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Albizia lebbeck Seed Protein Hydrolysates Inhibit α-Amylase and α-Glucosidase in vitro

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Abstract

Albizia lebbeck seeds have been found to contain a high proportion of proteins, which on hydrolysis have yielded hydrolysates which contained bioactive peptides that possessed antioxidant activities in earlier studies. Hence, this study investigated the potentials of these hydrolysates in inhibiting two carbohydrate – hydrolyzing enzymes, α-amylase and α-glucosidase. *Albizia lebbeck* seed proteins were hydrolyzed using the proteinases trypsin, chymotrypsin and papain. The hydrolysates obtained were evaluated for their inhibitory effects against α-amylase and α-glucosidase. The results revealed that *Albizia lebbeck* seed proteins were most susceptible to chymotrypsin hydrolysis (degree of hydrolysis of 62.43±1.685%) when compared to those of trypsin and papain, which had DH values of 42.200±2.071 % and 18.89±2.495% respectively. However, hydrolysates obtained from papain hydrolysis exhibited the highest inhibitory activities against α-amylase (70.453±1.619%) whereas tryptic digests inhibited α-glucosidase better than chymotrypsin and papain hydrolysates (55.354±0.808%). The result also suggest that proteinase specificity influenced the relative enzyme-inhibitory activities of the resulting hydrolysates, in terms of the nature of peptides released. The study concludes that *Albizia lebbeck* seed proteins, on proteolysis with appropriate enzymes, possess potentially therapeutic peptides which can be further characterized towards the development of provide peptide-based alternatives in the management of diabetes mellitus.

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Keyword
Albizia lebbeck; hydrolysates; α-amylase; α-glucosidase; diabetes mellitus

Introduction

The normalization of concentrations of glucose in the blood is vital to the management of diabetes mellitus. Changes in lifestyle and nutritional adjustments have been recommended as a non-pharmacologic intervention to improving glycaemic control. Also,carbohydrate-degrading enzymes, glucose transport proteins among others have been pharmacologic targets in the treatment of this metabolic disorder. Yu *et al.,* 2012, Sharifuddin *et al.,* 2015; Ekun *et al.,* 2022). A number of oral and injectable hypoglycemic drugs have formulated to this effect, but they are not without their disadvantages. The rising costs of procurement and certain undesirable effects of these medications such as nausea, abdominal problems, hepatic lesions, among other effects have been major drawbacks associated with their continued use (Olusola and Ekun, 2019[a,b]). Hence, the role of natural products as cost-

effective alternatives in diabetes management have since been considered in a variety of studies both *in vitro* and in animal models (Sharifuddin *et al.,* 2015; Daou et al., 2022; Silva *et al.,* 2022).

In the recent time, there have been increased emphasis on the exploration of peptides and peptide-tbased preparations for their therapeutic potentials (Majumder and Wu, 2015). These peptides and protein hydrolysates are frequently gotten from enzyme-assisted proteolysis or microbe-mediated fermentations of storage and structural proteins of plant and animal origins (Ulagesan *et al.,* 2018; Olusola and Ekun, 2019[a]). The biological activities of the resulting peptides are dependent on one or more conditions such as protease specificity, hydrolysis time, peptide size and sequence among other factors (Arise *et al.,* 2016[a]; Olusola *et al.,* 2018, Ekun, 2023). They have been found to possess a myriad of health promoting benefits. They have been demonstrated to possess antimicrobial properties (Ulagesan *et al.,* 2018, Ekun *et al.,* 2021), antihypertensive activities (Arise *et al.,* 2016[a]), antioxidant effects (Olusola *et al.,* 2018), antineoplastic properties (Gaspar *et al.,* 2013), other enzyme-inhibitory activities (Idowu *et al.,* 2021) among other numerous potentials.

Albizia lebbeck occurs as a leguminous tree plant which is a member of the Fabaceae family (Subfamily: Mimosoideae). Alternatively, this plant is known as the Siris tree or the Indian walnut, and it is indigenous to several locations in Asia, Australia and Sub-Saharan Africa (Verma *et al.,* 2013; Latif *et al.,* 2019). Various parts of the plant contain important secondary metabolites with potential health promoting properties. Its roots contain tannins and flavonoids (Verma *et al.,* 2013; Zia-Ul-Haq *et al,* 2013), whereas phytochemical analysis of its leaves have revealed that they have significant amounts of phytosterols (which have potentials in reducing cholesterol levels), in addition to tannins (Bobby et al., 2012). *A. lebbeck* seeds have been reported to contain abundant alkaloids, glycosides as well as proteins (Musa *et al.,* 2020; Ibrahim *et al.,* 2022).

