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Protein Hydrolysates from *Cucumeropsis mannii* Seed Inhibit Alpha-amylase *In-vitro*: Potential for Managing Postprandial Hyperglycemia

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Abstract

Diabetes mellitus is a significant global cause of mortality. A hallmark of diabetes pathophysiology is postprandial hyperglycaemia (PPH). PPH is defined as a sudden and exponential increase in blood glucose levels after meals, typically above 140 mg/dL, which does not return to pre-meal levels after two to three hours, leading to glucose toxicity, oxidative stress, cardiovascular risks, and diabetes complications. A management option for PPH is the inhibition of carbohydrate-metabolizing enzymes, such as alpha-amylase. However, current inhibitors are associated with gastrointestinal side effects. Therefore, the search for novel inhibitors is a rational research endeavor. Recent studies highlight plant-derived protein hydrolysates as inhibitors of alpha-amylase. In this study, proteins from *Cucumeropsis mannii* (*C. mannii*) were isolated using alkaline solubilization-acid precipitation method and enzymatically cleaved using pepsin and pancreatin to yield *C. mannii* seed protein hydrolysates. The α-amylase inhibitory property of the hydrolysates was investigated, using starch as the substrate. The IC_{50} values for α-amylase inhibition were 8.77 ± 0.35 mg/mL (pancreatin-derived) and 14.80 ± 0.50 mg/mL (pepsin-derived). Kinetic studies indicated uncompetitive inhibition for pancreatin-derived hydrolysate and mixed uncompetitive for pepsin-derived hydrolysate at 9 mg/mL. These results suggest that *C. mannii* seed protein hydrolysates may aid in postprandial hyperglycemia management through α-amylase inhibition.

Keywords: Diabetes Mellitus; Alpha-amylase Inhibition; Postprandial hyperglycaemia; Protein Hydrolysates; *Cucumeropsis mannii* Seed.

INTRODUCTION

One of the hallmarks of diabetes mellitus is Postprandial hyperglycaemia (PPH). It is characterized by a significant and swift increase in blood glucose levels, commonly exceeding 140 mg/dL, after a meal, which does not return to pre-meal levels for two to three hours (American Diabetes Association, 2024; Maffettone et al., 2018). Typically, PPH results from defective insulin secretion and/or action, leading to increased glucose levels after meals (Pinés Corrales et al., 2020). In PPH, chronic hyperglycaemia exacerbates oxidative stress, leading to glucose toxicity as well as the destruction of the pancreatic β-cells and vascular tissues, which lead to diabetic complications such as retinopathy and nephropathy (Campos, 2012). Furthermore, PPH is associated with increased cardiovascular risks, resulting from endothelial dysfunction, owing to the fluctuation in postprandial glucose levels (Gerich, 2013). Due to the pathophysiology of PPH, its management in diabetes mellitus has become a significant focus.

Effectively managing PPH involves inhibiting carbohydrate-metabolizing enzymes, such as alpha-amylase. Alpha-amylase is a key enzyme in carbohydrate metabolism, responsible for breaking down starch into simpler sugars, primarily in the mouth as salivary alpha-amylase and in the pancreas as pancreatic alpha-amylase (Butterworth et al., 2011). This breakdown process increases the availability of glucose, leading to a rise in blood sugar levels following a meal, known as postprandial hyperglycaemia. This postprandial spike in blood glucose can exacerbate oxidative stress, contributing to cellular and tissue damage and the progression of diabetic complications (Brownlee, 2005; Ceriello, 2005). Inhibiting alpha-amylase slows down the digestion of carbohydrates, thereby reducing the rapid increase in blood glucose levels after meals. This can help manage postprandial hyperglycaemia and mitigate oxidative stress, ultimately aiding in better overall

glucose control and reducing the risk of long-term complications (Nickavar & Yousefian, 2022).

Unfortunately, many commercially available alpha-amylase inhibitors are associated with gastrointestinal side effects such as bloating, flatulence, abdominal pain, and diarrhea (Kalinovskii et al., 2023; Liu et al., 2024). Therefore, the search for alternative inhibitors is a reasonable research endeavor. Fortunately, protein hydrolysates have been observed to possess alpha-amylase inhibitory properties without the side-effects associated with the current pharmaceutical collections. Protein hydrolysates are mixtures of amino acids, peptides, and oligopeptides obtained by breaking down proteins through enzymatic hydrolysis. This process involves the use of proteases to cleave the peptide bonds within protein molecules, resulting in smaller, more easily absorbed protein fragments (Clemente, 2000). Peptides, specifically known as bioactive peptides, present in protein hydrolysate are highly therapeutic and free of known side effects, making them deserving of significant attention (Korhonen & Pihlanto, 2006; Udenigwe & Aluko, 2012).

