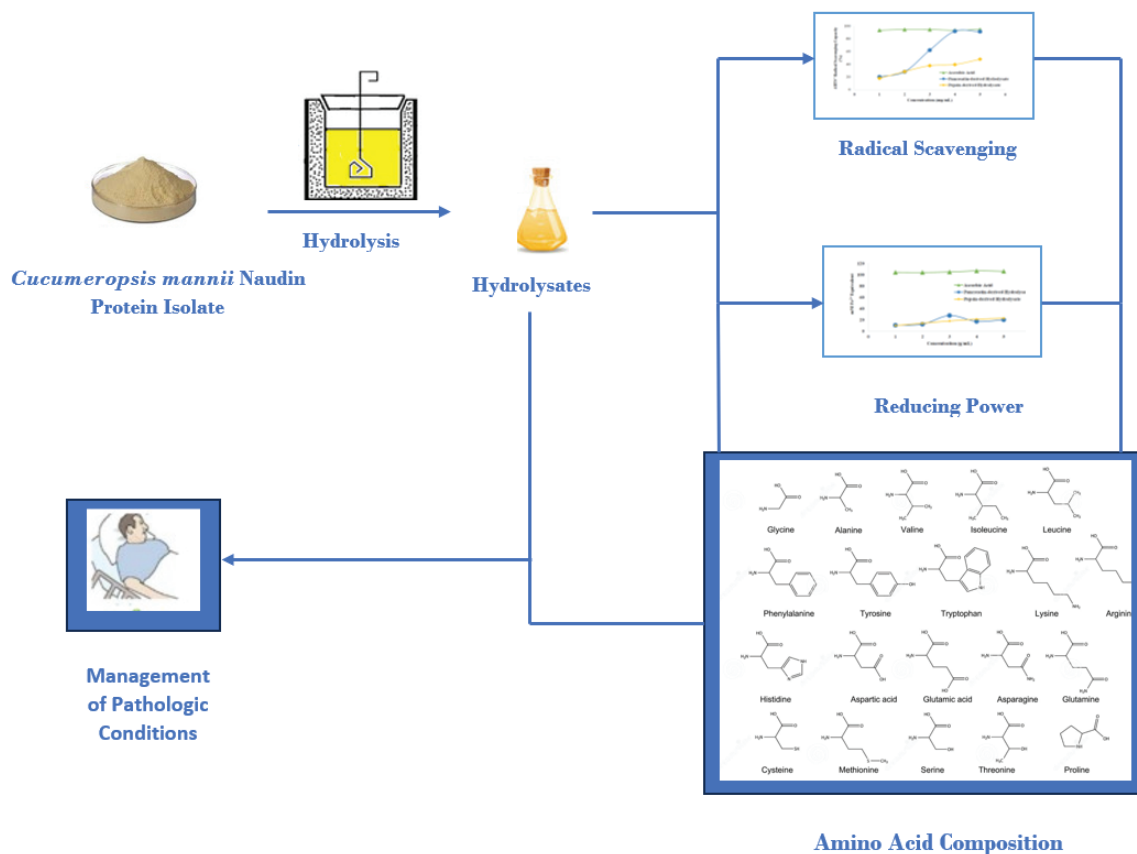


## RESEARCH ARTICLE

# Amino Acid Composition and Antioxidant Properties of Protein Hydrolysates from *Cucumeropsis mannii* Naudin Seed

O. T. Babatunde, C. O. Bewaji and A. Igunnu\*



## Highlights

- *Cucumeropsis mannii* Naudin seed protein hydrolysates possess a promising amino acid composition, containing both essential and non-essential amino acids.
- *Cucumeropsis mannii* Naudin seed protein hydrolysates exhibit strong free radical scavenging and reducing potentials.
- *Cucumeropsis mannii* Naudin seed protein hydrolysates have the potential to serve as an amino acid supplement for illness-related malnutrition and as antioxidant adjuvants in mitigating oxidative stress associated with various health conditions.

RESEARCH ARTICLE

## Amino Acid Composition and Antioxidant Properties of Protein Hydrolysates from *Cucumeropsis mannii* Naudin Seed

O. T. Babatunde<sup>1,2</sup>, C. O. Bewaji<sup>1</sup> and A. Igunnu<sup>1,3\*</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, P.M.B 1515, Ilorin, Kwara State, Nigeria

<sup>2</sup>Biochemistry Unit, Department of Chemical Sciences, Faculty of Science, Crown-Hill (Ojaja) University, P.M.B 1605, Eiyenkorin, Ilorin, Kwara State, Nigeria

<sup>3</sup>Department of Biochemistry, College of Pure and Applied Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria

Received: 23.12.2024; Accepted: 08.04.2025

**Abstract:** Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, driving various pathological conditions. Amino acid deficiency weakens these defenses, worsening cellular damage in a vicious cycle. Therefore, supplementing with amino acid-derived antioxidants can help restore the body's protective mechanisms. In this study, proteins were isolated via alkaline solubilization-acid precipitation and enzymatically cleaved using pancreatin and pepsin to yield *Cucumeropsis mannii* Naudin (*C. mannii*) seed protein hydrolysates. The amino acid composition of the hydrolysates was determined using spectrophotometric method. The hydrolysates were investigated for their antioxidant properties were evaluated using 2, 20-azinobis-3-ethylbenzothiazoline-6-sulphonate cation radical (ABTS<sup>+</sup>) scavenging, 1,1 diphenyl-1-picrylhydrazyl radical (DPPH) scavenging and ferric ion reducing antioxidant power (FRAP) assays. Following the experiments, the pancreatin- and pepsin-derived hydrolysate contained predominantly glutamate (10.61, 7.20 g/100g), leucine (9.32, 5.23 g/100g) and aspartate (7.11, 6.11 g/100g), respectively. The pancreatin- and pepsin-derived hydrolysates exhibited ABTS<sup>+</sup> scavenging activity with IC<sub>50</sub> values of 2.58 ± 0.01 mg/mL and 5.24 ± 0.03 mg/mL; DPPH scavenging activity with IC<sub>50</sub> values of 9.44 ± 0.69 mg/mL and 7.88 ± 0.24 mg/mL, respectively; and reducing power with 20.01 ± 1.20 mM Fe<sup>2+</sup> equivalent and 22.80 ± 1.45 mM Fe<sup>2+</sup> equivalent, respectively at 5 mg/mL. In conclusion, the hydrolysates have the potential to function as an amino acid supplement for illness-related malnutrition and as antioxidant adjuvants in mitigating oxidative stress linked to various health conditions.