Albizia lebbeck seeds, leaves and roots have been demonstrated to possess numerous ethnomedicinal benefits, as evidenced by both folkloric medicine and different studies. Its seeds for instance have been demonstrated to possess hypoglycemic, antioxidant and anti-inflammatory effects in certain studies (Kumar *et al,* 2007). Its leaves have been used in the treatment of insect bites and in the alleviation of gastric ulcer in addition to their reported antimicrobial activities (Yadav *et al.,* 2011). The root extract of the plant has been utilized in folkloric medicine as a diuretic and as an antipyretic; as it is also reported to alleviate urinary tract infections and feverish episodes (Yadav *et al.,* 2011; Bobby *et al.,* 2012). Studies have estimated the protein content of *A. lebbeck* seed proteins to be in the range of 27% - 43% (Adubiaro *et al.,* 2011, Zia-Ul-Haq *et al,* 2013, Ibrahim *et al.,* 2022). However, there is an apparent dearth of substantive reports about the identities of proteins present in the seed.

Notwithstanding, its high protein content gives an indication that it is an adequate source of therapeutic peptides on hydrolysis with suitable proteases (Ekun, 2022). Amino acid analysis of *A. lebbeck* seed proteins revealed that it is rich in a number of cationic amino acids, especially arginine, lysine and histidine, as well as the anionic amino acids glutamate and aspartate. Hydrophobic amino acids such as valine, leucine are present, in addition to tyrosine and phenylalanine, the aromatic aminoacyl residues (Zia -Ul-Haq *et al.,* 2013). However, *A. lebbeck* seeds, just like other legume seeds, are deficient in the sulfur-containing amino acids – cysteine and methionine (Zia -Ul-Haq *et al.,* 2013, Ekun, 2022). Adubiaro *et al.,* (2011) reported that *A. lebbeck* seeds are also abundant in important inorganic elements such as calcium, potassium, magnesium, and sodium.

Recently, *A. lebbeck* seed protein digests have been evaluated for their antioxidant properties (Ekun, 2022). However, there has been no studies relating to the abilities of *A. lebbeck* protein hydrolysates to inhibit key enzymes involved in diabetes mellitus. Therefore, this study is focused on evaluating the potentials of *A. lebbeck* protein hydrolysates to inhibit two carbohydrases - α-amylase and α-glucosidase, for the development of cost-effective peptide-based alternatives in diabetes mellitus management.

Materials and Methods

Collection of Albizia lebbeck Seeds

Dried *A. lebbeck* seeds in pods were harvested from Lebbeck trees in the premises of Adekunle Ajasin University in Akungba Akoko, Ondo State, Nigeria. Voucher samples were submitted at the University's Department of Plant Science and Biotechnology, after they have been identified.

Reagents and Chemicals

The proteolytic enzymes used in this study were trypsin (from bovine pancreas), papain (from *Carica papaya*) and chymotrypsin (from bovine pancreas). The carbohydrate-hydrolyzing enzymes used were α-amylase (from *Saccharomyces cerevisiae*) and α-glucosidase (human). Trypsin, papain and α-amylase were procured from Kem Light Laboratories, Mumbai, India; whereas chymotrypsin and α-glucosidase were products of Sigma-Aldrich Laboratories, United Kingdom. All other chemicals and reagents used in this study were of analytical grade.

Methods

Extraction of Protein from Albizia lebbeck seeds

A. lebbeck seeds were removed from their pods and they were dried. They were subsequently pulverized and defatted by suspending in n-hexane. The seed meal was extracted twice with the solvent in a 1:10 ratio. The defatted meal was then dried at 40^{o}C in an oven and blended again into powder, stored at -10^{o}C. The protein from the defatted seed meal was isolated using the method described by Ekun (2023). The previously defatted powder was suspended in 0.5 moles of sodium hydroxide solution at pH 12, ratio 1:10. The suspension was stirred continuously for an hour to achieve alkali-assisted solubilization.

The solubilized suspension was centrifuged at 18°C and 3000 g for 10 minutes. One additional extraction of the residue was performed by using the same volume of 0.5 moles of NaOH and the supernatants from each centrifugation round were collected together. To facilitate protein precipitation, the pH of the supernatant was reduced to 4.0, using 0.5 moles of hydrochloric acid, and the resulting precipitate was recovered by means of centrifugation. The precipitate obtained was then washed with distilled water, adjusted to pH 7.0 using 0.1 M NaOH, freeze-dried and the protein isolate was then stored at -10°C until required for further analysis. After freeze-drying, The precipitate was analyzed for protein content using the Biuret assay method, with the use of bovine serum albumin as standard. In addition, the protein yield of isolation was computed as the ratio of the weight of freeze-dried protein isolate to the weight of the defatted seed meal expressed in percentage.