One plant seed, whose attributes make it a probable source of bioactive peptides, is *Cucumeropsis mannii* seed. The properties that make this plant seed outstanding include its folkloric use as an antidiabetic regimen, its protein content (33.8% to 38.17% of the dry weight), and its amino acid composition, consisting of nearly all essential and non-essential amino acids (Besong et al., 2011; Ezuruike & Prieto, 2014). Despite these promising attributes, there has been minimal scientific investigation to establish its therapeutic significance conclusively. To the best of our knowledge, there are no published articles on the therapeutic benefits of protein hydrolysates from *Cucumeropsis mannii* seed. Consequently, this study examined the alpha-amylase inhibitory property of protein hydrolysates derived from *Cucumeropsis mannii* seed and its kinetics to understand the mechanism of inhibition.

MATERIALS AND METHODS

Materials

Fully developed seeds of *C. mannii* were acquired from a local market situated in Mopa, Kogi State, Nigeria; and were authenticated at the Department of Plant Biology Herbarium, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria, and assigned a voucher number UILH/003/1420/2021. Analytically graded chemicals and reagents were used without further purification. Pepsin (from porcine gastric mucosa), pancreatin, α-amylase (*Penicillium fellutanum*), and bovine serum albumin were products of Sigma-Aldrich Chemicals, USA, while maltose, dinitrosalicylic acid, and starch were products of Loba-Chemie Biotech., India. 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* Seed Protein

The seeds of *C. mannii* were dried, ground into powder, and stored in an air-tight container at 4°C. The resulting powder was then defatted using n-hexane, following the procedure described by (Wani et al., 2011). The defatted seed obtained was subsequently dried at room temperature and ground to fine powder. The fine powder was refrigerated for future use. The method described by (Alashi et al., 2014) was employed for protein extraction from the defatted seed. In this process, the defatted *C. mannii* seed 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 facilitate alkaline solubilization. Next, the mixture underwent centrifugation at 18°C and 3000 rpm for 10 minutes. Subsequently, two more extractions of the residue from the centrifugation process were conducted using an equivalent volume of 0.1 M NaOH, and the resultant supernatants were pooled together. The pH of the combined supernatant was adjusted to 4.0 to facilitate acid-induced protein precipitation, using 1 M HCl solution. The formed precipitate was recovered through centrifugation, washed with distilled water, adjusted to pH 7.0 using 1 M NaOH, freeze-dried, and the resulting protein isolate was refrigerated until needed for further analysis. Following isolation, the protein yield was determined as described by (Arise et al., 2015) using equation 1.

$$\%\ \text{Protein Yield} = \frac{\text{Mass of protein isolate (g)}}{\text{Mass of defatted } C.mannii \text{ seed meal (g)}} \times 100\ (\%) \qquad \text{(Equation 1)}$$

Preparation of *Cucumeropsis mannii* Seed Protein Hydrolysates

The protein isolate was hydrolyzed according to the method of (Udenigwe et al., 2009), as documented by (Onuh et al., 2015). Separate hydrolysis of the protein isolate was conducted utilizing pancreatin (at pH 8.0 and 37 ºC) and pepsin (at pH 2.2 and 37 ºC). The protein isolate (5% w/v) was dissolved in distilled water, forming distinct protein slurries for pancreatin and pepsin. Enzymes were introduced to the respective slurries at an enzyme-substrate ratio (E: S) of 1:100. Digestions were carried out under the specified

conditions for 6 hours with continuous stirring, followed by enzyme inactivation through immersion of the reaction vessels in boiling water (95 – 100 ºC) for 15 minutes. Undigested proteins were precipitated by adjusting the pH to 4.0 with 2 M HCl/2 M NaOH, succeeded by centrifugation at 4000 rpm for 30 minutes. The supernatant, containing target peptides, was collected and subjected to analysis for degree of hydrolysis and percentage peptide yield. This supernatant was refrigerated until further analysis. The protein content of the samples was determined using the biuret assay method of (Gornall et al., 1949), with bovine serum albumin (BSA) serving as the standard.