**Keywords:** *Cucumeropsis mannii* Naudin; Protein Hydrolysates; Amino Acid Composition; Radical Scavenging Capacity; Reducing Antioxidant Property

### INTRODUCTION

Oxidative stress is a condition characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defense mechanisms. This condition disrupts cellular functions and has been implicated in several diseases, particularly neurodegenerative disorders, cardiovascular diseases, cancer, and diabetes mellitus. In neurodegenerative disorders, oxidative stress leads to neuronal damage and inflammation, resulting in the

distortion of neural coordination (Singh et al., 2024). Furthermore, in cardiovascular conditions, oxidative stress disrupts the action potentials of cardiac myocytes, causing arrhythmias, a particularly concerning cardiovascular state (Mahapatra et al., 2024). Excessive ROS production can also damage biomolecules such as DNA, proteins, and lipids, altering cellular proliferation processes and potentially leading to cancer (Tusa et al., 2023). In addition, oxidative stress plays a critical role in postprandial hyperglycemia associated with diabetes mellitus, altering the structure and function of proteins, lipids, and nucleic acids, impairing insulin secretion, and contributing to insulin resistance, a central pathology in diabetes mellitus (Bhatti et al., 2022).

Beyond its direct involvement in disease progression, oxidative stress is closely linked to amino acid malnutrition, creating a vicious cycle that exacerbates cellular damage. Amino acids, particularly sulfur-containing ones like methionine and cysteine, are essential for the synthesis of glutathione, a major antioxidant that neutralizes ROS (Bin et al., 2017). A deficiency in these amino acids weakens antioxidant defenses, increasing susceptibility to oxidative stress (Bin et al., 2017). In turn, heightened oxidative stress depletes amino acids and disrupts protein synthesis, further worsening malnutrition (Ling & Soll, 2010). Therefore, maintaining adequate amino acid intake is therefore crucial for preserving antioxidant capacity, preventing oxidative damage, and supporting overall cellular function.

In general, the body possesses specific defense mechanisms against ROS, including the utilization of innate antioxidant enzymes and molecules. The main innate antioxidant enzymes include, superoxide dismutase, catalase, glutathione peroxidase, which work in concert to ensure the effective detoxification of ROS (Brigelius-Flohé & Maiorino, 2013; Chelikani et al., 2004; Weydert & Cullen, 2010). Furthermore, the innate antioxidant molecules include, glutathione, uric acid, and bilirubin, which work in conjunction with innate antioxidant enzymes to scavenge ROS (Lu, 2013; Sautin & Johnson, 2008; Sedlak & Snyder, 2004). Oxidative stress ensues when these innate

\*Corresponding Author's Email: [doyinigunnu@unilorin.edu.ng](mailto:doyinigunnu@unilorin.edu.ng)



mechanisms are overwhelmed, requiring the ingestion of antioxidant molecules to augment the body's antioxidant defense mechanisms. In this regard, protein hydrolysates have been recognized for their potent antioxidant properties (Arise et al., 2019; Arise et al., 2016; Babatunde et al., 2022a,b; James et al., 2020a,b). Protein hydrolysates are mixtures of amino acids, peptides, and oligopeptides produced through the enzymatic cleavage of proteins. This process involves the breakdown of peptide bonds, yielding peptide sequences with significant biological and therapeutic relevance. One particularly promising plant seed for the production of protein hydrolysates is *Cucumeropsis mannii* Naudin. In Nigeria, it is commonly referred to as white melon in English, *Egushi itoo* in Yoruba, *Agushi* in Hausa, and *Ahu-ilu* in Igbo (Koffi et al., 2008; Ileola et al., 2020). The plant produces climbing vines up to 4 meters long. The seeds are egg-shaped or an elongated ovate shape, up to about 19 cm long and 8 cm wide, and cream in colour with green streaks (Loukou et al., 2007). The seed's protein content (33.8% to 38.2% of its dry weight) is highly encouraging (Besong et al., 2011). Primarily, it is cultivated for food and as a source of oil in the tropical region of Africa (Agu et al., 2022). However, *Cucumeropsis mannii* Naudin seed oil has been proven to exhibit renoprotective properties against cisplatin-induced nephrotoxicity in Wistar rats (Ale et al., 2024). Its phenolic extracts and amino acid content have been shown to have erectile dysfunction management properties (Jimoh et al., 2017). Furthermore, *Cucumeropsis mannii* Naudin seed oil possesses a diverse nutritional composition, comprising essential minerals such as sodium, phosphorus, potassium, magnesium, calcium, iron, copper, manganese, selenium, and zinc. In addition, it contains carbohydrates and a range of amino acids (Besong et al., 2011). Still more, Ijarotimi et al. (2022) reported that *Cucumeropsis mannii* Naudin seed flour exhibits antioxidant activity and the ability to inhibit angiotensin-1-converting and carbohydrate-hydrolyzing enzymes. However, to the best of our knowledge, no scientific studies have been conducted to validate the biological significance of protein hydrolysates, a highly promising compound derived from *Cucumeropsis mannii* Naudin seed proteins.