Protein Hydrolysis and Hydrolysate Preparation

The extracted protein was digested by utilizing the method reported by Ekun *et al.,* (2022). The conditions for hydrolysis were tailored to suit the optimal activity of each

protease. Enzymatic digestion was carried out by employing trypsin (pH 8.0, 37ºC), chymotrypsin (pH 8.0, 37ºC and papain (pH 6.0, 60ºC). Also, the isolated protein (5% w/v) was suspended in the requisite buffer (phosphate buffer, pH 8.0 for trypsin and chymotrypsin; as well as phosphate buffer, pH 6.0 for papain). Each enzyme was added to the suspension at an enzyme-substrate (E-S) ratio of 1:50. Proteolysis of the protein suspension in the was done for each of the enzymes at the stated conditions for eight (8) hours. Each enzyme activity was stopped by suspension in boiling in water bath (100^{o}C) for fifteen (15) minutes and undigested proteins were removed by precipitation by adjusting the pH to 4.0 with 2 M HCl and/or 2 M NaOH followed by centrifugation at 7000 g for 30 minutes. The resulting supernatants were collected (which contained the protein hydrolysates) after which the protein concentration of the samples were evaluated by means of the Biuret assay method.

Evaluation of Extent of Protein Hydrolysis

The method reported by Olusola and Ekun, (2019[a]) was used to evaluate the extent of enzyme-assisted hydrolysis. Summarily, 1 ml of the hydrolysate was added to 1 ml of 20% trichloroacetic acid, to obtain a 10% soluble mixture. The resulting suspensions were made to stand for half an hour to facilitate precipitation, after which they were centrifuged at a speed of 4000g for 20 minutes. The supernatants obtained were evaluated for protein concentration. The extent of hydrolysis was calculated as the ratio of hydrolysate mass dissolved in 10% trichloroacetic acid (in mg) to the total protein content of isolate (in mg), and the result expressed in percentage.

Determination of α-amylase Inhibition

An assay for α-amylase inhibition was conducted utilizing the methodology described by Ekun (2023). with a few minor adjustments. To summarize, test tubes were filled with 125 µL of hydrolysate (0.5 to 2.0 mg mL–1) and 250 µL of 20 mM sodium phosphate buffer (pH 6.9, with 6 mM NaCl) that included 0.5 mg/mL of α-amylase solution. Following a 10-minute pre-incubation period at 25 °C, 125 µL of 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9, with 6 mM NaCl) was added to each tube at predetermined intervals. For ten minutes, the reaction mixtures were incubated at 25 °C. After adding 250 µL of dinitrosalicylic acid (DNS) color reagent to stop the reaction, it was further incubated in boiling water for five minutes. After incubation, the mixture was allowed to cool till it reached room temperature. For the purpose of dilution, 2.5 mL of distilled water was added to each test tube and the absorbance at 540 nm was recorded. The same process was used to prepare a control, but distilled water was used in place of the hydrolysate. The percentage inhibitory activity of α-amylase was computed by dividing the ratio of the difference in absorbance of control and sample by absorbance of control and expressing the result in percentage. Using a web-based technique (https://www.aatbio.com/tools/ic50-calculator), the concentration of hydrolysate which caused a 50% inhibition of α-amylase activity (IC_{50}) was calculated from a graph of percentage inhibition against hydrolysate concentrations.

Determination of α-glucosidase Inhibition

The method outlined by Kim et al. (2005) was used to determine the inhibitory impact of the hydrolysates on α-glucosidase activity. Phosphoryl glucopyranoside (pNPG) substrate solution was made in 20 mM phosphate buffer at pH 6.9. 50 μL of the various hydrolysate concentrations were pre-incubated for 10 minutes with 100μL of Saccharomyces cerevisiae α glucosidase (1.0 U/mL). The reaction was then started by adding 50μL of 3.0 mM (pNPG) as a substrate that had been dissolved in 20 mM phosphate buffer (pH 6.9). After 20 minutes of

incubation at 37◦C, the reaction mixture was stopped by adding 2 mL of 0.1 M sodium trioxocarbonate (iv) solution. By measuring the yellow-colored para-nitrophenol produced from p-NPG at 405 nm, the activity of α-glucosidase was ascertained. The inhibitory influence of the hydrolysate on α-glucosidase was computed by dividing the ratio of the difference in absorbance of control and sample by absorbance of control and expressing the result in percentage.