Determination of Degree of Hydrolysis

The degree of hydrolysis was evaluated by calculating the ratio of soluble peptides in 10% trichloroacetic acid (TCA) to the total protein content of the protein isolate, using a technique detailed by (Hoyle, 1994) with slight modifications. Five hundred microliters (500 µL) of protein hydrolysates were combined with 500 µL of 20% TCA to generate 10% TCA soluble material. After allowing the mixtures to stand for 30 minutes to induce precipitation, centrifugation was performed (3500 rpm for 15 minutes). The supernatant was then examined for protein content using the biuret assay method of (Gornall et al., 1949), utilizing bovine serum albumin (BSA) as a standard. The experiment was conducted in triplicates. The degree of hydrolysis (DH) was calculated using Equation 2.

$$\text{Degree Hydrolysis (\%)} = \frac{\text{Soluble peptide in 10\% TCA (mg mL}^{-1})}{\text{Total protein content of isolate (mg mL}^{-1})} \times 100 \qquad \text{(Equation 2)}$$

Determination of Peptide Yield

The percentage yield of peptides was estimated following the procedure described by (Girgih et al., 2011). The experiment was conducted in triplicates. The peptide yield (%) of the hydrolysates was computed as the ratio of the weight of peptides in each hydrolysate to the protein weight of the protein isolate, expressed by Equation 3:

$$\text{Peptide Yield (\%)} = \frac{\text{Peptide weight of each hydrolysate (mg mL}^{-1})}{\text{Protein weight of lyophilized isolate (mg mL}^{-1})} \times 100 \qquad \text{(Equation 3)}$$

Determination of α-Amylase Inhibition of the Hydrolysates

The alpha-amylase inhibitory assay was carried out according to the method of (Bernfeld, 1951), as documented by (Oboh et al., 2011). In test tubes, 125 µL of hydrolysate (pancreatin/pepsin) at concentrations ranging from 1.0 to 9.0 mg/mL were mixed with 125 µL of 20 mM sodium phosphate buffer (pH 6.9, with 6 mM NaCl) containing α-amylase solution (0.5 mg/mL). Following pre-incubation at 25 °C for 10 minutes, 125 µL of 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9, with 6 mM NaCl) was added at specific intervals. The reaction mixtures were then incubated at 25 °C for 10 minutes. Termination of the reaction occurred with the addition of 250 µL of dinitrosalicylic acid (DNS) colour reagent, followed by further incubation in boiling water for 5 minutes and subsequent cooling to room temperature. The contents of each test tube were diluted with 2.5 mL of distilled water, and the absorbance was recorded at 540 nm. A control was also prepared following the same procedure, but substituting the hydrolysate with distilled water. The experiment was carried out in triplicate, and the α-amylase inhibitory activity was calculated using equation 4.

$$\text{\% Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100\ (\%) \qquad \text{(Equation 4)}$$

The determination of the concentration of hydrolysate leading to a 50% inhibition of α-amylase activity (IC_{50}) involved plotting the percentage inhibition (y-axis) against concentrations (x-axis) using Microsoft Excel Version 2016, employing linear regression. The intercept (C) and the gradient (M) were obtained from the line of

best fit curve. The 50% Inhibitory concentration (IC_{50}) was mathematically derived according to equation 5.

$$50\%\ \text{Inhibitory concentration (IC50)} = \frac{50 - \text{Intercept}}{\text{Gradient}}(\text{mg/mL}) \quad \text{(Equation 5)}$$

Determination of Kinetic Parameters of Alpha-amylase Inhibition of the Hydrolysates

The kinetic investigation of α-amylase inhibition by the hydrolysates was carried out following the procedure described by (Ali et al., 2006) with minor adjustments. In one set of tubes, 125 µL of the hydrolysates (9 mg/mL) was pre-incubated with 125 µL of α-amylase solution for 10 minutes at 25 ºC. Simultaneously, in another set of tubes, 250 µL of phosphate buffer (pH 6.9) was pre-incubated with 125 µL of α-amylase solution. Subsequently, starch solution (125 µL) at varying concentrations (1.0 – 8.0 mg/mL) was introduced into both sets of reaction mixtures to initiate the reaction. After a 10-minute incubation at 25 ºC, the reaction was terminated by boiling for 5 minutes following the addition of 250 µL of DNS reagent. The amount of reducing sugars released was quantified spectrophotometrically using a maltose standard curve and converted to reaction velocities using equation 6.

$$\text{Specific Activity (µmol/ /min)} = \frac{[\text{Maltose released}]}{\text{Incubation time X } M_E} \quad \text{(Equation 6)}$$

M_E = Amount (in mg) of enzyme in the reaction mixture
Incubation time: 10 minutes

A reciprocal plot was generated, depicting 1/[Specific Activity] on the y-axis against 1/[Starch] on the x-axis. The mode of inhibition and the kinetic parameters (K_m and V_{max}) associated with α-amylase inhibition by the hydrolysates were elucidated through analysis of the double reciprocal plot using Microsoft Excel Version 2016.