Therefore, this study examined the amino acid composition and antioxidant properties of protein hydrolysates obtained from *Cucumeropsis mannii* seed proteins. These findings seek to offer a beneficial amino acid supplement to address illness-related malnutrition and fulfill essential nutritional requirements. In addition, they will expand the reservoir of antioxidant molecules available to counteract oxidative stress, which plays a role in numerous pathological conditions.

## MATERIALS AND METHODS

### Materials

Mature seeds of *Cucumeropsis mannii* Naudin were procured from a local market in Mopa, Kogi State, Nigeria. The seeds were authenticated at the Herbarium of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria, and assigned the

voucher number UILH/003/1420/2021. This research was submitted to the University Ethical Review Committee (UERC) and received full approval, with UERC Approval Number: UERC/ASN/2023/2463.

### Methods

#### Extraction of *Cucumeropsis mannii* Naudin seed protein

The seeds of *Cucumeropsis mannii* Naudin were dried, ground into a powder, and stored in an airtight container at 4°C. The powdered seeds were defatted using n-hexane, following the method outlined by Wani et al. (2011). The defatted seeds were dried at room temperature, ground into a fine powder, and refrigerated for future use (Wani et al., 2011). Protein extraction was performed using the procedure described by Alashi et al. (2014). The defatted seed powder was mixed with 0.1 M NaOH (pH 12.0) at a ratio of 1:10 (w/v; meal to solvent) and stirred for 1 hour to solubilize the protein under alkaline conditions. The mixture was then centrifuged at 18°C and 3000 rpm for 10 minutes. Two additional extractions were conducted on the residue using fresh 0.1 M NaOH, and the supernatants from all extractions were combined. The pH of the pooled supernatant was adjusted to 4.0 using 1 M HCl to precipitate the proteins. The resulting precipitate was collected by centrifugation, washed with distilled water, and its pH was neutralized to 7.0 using 1 M NaOH (Alashi et al., 2014). Finally, the protein isolate was freeze-dried and stored under refrigeration for subsequent analyses.

#### Preparation of *Cucumeropsis mannii* Naudin seed protein hydrolysates

The protein isolate was hydrolyzed using the method described by Udenigwe et al. (2009) and documented by Onuh et al. (2015). Hydrolysis was carried out separately with pancreatin (at pH 8.0, 37°C) and pepsin (at pH 2.2, 37°C). A 5% (w/v) solution of the protein isolate was prepared in distilled water to form distinct slurries for each enzyme. Enzymes were added to their respective slurries at an enzyme-to-substrate ratio (E:S) of 1:100. The digestion process was maintained under the specified conditions for 6 hours with continuous stirring. Enzyme activity was terminated by placing the reaction vessels in boiling water (95–100°C) for 15 minutes. Undigested proteins were precipitated by adjusting the pH of the mixture to 4.0 using 2 M HCl or 2 M NaOH, followed by centrifugation at 4000 rpm for 30 minutes (Onuh et al., 2015; Udenigwe et al., 2009). The supernatant, containing the target peptides, was collected for analysis of the degree of hydrolysis and peptide yield. It was then stored under refrigeration until further analyses.

#### Determination of the amino acid composition of *Cucumeropsis mannii* Naudin seed protein hydrolysates

The Amino Acid profile of the hydrolysates was determined using method described by (AOAC, 2006) with modifications. Two hundred and fifty milligram (250 mg) of the defatted hydrolysates was placed into a glass ampoule. To this, 7 mL of 6N HCl was added, and nitrogen was introduced to expel oxygen (to prevent potential oxidation of certain amino acids like methionine and cysteine during hydrolysis). The glass ampoule was sealed using a Bunsen

burner flame and placed in an oven set at  $105\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for 22 hours. After cooling, the ampoule was carefully opened at the tip, and the contents were filtered. The filtrate was then evaporated to dryness using a rotary evaporator. The resulting residue was dissolved in 5 mL of acetate buffer (pH 2.0), transferred to plastic specimen bottles, and stored in the freezer. Afterward, sixty microliters (60  $\mu\text{L}$ ) of the hydrolysate were introduced into the cartridge of the analyzer (manufacturer: Applied Biosystem Inc., model: 120A; country: USA 4347131), specifically designed for the separation and analysis of free acidic, neutral, and basic amino acids present in the hydrolysate (AOAC, 2006). The attached integrator to the analyzer computes the peak area, which is proportional to the concentration of each amino acid.

## 2, 20-Azinobis-3-ethylbenzothiazoline-6-sulphonate cation radical scavenging capacity of the hydrolysates

The effect of the hydrolysate on 2, 20-azinobis-3-ethylbenzothiazoline-6-sulphonate Cation (ABTS<sup>+</sup>) radical was determined by the ABTS cation decolourization assay described by (Re et al., 1999). The ABTS<sup>+</sup> radical was produced by combining 7 mM ABTS in water with 2.45 mM potassium persulfate (in a 1:1 ratio), and this mixture was kept in the dark at room temperature for 12-16 hours prior to use. Subsequently, the ABTS<sup>+</sup> solution was diluted with methanol to reach an absorbance of 0.7 at 734 nm. Following the addition of 5  $\mu\text{L}$  of hydrolysates (1.0 – 5.0 mg/mL) to 4 mL of diluted ABTS<sup>+</sup> solution, the absorbance was measured after 30 minutes. A suitable solvent blank was included in each assay. The experiment was conducted in triplicates. The hydrolysates' ability to neutralize the ABTS<sup>+</sup> radical was determined using the equation 9:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100 \quad (1)$$

