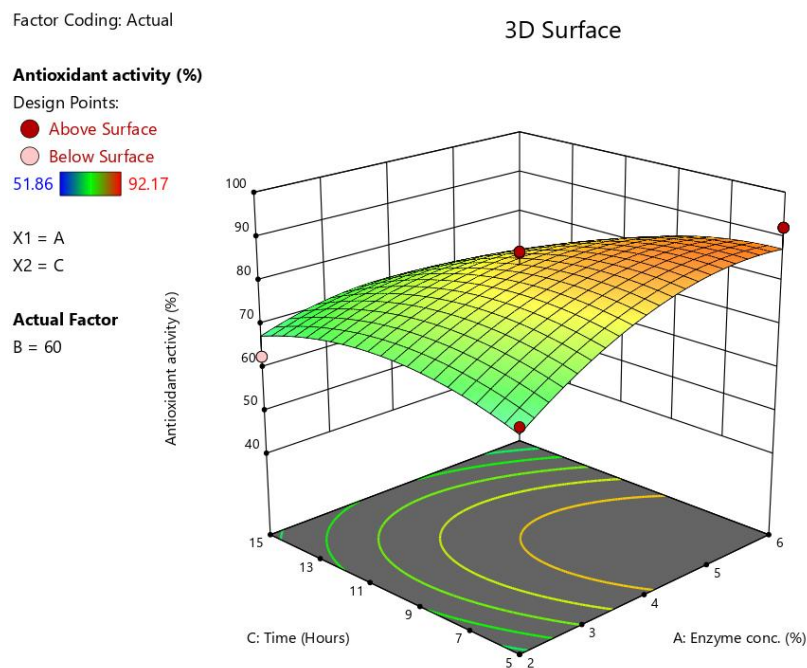


Figure 3: Interaction Effect Enzyme Concentration and Hydrolysis Time on Antioxidant Activity of Hydrolyzed Tilapia Protein

Factor Coding: Actual

3D Surface

Antioxidant activity (%)

Design Points:

● Above Surface

○ Below Surface

51.86  92.17

X1 = B

X2 = C

Actual Factor

A = 4

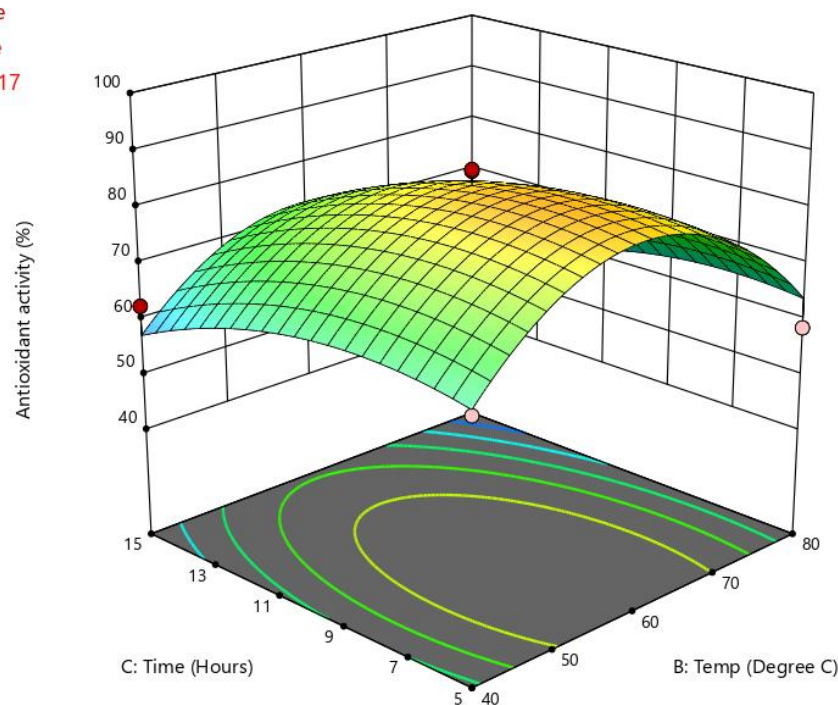


Figure 4: Interaction Effect Temperature and Hydrolysis Time on Antioxidant Activity of Hydrolyzed Tilapia Protein

IV. DISCUSSION

From Figure 1 it presents the model identifies a distinct optimum for each factor. Maximum antioxidant activity was achieved at intermediate levels within the studied design space: specifically at an enzyme concentration of 4%, temperature of 60 °C, and a hydrolysis time of 5 h. The response surface indicates that deviation from these optimal levels, either increasing or decreasing any single factor while holding others constant, results in decrease in antioxidant activity. This suggests a non-linear, concave-down relationship for each factor, where the biological system is sensitive to both insufficient and excessive conditions.

Illustrates from figure 2 response surface plot for the interaction effect of enzyme concentration (A) and

hydrolysis time (C) on the antioxidant activity of hydrolyzed tilapia protein. Analysis of Variance (ANOVA) for the fitted model indicates a statistically non-significant interaction term (AC), $p > 0.05$. Consequently, the main effects of enzyme concentration and hydrolysis time operate independently; the magnitude of the antioxidant response elicited by enzyme concentration is not dependent on the duration of hydrolysis. The response surface is thus characterized by an additive main-effects topology. Within the evaluated design space, maximum predicted antioxidant activity occurred at an enzyme concentration of approximately 4% and a hydrolysis time of 5 h. Because the main effects function independently, concurrently applying these individual factor optima yields the highest cumulative antioxidant activity.

Analysis of Variance (ANOVA) for the response surface in Figure 3 reveals that the temperature x hydrolysis time interaction term (AC) is non-significant ($p > 0.05$), establishing that both variables exert independent main effects on the antioxidant activity of tilapia protein hydrolysates. Characterized by an additive response topology, the model predicts peak antioxidant activity when temperature (60 °C) and hydrolysis time (5 h) are set concurrently to their respective individual optimum levels.

V. CONCLUSION

The model predicts peak antioxidant activity when temperature (60 °C) and hydrolysis time (5 h) are set concurrently to their respective individual. It can also be concluded that temperature and hydrolysis have independent effect on the activity of hydrolysed tilapia protein.

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