Calculations:

The maximum velocity (V_{max}) and Michaelis constant (K_m) were derived from the double reciprocal plot of 1/ [Specific Activity] (y-axis) against 1/ [Starch] (x-axis) using Microsoft Excel Version 2016. From the line of best fit, the intercept (C) and the gradient (M) were obtained for the presence and absence of hydrolysate. The values of the V_{max} and K_m were derived according to equations 7 and 8, respectively.

$$\text{Maximum velocity (Vmax)} = \frac{1}{\text{Intercept on the y-axis}} \quad \text{(Equation 7)}$$

$$\text{Michaelis Constant (Km)} = V_{max} \times \text{Gradient} \quad \text{(Equation 8)}$$

Data Analysis

Data were expressed as the mean of three replicates ± standard deviation (SD). The data were subjected to Analysis of Variance and Tukey's multiple range tests, utilizing GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). The IC_{50} values were determined using Microsoft Excel Version 2016, employing linear regression. Statistical significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Protein Yield of *Cucumeropsis mannii* Seed and Protein Content, Degree of Hydrolysis and Peptide Yield of *Cucumeropsis mannii* Seed Protein Isolate

Following the extraction of protein from *C. mannii* seeds, a protein yield of 22.8% was obtained, comprising 94.8% protein content. Table 1 illustrates the degree of hydrolysis and the resultant peptide yield from the protein hydrolysis of *C. mannii* seeds. The degree of hydrolysis obtained using pancreatin was significantly

higher ($p < 0.05$) compared to pepsin. However, no significant difference ($p > 0.05$) was observed in the peptide yields between the two enzymes utilized for the hydrolysis of *C. mannii* seed protein.

Percentage Alpha-Amylase Inhibitory Effects of *Cucumeropsis mannii* Protein Hydrolysates

The percentage α-amylase inhibition and 50% α-amylase inhibitory concentration (IC_{50}) of pancreatin-derived and pepsin-derived hydrolysates is shown in Figure 1. In the case of pancreatin-derived hydrolysate, its α-amylase inhibitory effect increased progressively from 1.0 mg/mL to 9.0 mg/mL. Although, the inhibitory effect of the hydrolysate was not significantly different ($p > 0.05$) between 1.0 mg/mL and 3.0 mg/mL (4.21 ± 0.37% and 5.75 ± 1.64% respectively), there was a significant difference ($p < 0.05$) as the concentration progressed from 3.0 mg/mL to 9.0 mg/mL (15.83 ± 4.44% to 54.50 ± 1.82%).

In a similar vein, the α-amylase inhibitory effect of pepsin-derived hydrolysate increased progressively, with an increase in concentration from 5 mg/mL to 9.0 mg/mL. Although, there was no inhibitory effect observed at 1.0 and 3.0 mg/mL, there was observable inhibitory effect as the concentration progressed from 5 mg/mL to 6.0 mg/mL and 9 mg/mL (7.57 ± 1.86%, 16.74 ± 2.70% and 24.43 ± 2.32%). The inhibitory effect of pancreatin-derived hydrolysate from 5 mg/mL to 9 mg/mL was significantly higher ($p < 0.05$) than that of pepsin-derived hydrolysate. The 50% α-amylase inhibitory concentration (IC_{50}) values were 8.767 ± 0.35 mg/mL and 14.80 ± 0.50 mg/mL for pancreatin-derived and pepsin-derived hydrolysates, respectively (Figure 1).

Kinetics of α-amylase Inhibition of *Cucumeropsis mannii* Protein Hydrolysates

The Lineweaver-Burk plots of α-amylase-catalyzed hydrolysis of starch in the absence and presence of pancreatin-derived and pepsin-derived hydrolysates are shown in Figures 2 and 3, respectively. The modes of inhibition exhibited by pancreatin-derived and pepsin-derived hydrolysates were uncompetitive and mixed uncompetitive inhibitions, respectively. The estimation of kinetic parameters, maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) are shown in Table 2.