## 2,2-Diphenyl-1-picrylhydrazine radical scavenging capacity of the hydrolysates

The effect of the hydrolysates on 2, 2-diphenyl-1-picrylhydrazine (DPPH) radical was determined according to the method described by (Brand-Williams et al., 1995) with minor adjustments. A DPPH radical solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. To this solution, 5  $\mu\text{L}$  of the hydrolysates (1.0 – 5.0 mg/mL) were added to 4 mL of DPPH dissolved in methanol. The mixture was vigorously agitated and allowed to stand at room temperature for 30 minutes in darkness. The absorbance of the reaction mixture was then determined spectrophotometrically at 515 nm. Additionally, the absorbance of the DPPH radical without any antioxidant (blank) was measured. The experiment was repeated in triplicate, and the ability of the hydrolysates to neutralize the DPPH radical was computed using equation 10:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100 \quad (2)$$

## Ferric ion reducing antioxidant power of the hydrolysates

The reducing capacity of the hydrolysates on ferric ion was determined according to the method described by (Benzie

& Strain, 1996). The ferric ion reducing antioxidant power (FRAP) reagent utilized in the FRAP assessment was created by combining 300 mM acetate buffer, 10 mL of TPTZ in 40 mM HCl, and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a ratio of 10:1:1 at 37  $^{\circ}\text{C}$ . The freshly prepared working FRAP reagent (3.995 mL) was mixed with 5  $\mu\text{L}$  of the hydrolysates (1.0 – 5.0 mg/mL). A vibrant blue color complex was generated as the ferric tripyridyl triazine ( $\text{Fe}^{3+}$  TPTZ) complex was reduced to the ferrous ( $\text{Fe}^{2+}$ ) form. The absorbance at 593 nm was measured against a reagent blank after 30 minutes of incubation at 37  $^{\circ}\text{C}$ . The calibration curve, correlating absorbance at 593 nm with different concentrations of  $\text{FeSO}_4$ , was established. The experiment was conducted in triplicates, and the concentrations of  $\text{FeSO}_4$  produced by the standard (ascorbate) and hydrolysates were extrapolated from the calibration curve, expressed in mM  $\text{Fe}^{2+}$  equivalent.

## Data presentation and statistical analysis

The data were presented as the mean  $\pm$  standard deviation (SD) of three replicates. Statistical analysis was performed using Analysis of Variance (ANOVA) followed by Tukey's multiple range test with GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). IC50 values were calculated using Microsoft Excel 2016 through linear regression. A significance level of  $p < 0.05$  was considered statistically significant.

## RESULTS

### Amino acid profile and classification of *Cucumeropsis mannii* Naudin protein hydrolysates

Tables 1 and 2 present the amino acid composition and categorization of hydrolysates derived from *C. mannii* seeds using pancreatin and pepsin, respectively. As can be observed in Table 1, the most abundant amino acid and the least abundant amino acid in the two hydrolysates were glutamic acid and tryptophan, respectively. However, asparagine and glutamine were absent in the two hydrolysates. Based on the chemical nature of the side chain, the most abundant and the least abundant classes of amino acids in the hydrolysates were hydrophobic amino acids and positively charged (acidic) amino acids, respectively (Table 2). Based on the chemical structure of the side chain, the most abundant and the least abundant classes of amino acid in the hydrolysates were branched-chain amino acids and sulphur-containing amino acids, respectively (Table 2). Based on the nutritional requirements of the amino acids, essential amino acids were slightly more abundant than non-essential amino acids in the hydrolysate derived using pancreatin. Conversely, in the hydrolysate obtained using pepsin, non-essential amino acids were slightly more prevalent than essential amino acids (Table 2).

### 2, 20-Azinobis-3-ethylbenzothiazoline-6-sulphonate cation radical scavenging capacity of *Cucumeropsis mannii* Naudin seed protein hydrolysates

The 2, 20-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS<sup>+</sup>) radical scavenging capacities of ascorbic acid, pancreatin-derived hydrolysate, and pepsin-derived hydrolysate are shown in Figure 1. Ascorbic acid exhibited

significantly higher ( $p < 0.05$ ) ABTS<sup>+</sup> radical scavenging capacity compared to the hydrolysates across the tested concentrations (1.0 to 5.0 mg/mL).

Notably, the ABTS<sup>+</sup> radical scavenging capacity of ascorbic acid remained consistent across these concentrations ( $p > 0.05$ ). In contrast, the ABTS<sup>+</sup> radical scavenging capacity of pancreatin-derived hydrolysate showed a significant increase ( $p < 0.05$ ) from 1.0 to 4.0 mg/mL, reaching a peak at 4.0 mg/mL and 5.0 mg/mL. The highest observed ABTS<sup>+</sup>

radical scavenging capacity was at 4.0 mg/mL, with a value of  $91.71 \pm 0.45\%$ , while the lowest was at 1.0 mg/mL, with a value of  $19.97 \pm 0.36\%$ . In the case of pepsin-derived hydrolysate, its ABTS<sup>+</sup> radical scavenging capacity increased significantly ( $p < 0.05$ ) from 1.0 to 3.0 mg/mL. There was no significant difference in radical scavenging activity between 3.0 mg/mL to 4.0 mg/mL but it increased afterwards at the highest concentration considered (5 mg/mL). Consequently, the highest ABTS<sup>+</sup> radical scavenging

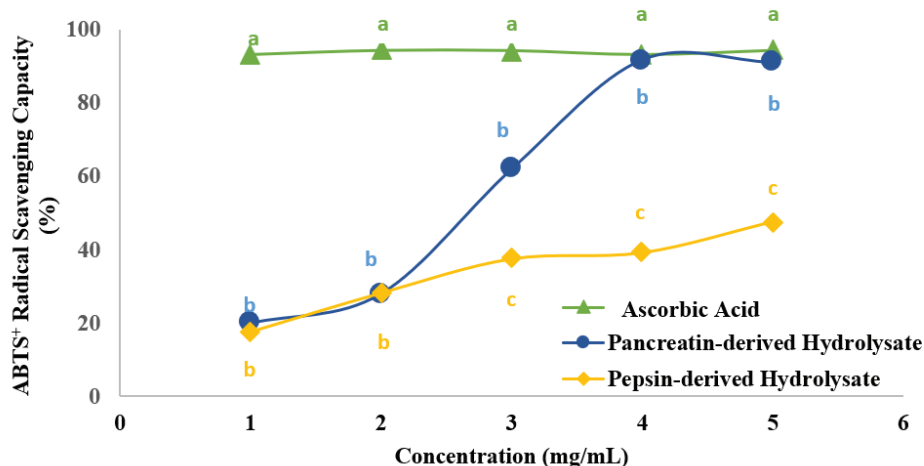
**Table 1:** Amino acid profile of *Cucumeropsis mannii* Naudin seed protein hydrolysates

| Amino Acids   | Pancreatin-derived Hydrolysate (g/100g protein) | Pepsin-derived Hydrolysate (g/100g protein) |
|---------------|-------------------------------------------------|---------------------------------------------|
| Leucine       | 5.23                                            | 9.32                                        |
| Lysine        | 3.64                                            | 4.19                                        |
| Isoleucine    | 3.90                                            | 3.31                                        |
| Phenylalanine | 1.95                                            | 2.91                                        |
| Tryptophan    | 0.89                                            | 1.58                                        |
| Valine        | 3.16                                            | 4.09                                        |
| Methionine    | 1.61                                            | 2.27                                        |
| Proline       | 3.25                                            | 3.05                                        |
| Arginine      | 5.33                                            | 6.37                                        |
| Tyrosine      | 2.93                                            | 3.61                                        |
| Histidine     | 3.80                                            | 2.23                                        |
| Cysteine      | 1.64                                            | 2.12                                        |
| Alanine       | 3.80                                            | 5.32                                        |
| Glutamic Acid | 7.20                                            | 10.61                                       |
| Glycine       | 3.35                                            | 4.33                                        |
| Threonine     | 3.61                                            | 3.70                                        |
| Serine        | 3.13                                            | 4.49                                        |
| Aspartic Acid | 6.11                                            | 7.11                                        |
| Glutamine     | ND                                              | ND                                          |
| Asparagine    | ND                                              | ND                                          |

ND: Not Detected

**Table 2:** Amino acid classification of *Cucumeropsis mannii* Naudin seed protein hydrolysates

| Classification                                                          | Pancreatin-derived Hydrolysate (g/100 g protein) | Pepsin-derived Hydrolysate (g/100 g protein) |
|-------------------------------------------------------------------------|--------------------------------------------------|----------------------------------------------|
| <b>Based on the Chemical structure of the side chain amino acid</b>     |                                                  |                                              |
| Aromatic amino acids                                                    | 5.77                                             | 8.1                                          |
| Branched chain amino acids                                              | 12.29                                            | 16.72                                        |
| Sulphur containing amino acids                                          | 3.25                                             | 4.39                                         |
| <b>Based on the chemical nature of the side chain of the amino acid</b> |                                                  |                                              |
| Hydrophobic amino acids                                                 | 26.72                                            | 35.46                                        |
| Positively charged amino acids (Acidic)                                 | 12.77                                            | 12.79                                        |
| Negatively charged amino acids (Basic)                                  | 13.31                                            | 17.72                                        |
| <b>Based on the nutritional requirement of the amino acid</b>           |                                                  |                                              |
| Essential amino acids                                                   | 33.12                                            | 39.97                                        |
| Non-essential amino acids                                               | 31.41                                            | 40.64                                        |



**Figure 1:** 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS<sup>+</sup>) radical-scavenging capacity of *Cucumeropsis mannii* Naudin seed protein hydrolysates. Values represent the mean of triplicate determinations  $\pm$  standard deviation (SD). Dots representing different protein hydrolysates at the same concentration, but marked with different letters, indicate a significant difference at  $p < 0.05$

capacity was observed at 5.0 mg/mL with  $47.68 \pm 0.51\%$ , while the least was observed at 1.0 mg/mL with  $17.63 \pm 0.46\%$ .

At the same concentrations, the ABTS<sup>+</sup> radical scavenging activities of the hydrolysates were significantly different ( $p < 0.05$ ) at 1.0 mg/mL, 3.0 mg/mL, 4.0 mg/mL, and 5.0 mg/mL, but no significant difference was observed at 2.0 mg/mL. In instances of significant difference, the ABTS<sup>+</sup> radical scavenging activity of the pancreatin-derived hydrolysate surpassed that of the pepsin-derived hydrolysate. The 50% inhibitory concentration ( $IC_{50}$ ) values of the hydrolysates in scavenging ABTS<sup>+</sup> radicals are illustrated in Figure 4. Notably, the  $IC_{50}$  of pancreatin-derived hydrolysate ( $2.58 \pm 0.01$  mg/mL) was significantly lower ( $p < 0.05$ ) than that of pepsin-derived hydrolysate ( $5.24 \pm 0.03$  mg/mL).