Table 1. The degree of hydrolysis and peptide yield following the hydrolysis of Cucumeropsis mannii seed proteins

Proteolytic Enzyme	Degree of Hydrolysis (%)	Peptide Yield (%)
Pancreatin	15.63 ± 0.37[a]	19.55 ± 0.08[a]
Pepsin	10.36 ± 0.46[b]	18.73 ± 0.66[a]

Values represent the mean of triplicate determinations ± standard deviation (SD). Values with different letters across the column are significantly different at $p < 0.05$.

Table 2. Kinetic parameters of α-amylase-catalyzed hydrolysis of starch in the absence (control) and presence of *Cucumeropsis mannii* seed protein hydrolysates

Kinetic Parameter	Control (No Hydrolysate)	Pancreatin-derived Hydrolysate (9.0 mg/mL)	Pepsin-derived Hydrolysate (9.0 mg/mL)
V_{max} (μmol/mg/min)	46.08	6.48	10.53
K_m (mg/mL)	20.10	2.48	2.28

V_{max}: Maximum reaction rate in the presence and absence of hydrolysates

K_m: Michaelis Constant in the presence and absence of hydrolysates

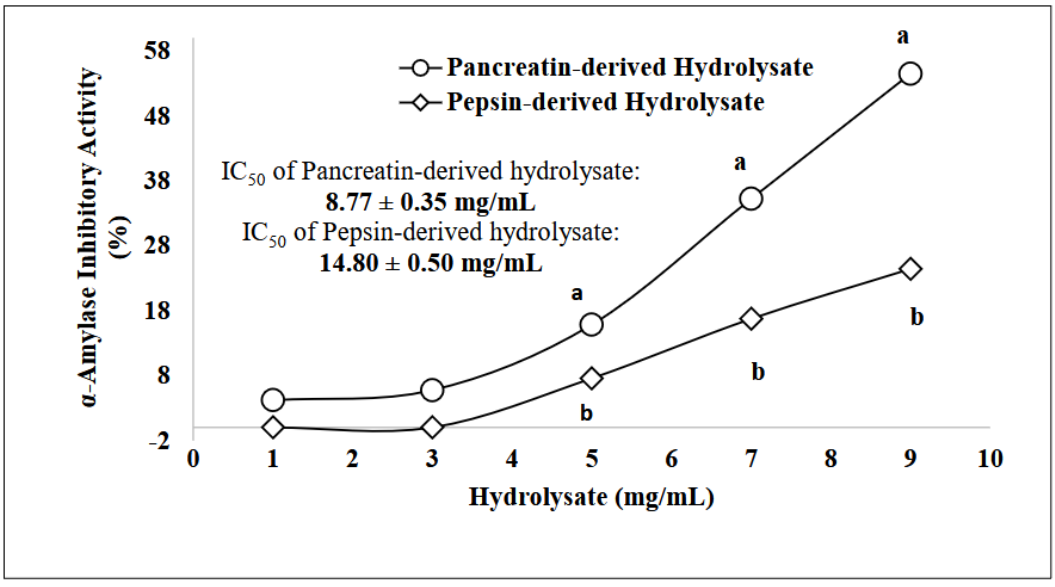

Figure 1. Alpha-amylase inhibitory activity of Cucumeropsis mannii seed protein hydrolysates. Values represent the mean of triplicate determinations ± standard deviation (SD). Inset: Fifty percent α-amylase-inhibitory concentration (IC_{50}) values of Cucumeropsis mannii seed protein hydrolysates. Values represent the mean of triplicate determinations ± standard deviation (SD). Dots representing different protein hydrolysates at the same concentration, but marked with different letters, indicate a significant difference at $p < 0.05$.