#### 1, 1 Diphenyl-1-picrylhydrazyl radical scavenging capacity of *Cucumeropsis mannii* Naudin seed protein hydrolysates

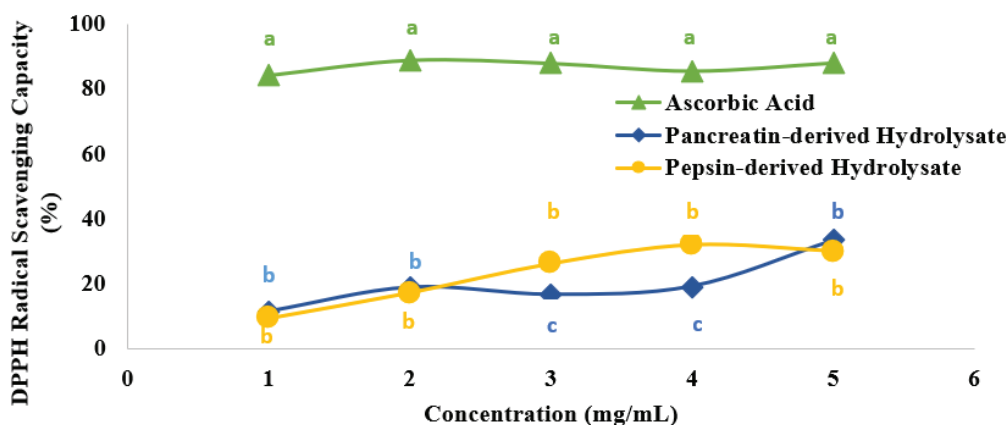
The 1, 1 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacities of ascorbic acid, pancreatin-derived and pepsin-derived hydrolysates are shown in Figure 2. The DPPH radical scavenging capacity of ascorbic acid was significantly higher ( $p < 0.05$ ) than that of the hydrolysates across the examined concentrations (1.0 to 5.0 mg/mL). However, the DPPH radical scavenging capacity of ascorbic acid at these concentrations exhibited no significant variation ( $p > 0.05$ ). In the case of pancreatin-derived hydrolysate, its DPPH radical scavenging capacity exhibited a gradual increase from 1.0 to 5.0 mg/mL. Consequently, the highest DPPH radical scavenging capacity was observed at 5.0 mg/mL, amounting to  $33.52 \pm 0.11\%$ , while the lowest was noted at 1.0 mg/mL, with  $11.43 \pm 3.27\%$ . Conversely, in the case of pepsin-derived hydrolysate, its DPPH radical scavenging capacity significantly increased ( $p < 0.05$ ) from 1.0 to 3.0 mg/mL, and stabilized thereafter until the highest concentration considered (5.0 mg/mL); no significant difference ( $p >$

0.05) in the DPPH radical scavenging capacity of the hydrolysate was observed from 3.0 to 5.0 mg/mL. The highest DPPH radical scavenging capacity was recorded at 4.0 mg/mL, amounting to  $31.81 \pm 0.42\%$ , while the lowest was observed at 1.0 mg/mL, with  $9.43 \pm 2.32\%$ .

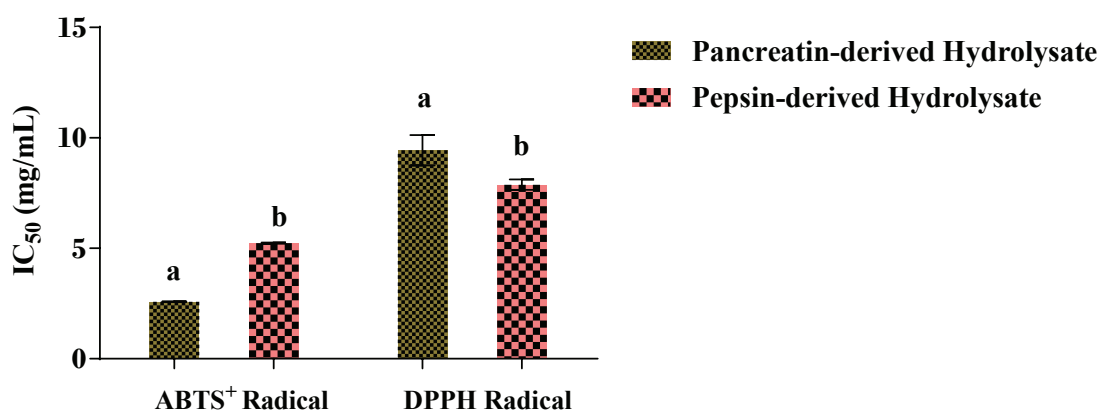
When comparing the hydrolysates at equivalent concentrations, the DPPH radical scavenging capacity of the hydrolysates did not display significant differences ( $p > 0.05$ ) at 1.0 mg/mL, 2.0 mg/mL, and 5.0 mg/mL, but did exhibit significance at 3.0 and 4.0 mg/mL. At these points of notable difference, specifically at 3.0 and 4.0 mg/mL, the pepsin-derived hydrolysate was significantly higher ( $p < 0.05$ ) compared to the pancreatin-derived hydrolysate. The 50% inhibitory concentration ( $IC_{50}$ ) values of *C. mannii* seed protein hydrolysates in scavenging DPPH radicals are illustrated in Figure 3. As observed, the  $IC_{50}$  of pancreatin-derived hydrolysate ( $9.44 \pm 0.69$  mg/mL) was significantly higher ( $p < 0.05$ ) than that of pepsin-derived hydrolysate ( $7.88 \pm 0.24$  mg/mL).

#### Ferric ion reducing antioxidant power of *Cucumeropsis mannii* Naudin seed protein hydrolysates

The ferric ion reducing antioxidant abilities of ascorbic acid, pancreatin-derived, and pepsin-derived hydrolysates are shown in Figure 4. The ferric ion reducing antioxidant power exhibited by ascorbic acid was significantly higher ( $p < 0.05$ ) compared to that of the hydrolysates within the concentrations evaluated (1.0 – 5.0 mg/mL). However, there were no significant differences ( $p > 0.05$ ) observed in the ferric ion reducing antioxidant power of ascorbic acid across these concentrations. In the case of the pancreatin-derived hydrolysate, although no significant differences ( $p > 0.05$ ) were noted in the ferric ion reducing antioxidant power at 1.0 and 2.0 mg/mL, a significant difference ( $p < 0.05$ ) was observed from 3.0 to 5.0 mg/mL. The peak ferric ion reducing antioxidant power of the hydrolysate was registered at 3.0 mg/mL, measuring  $27.50 \pm 1.48$  mM  $Fe^{2+}$  equivalent, while the lowest was observed at 1.0 mg/



**Figure 2:** 1, 1 diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity of *Cucumeropsis mannii* seed protein hydrolysates. Values represent the mean of triplicate determinations  $\pm$  standard deviation (SD). Dots representing different protein hydrolysates at the same concentration, but marked with different letters, indicate a significant difference at  $p < 0.05$



**Figure 3:** Fifty percent inhibitory concentration ( $IC_{50}$ ) values of *Cucumeropsis mannii* Naudin seed protein hydrolysates in scavenging 2, 2 diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS<sup>+</sup>) radicals. Values represent the mean of triplicate determinations  $\pm$  standard deviation (SD). Values with different letters are significantly different at  $p < 0.05$

mL, with  $10.38 \pm 0.82$  mM  $Fe^{2+}$  equivalent. Conversely, for the pepsin-derived hydrolysate, the ferric ion reducing antioxidant power progressively increased from 1.0 to 5.0 mg/mL.

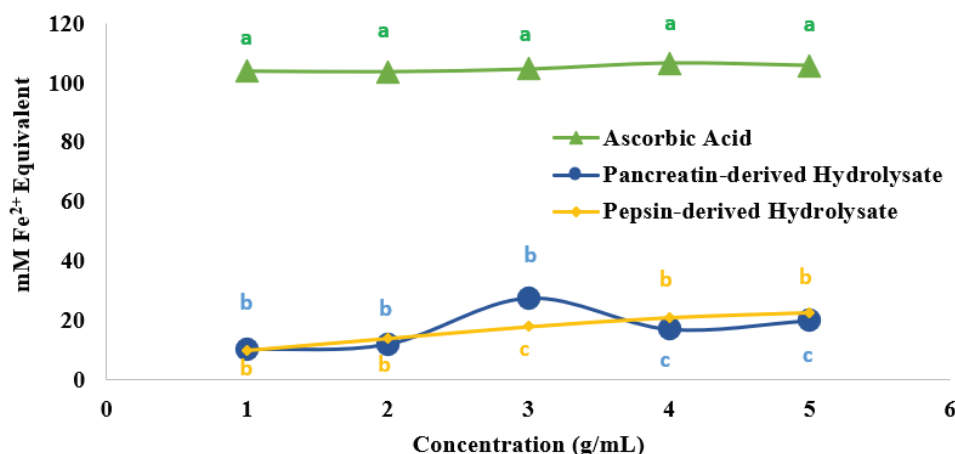
Significant differences ( $p < 0.05$ ) were noted in the ferric ion reducing antioxidant power of the hydrolysate from 1.0 – 3.0 mg/mL, but no significant difference ( $p > 0.05$ ) was observed between 4.0 and 5.0 mg/mL. The highest ferric ion reducing antioxidant power was achieved at the highest concentration, at 5.0 mg/mL, with  $22.80 \pm 1.45$  mM  $Fe^{2+}$  equivalent, while the lowest was observed at 1.0 mg/mL with  $9.80 \pm 1.10$  mM  $Fe^{2+}$  equivalent.

At equivalent concentrations, the ferric ion reducing antioxidant power of both pancreatin-derived and pepsin-derived hydrolysates did not show significant differences ( $p > 0.05$ ) at 1.0 mg/mL and 2.0 mg/mL. However, significant differences ( $p < 0.05$ ) were observed between 3.0 and 5.0 mg/mL. At these points of notable disparity, the pepsin-derived hydrolysate demonstrated a higher

ferric ion reducing antioxidant power compared to the pancreatin-derived hydrolysate at two concentrations (4.0 mg/mL and 5.0 mg/mL). Conversely, at 3.0 mg/mL, the pancreatin-derived hydrolysate exhibited a higher ferric ion reducing antioxidant power compared to the pepsin-derived hydrolysate.

## DISCUSSION

The amino acid composition of protein hydrolysates is closely linked to their bioactivity (Chauhan & Kanwar, 2020). The two protein hydrolysates (Tables 1 and 2) had all the essential amino acids in amounts comparable with the FAO/WHO (1991) benchmark (23.20 g/100 g) (FAO/WHO, 1991). Essential amino acids, which include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, must be obtained through diet as they cannot be produced by the human body (Sanz et al., 2019; Lopez & Mohiuddin, 2024). The nine essential amino acids are vital for various



**Figure 4:** Ferric-reducing antioxidant power of *Cucumeropsis mannii* Naudin seed protein hydrolysates. Values represent the mean of triplicate determinations  $\pm$  standard deviation (SD). Dots representing different protein hydrolysates at the same concentration, but marked with different letters, indicate a significant difference at  $p < 0.05$

physiological functions, including protein synthesis and metabolic regulation (Lopez & Mohiuddin, 2024). Furthermore, amino acid supplements are popular among athletes for their role in stimulating muscle protein synthesis and reducing exercise-induced muscle damage (Sanz et al., 2019; Kaspy et al., 2024). Compositions containing essential amino acids can aid in recovery from malnutrition and support individuals with specific dietary needs, such as infants or those recovering from illness (Jini et al., 2020; Negro et al., 2024). Still more, amino acids, especially sulfur-containing ones such as methionine and cysteine, play a crucial role in the production of glutathione, a key antioxidant responsible for neutralizing reactive oxygen species (ROS) (Bin et al., 2017). Therefore, the abundant presence of both essential and non-essential amino acids (Tables 1 and 2) in the hydrolysates highlights their potential as a valuable nutraceutical. Indeed, the radical scavenging capacity and ferric-ion reducing ability observed in this study can be attributable to the amino acid composition of the hydrolysates (Ngoh et al., 2017; Udenigwe & Aluko, 2011).