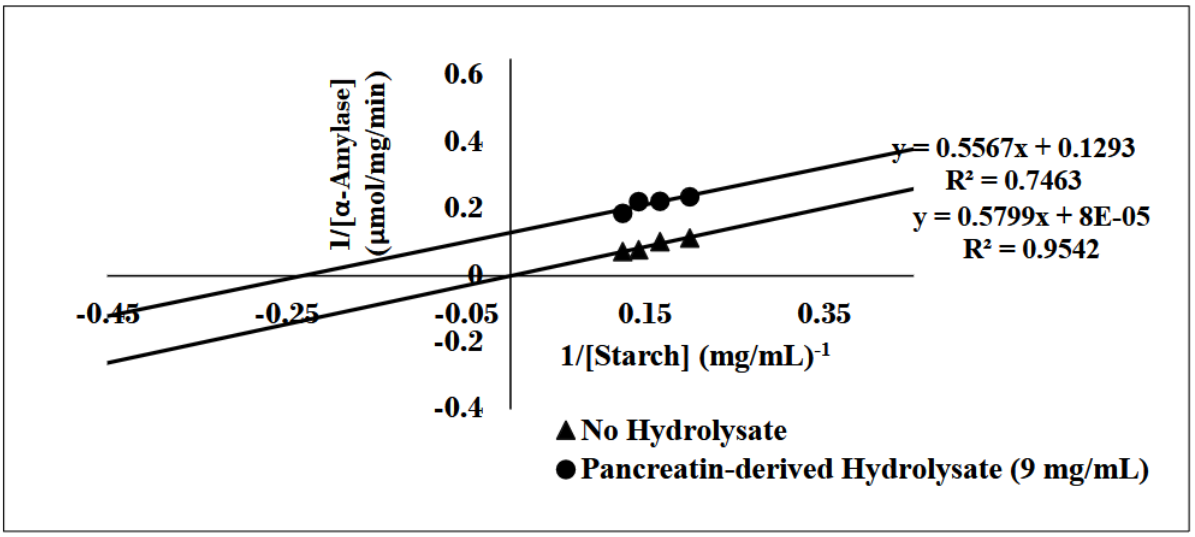

Figure 2. Lineweaver-Burk plot of the effect of *Cucumeropsis mannii* seed hydrolysate derived from pancreatin digestion on α-amylase-catalyzed hydrolysis of starch.

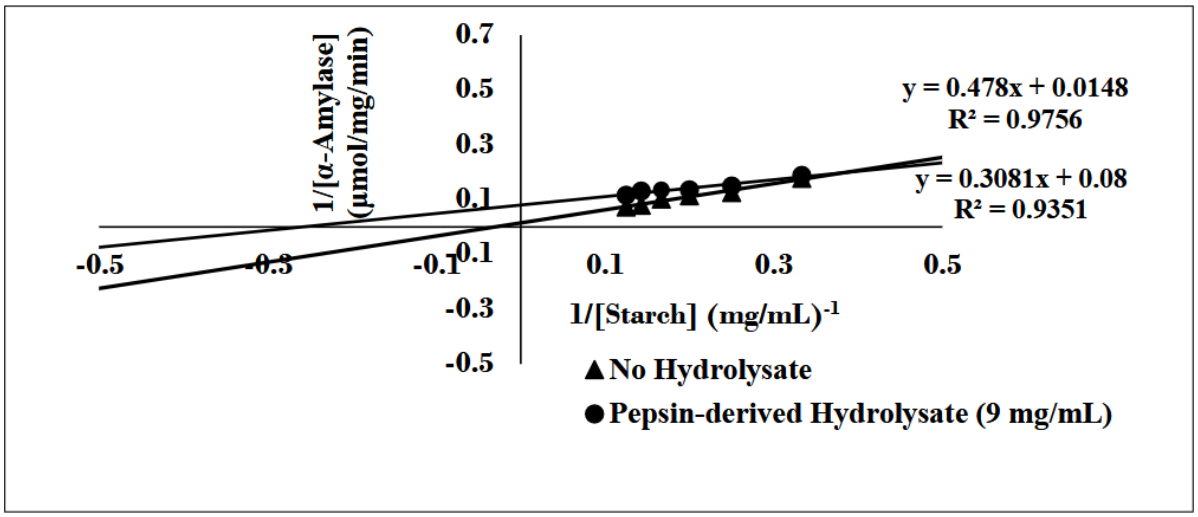

Figure 3. Lineweaver-Burk plot of the effect of *Cucumeropsis mannii* seed hydrolysate derived from pepsin digestion on α-amylase-catalyzed hydrolysis of starch.

The maximum velocity (V_{max}) of starch hydrolysis catalyzed by α-amylase in the presence of pancreatin- and pepsin-derived hydrolysates decreased by roughly 7-fold and 4-fold, respectively, compared to the control (Table 2). In addition, the Michaelis-Menten constant (K_m) of starch hydrolysis catalyzed by α-amylase in the presence of pancreatin- and pepsin-derived hydrolysates decreased by approximately 8-fold and 9-fold, respectively, compared to the control (Table 2).

Discussion

Protein Yield of *Cucumeropsis mannii* Seed and Protein Content, Degree of Hydrolysis and Peptide Yield of *Cucumeropsis mannii* Seed Protein Isolate

The percentage protein yield accounts for the amount of protein obtained in a protein extraction procedure. It is often a function of the extraction method employed (Boye et al., 2010). In this study, the alkaline solubilization-acid precipitation method which extracts the glutelin fraction of seed storage proteins was utilized (Osborne, 1924). The percentage protein yield (22.8%) of *C. mannii* seed obtained in this study was higher than that of pawpaw seed (5.13%), neem seed (12.52%), moringa seed (18.60%), watermelon seed (18.91%), *Polyalthia longifolia* seed (2.23%), and *Annona muricata* seed (7.1%) which utilized the same method of protein extraction (Arise et al., 2019; Arise et al., 2016; Babatunde et al., 2022a; Babatunde et al., 2022b; James et al., 2020a; Jamesa et al., 2020b; Olusola et al., 2018). Previous studies have credited hydrolysates derived from the glutelin fractions of seed proteins with incredible biological activities (Arise et al., 2019; Arise et al., 2016;

Babatunde et al., 2022a; Babatunde et al., 2022b; James et al., 2020a; Jamesa et al., 2020b; Olusola et al., 2018). The high percentage protein content of the isolate (94.8%) obtained in this study indicates the effectiveness of the extraction process. Indeed, alkaline solubilization-acid precipitation method often results in protein isolate with high protein content. Previous studies on the extraction of protein from watermelon seed and Bambara groundnut using the acid precipitation method resulted in isolates with relatively high protein content. However, the value obtained in this study was higher than that of watermelon seed (74%) and Bambara groundnut (79%) (Arise et al., 2015; Arise et al., 2016).

The degree of hydrolysis (DH) accounts for the amount of peptide bonds cleaved during protein hydrolysis. It is influenced by the type of enzyme used, the enzyme/substrate (E/S) ratio, pH value, temperature, and time of hydrolysis (Benítez et al., 2008). Concerning the type of enzyme used, pancreatin effectively hydrolyzed *C. mannii* protein isolate more than pepsin (Table 1). The effectiveness of pancreatin may be attributable to its protease mix, which includes endopeptidases (trypsin, chymotrypsin and elastase) and exopeptidases (carboxypeptidases A and B) (Andriamihaja et al., 2013). With such a collection of proteases, it is no surprise that it yielded a higher DH than pepsin, which is a single enzyme. The DH value obtained from the hydrolysis of *C. mannii* seed protein using pancreatin (E/S of 2:100) (Table 1) was found to be lower than the DH value obtained from the hydrolysis of black cumin protein (at 20%) using the same enzyme (E/S 2:100) (Shahi et al., 2020). In addition, the value of DH obtained from the hydrolysis of *C. mannii* seed protein by pepsin (E/S of 2: 100) (Table 1) was lower than that obtained from the hydrolysis of moringa seed protein (26.93%) by the same enzyme (E/S of 2: 100) (Olusola et al., 2018). The difference in the DH observed with the use of the same enzyme and E/S ratio might be attributable to the difference in the amino acid composition of the protein isolates. Hence, black cumin protein has more amino acid residues specific for pancreatin than *C. mannii* seed protein; also, moringa seed protein has more amino acid residues specific for pepsin than *C. mannii* seed protein.

The peptide yield provides information on the number of peptides produced after protein hydrolysis and the efficiency of the hydrolytic process (Alashi et al., 2014). In this study, the difference in the peptide yield observed with the use of pancreatin and pepsin was not significant (Table 1). Hence, it is clear that the higher DH observed with the use of pancreatin compared to pepsin did not significantly affect the peptide yield. It can be rationalized that the carboxypeptidases present in pancreatin did not influence the peptide yield because they cleave the C-terminal amino acids resulting in the production of free amino acids rather than peptides (Naik, 2012). Therefore, the peptide yield resulting from the use of pancreatin might be due to the three endopeptidases in it, and the ability of pepsin to produce peptides was at par with that of the proteases in pancreatin.