The 2, 20-azino-bis-3-ethylbenzothiazoline-6-sulphonate (ABTS) cation radical and 1, 1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods are uniquely rapid means of estimating the free radical scavenging abilities of bioactive compounds (Babatunde et al., 2022b). The ABTS<sup>+</sup> radical scavenging activity of a bioactive compound correlates strongly with its DPPH radical scavenging capacity because both radicals can accept electrons and H<sup>•</sup> from available antioxidants (Braca et al., 2003; Ismail et al., 2004). Although these radicals do not exist in biological systems, their usefulness in the ranking of novel antioxidants has received wide acceptability (Santos-Sánchez et al., 2019). Free radical production facilitates lipid peroxidation, non-enzymatic glycation of proteins, and oxidation of glucose, which aggravates diabetes mellitus and its complications (Asmat et al., 2016).

Fortunately, protein hydrolysates and isolated bioactive peptides have shown encouraging free radical scavenging properties (Lorenzo et al., 2018). In this study, ascorbate

yielded higher ABTS<sup>+</sup> radical and DPPH radical scavenging capacities than the hydrolysates (Figures 1 and 2). However, the radical scavenging activities of the hydrolysates were quite encouraging. The ability of the hydrolysates to scavenge free radicals can be partly attributed to their amino acid composition (Hwang & Winkler-Moser, 2017; Wang et al., 2020). In particular, a high level of hydrophobic amino acids (alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, and phenylalanine) has been correlated with radical scavenging capacity through hydrophobic interaction between free radicals and active functional groups on these amino acids, thereby, making free radicals unavailable for interaction with other molecules (Udenigwe & Aluko, 2011). Furthermore, negatively charged amino acids (glutamate and aspartate) have been suggested to confer radical scavenging activity to hydrolysates due to the abundance of electrons that can be donated to quench free radicals (He et al., 2013). In addition, aromatic amino acids (tyrosine, tryptophan, and phenylalanine) have been suggested to potentiate the radical scavenging ability of hydrolysates by donating protons to stabilize electron-deficient radicals while retaining their stability through resonance structures (Sarmadi & Ismail, 2010).

Benzie and Strain (1996) introduced the ferric ion reducing antioxidant power method, to assess the antioxidant capacity of bioactive compounds (Benzie & Strain, 1996). It involves the reduction of ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>), forming a blue complex (Santos-Sánchez et al., 2019). This method augments other methods of antioxidant estimation and mainly involves the electron transfer ability of novel antioxidants. Due to its acceptability, the ability of bioactive compounds to scavenge free radicals produced associated with several pathologic conditions can be estimated by this method. Consequently, several studies have chronicled the ferric ion reducing antioxidant power of hydrolysates and isolated bioactive peptides (Lorenzo et al., 2018).

In this study, ascorbic acid yielded more overwhelming reducing power than the hydrolysates (Figure 4).

Nevertheless, the hydrolysates exhibited reducing abilities worthy of note (Figure 4). Several studies have indicated that both the amino acid composition and the size of peptides are significant factors contributing to the ferric ion reducing ability of hydrolysates (Ajibola et al., 2011; Carrasco-Castilla et al., 2012; Li et al., 2008; Udenigwe & Aluko, 2011; Vaštag et al., 2011). In particular, Udenigwe and Aluko (2011) stated that amino acid residues with sulfur and acidity positively influence the ferric ion reducing properties of peptides (Udenigwe & Aluko, 2011). In addition, studies by Li et al. (2008) and Ajibola et al. (2011) have shown that peptides of smaller size display superior reducing abilities compared to those with higher molecular weights. Therefore, the reducing power observed in this study may be attributable to the amino acid composition and size of the peptides present in the hydrolysates (Tables 2 and 3). The implicated amino acids residues have similar abundance in the hydrolysates; therefore, the slight discrepancy in the reducing power of the hydrolysates may be because of the differences in the arrangement of the amino acids.

## CONCLUSIONS

This study demonstrates that protein hydrolysates obtained from *Cucumeropsis mannii* seeds contain significant amounts of both essential and non-essential amino acids, along with promising antioxidant properties. Consequently, these hydrolysates have the potential to function as an amino acid supplement for illness-related malnutrition and as antioxidant adjuvants in mitigating oxidative stress linked to various health conditions. Additional research is needed to isolate and identify the specific bioactive peptides in the hydrolysates and evaluate their biological activity, especially their ability to scavenge free radicals.

## DECLARATIONS OF CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

## ACKNOWLEDGMENTS

The authors thank the Central Research and Diagnostic Laboratory, led by Dr. Nnemeka Asogwu, for offering the laboratory space and equipment necessary to conduct this research.

## FUNDING INFORMATION

No funding received for this work.

## AUTHOR CONTRIBUTIONS

All authors have accepted responsibility for the content of the manuscript, reviewed all results, and approved the final version. Conceptualization, Funding acquisition: **O.T.B.; C.O.B.; A.I.**;

Data collection and Statistical analysis: **O.T.B.**; Data interpretation: **O.T.B.**; Writing the first draft: **O.T.B.**; Manuscript editing and reviewing: **O.T.B., A.I.**; Project administration: **C.O.B.; A.I.**

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