Cucumeropsis mannii Protein Hydrolysates Inhibited Alpha-amylase-catalyzed Reactions: Potential for Postprandial Hyperglycaemia Management

The inhibition of α-amylase is an approach utilized in the management of postprandial hyperglycemia associated with type 2 diabetes mellitus (Qin et al., 2011). Although there are commercially available α-amylase inhibitors, however, they are associated with gastrointestinal side effects such as bloating, diarrhea, and abdominal pain (Chiasson et al., 2002). Fortunately, plant-derived protein hydrolysates have demonstrated therapeutic efficiency with limited side effects (Arise et al., 2019; Arise et al., 2016; Babatunde et al., 2022a; Babatunde et al., 2022b; Ishida et al., 2011; Ismail et al., 2004; James et al., 2020a; Jamesa et al., 2020b; Olusola et al., 2018). The results obtained in this study further reinforces the assertion of many researchers that protein hydrolysates have promising therapeutic abilities (Nasri, 2017). The hydrolysates exhibited concentration-dependent inhibitory effects against α-amylase (Figure 1). Leucine and proline, both hydrophobic amino acids, along with arginine, a hydrophilic amino acid, have been identified in previous studies as contributors to the inhibition of α-amylase (Jiang et al., 2018; Ramadhan et al., 2017; Ren et al., 2016; Wang et al., 2020; Zhang et al., 2007). Specifically, Wang *et al.* (2020) emphasized the importance of these three amino acids, either individually or in synergy within peptides, in the inhibition of α-amylase. Therefore, the inhibitory effect of the hydrolysates on α-amylase can be attributed to the abundance of these amino acids within them.

Meanwhile, there is a possibility that other hydrophobic amino acids such as alanine, phenylalanine, valine, and glycine, as well as hydrophilic amino acids like cysteine, histidine, and serine, played no mean role in potentiating the α-amylase inhibitory abilities of the hydrolysates. This notion is plausible because of an earlier study that illustrated the interaction of hydrophobic and hydrophilic amino acids with the active sites of human salivary α-amylase, resulting in its inhibition through hydrophobic and hydrogen bonds (Ngoh et al., 2017). The observed range of inhibition in this study, as depicted in Figure 1, is considered appropriate, as excessive inhibition of α-amylase is undesirable. Kumar et al. (2011) emphasized that an excessive inhibition of α-amylase could lead to abnormal bacterial fermentation of undigested carbohydrates in the colon (Kumar et al., 2011). Hence, mild α-amylase inhibition effects are deemed more beneficial. The 50% inhibitory concentration (IC_{50}) for the inhibitory effects observed in this study (Figure 1) was higher than that of most hydrolysates studied previously (Arise et al., 2019; Arise et al., 2016; James et al., 2020a; Jamesa et al.,

2020b; Olusola et al., 2018). This implies that higher concentrations of the hydrolysates are needed to achieve desirable inhibitory effects. Notwithstanding the higher IC_{50} values, the inhibition afforded by the hydrolysates is promising. More so, peptides can be administered at high doses without eliciting grievous side effects (Ishida et al., 2011; Ismail et al., 2004).

Kinetic parameters derived from the Lineweaver-Burk plots of α-amylase inhibition by the hydrolysates were employed in determining the mechanism of the inhibition. Pancreatin-derived hydrolysate exhibited uncompetitive inhibition pattern, while pepsin-derived hydrolysate exhibited mixed inhibition pattern (Figures 2 and 3). In uncompetitive inhibition, there is a reduction in the K_m and V_{max} of an enzyme-catalyzed reaction (Nelson & Cox, 2008). Hence, this study suggests that pancreatin-derived hydrolysate enhanced the binding of the substrate (starch) to the enzyme and was bound to the enzyme-substrate (E/S) complex formed. Similarly, in mixed uncompetitive inhibition, the inhibitor binds to the enzyme-substrate (E/S) complex (Nelson & Cox, 2008); hence the reduction in the K_m and V_{max} (Table 2) of the α-amylase-catalyzed reaction by pepsin-derived hydrolysate can be caused by the binding of the hydrolysate to the enzyme-substrate complex, in that case enhancing substrate binding. The inhibition pattern expressed by pancreatin-derived hydrolysate at 9.0 mg/mL was similar to that of trypsin-derived hydrolysate from *Moringa oleifera* seed protein at 1.0 mg/mL (Olusola et al., 2018); however, the V_{max} and K_m of the former were higher than that of the latter. In addition, the inhibition pattern expressed by pepsin-derived hydrolysate at 9.0 mg/mL was similar to that of pepsin-derived hydrolysate from Moringa oleifera seed protein at 1.0 mg/mL (Olusola et al., 2018), with the latter having a lower V_{max} and K_m than the former.

CONCLUSIONS

The study findings indicate that *Cucumeropsis mannii* seed protein hydrolysates display encouraging alpha-amylase inhibitory properties. This implies that the hydrolysates could potentially aid in controlling postprandial hyperglycemia associated with diabetes mellitus through α-amylase inhibition. Further research is necessary to fractionate the hydrolysates, characterize their effects, and identify the specific bioactive peptides they contain.

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