From the plotting the percentage of inhibition against the hydrolysate concentrations, a web-based algorithm (https://www.aatbio.com/tools/ic50-calculator) was used to determine the concentration of hydrolysate that achieved a 50% inhibition of α-glucosidase activity.

Statistical Analysis

The means ± standard error of means of the three duplicate observations were used to display the results. Two statistical tests were used to analyze the data: One Way Analysis of Variance (ANOVA), which was followed by Tukey's Post-hoc tests. GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used to determine significance for differences between means of values at $p<0.05$.

Results and Discussion

Yield of *Albizia lebbeck* seed Protein Isolate

The percentage protein yield of isolation of *A. lebbeck* seed proteins was found to be 19.165%. Protein yield of isolation indicates an estimate of the amount of extractable protein from a protein-containing substance (Arise *et al.,* 2016[b]). A number of methods exist for the isolation of proteins, depending on the source of protein, either plant or animal origin; and each method has a bearing on the overall yield of the protein (Olusola and Ekun, 2019[a], Acho *et al.,* 2023). In this study, alkaline extraction followed by acid-induced, isoelectric precipitation was employed. In spite of certain studies which reported that the protein content of *A. lebbeck* seeds was in the range of 27% - 43% the protein yield in this study was lower than what was reported in previous reports (Adubiaro *et al.,* 2011, Zia-Ul-Haq *et al,* 2013, Ibrahim *et al.,* 2022),. However, the yield of protein isolation obtained was slightly higher than 18.60% and 18.91% determined for *Moringa oleifera* seed protein (Olusola *et al.,* 2018) and *Citrullus lanatus* seed protein (Arise *et al.,* 2016[b]) respectively. Certain proteins such as plant albumins are likely soluble in dilute acid, may have been lost during the precipitation process, leaving behind the globulins which are relatively insoluble to constitute the major mass of the protein precipitate. This may be the reason why protein yield obtained in this study is reduced.

Degree of Hydrolysis

The degrees of hydrolysis (DH) of *A. lebbeck* seed proteins as hydrolyzed by trypsin, chymotrypsin and papain were 42.200±2.071 %, 62.43±1.685% and 18.89±2.495% respectively, and they are illustrated in Figure 1. The degree of chymotrypsin hydrolysis was significantly higher($p<0.05$) than those of trypsin and papain; and trypsin had a higher ($p<0.05$) DH value than papain. The amount of amide (peptide) bonds broken in polypeptides and proteins is determined by the degree of hydrolysis. The extent to which the degree of hydrolysis (DH) influences the amino acid composition and sequences of the resulting peptides is important as it determines their eventual bioactivities (Jamdar *et al.,* 2010, Olusola *et al.,* 2018). The DH obtained with trypsin (42.200 ±2.071%) at an enzyme substrate-ratio of 1:50 was higher than 10.31 ± 0.07 %, 9.00 ± 0.63 % and 21.16 ± 0.76 % gotten for tryptic

hydrolyses of *M. oleifera* seed protein digests (Olusola *et al.,* 2018), cowpea seed protein hydrolysates (Olusola and Ekun, 2018) and *Cryptozona bistrialis* protein hydrolysates (Ulagesan *et al.,* 2018) at the same E-S ratio. The relatively high degree of tryptic hydrolysis could be attributed to the relative abundance of cationic aminoacyl residues – arginine and lysine – in the protein, which are sites for trypsin-catalyzed proteolysis, despite the high cleavage specificity of trypsin (Zia-Ul-Haq *et al.,* 2013; Voet *et al.,* 2016).

The degree of hydrolysis of *A. lebbeck* by Chymotrypsin (62.43±1.685%) which was the highest in this study was higher than the duo of chymotryptic digestions of whey protein with values of 38.66% and 57.34% respectively at E/S ratios of 1:100 and 1:50 respectively (Galvao *et al.,* 2001).

It was greater than the 11.2 ± 1.4% DH value found for buffalo casein chymotrypsin hydrolysates at a 1:100 E/S ratio (Shanmugam et al., 2015). Because chymotrypsin hydrolyzes aromatic aminoacyl residues preferentially at their C-terminal peptide links, it is possible that the observed variations result from distinct protein sources (animal proteins as against proteins from plant origin). This would explain why various numbers of sites may be susceptible to hydrolysis by chymotrypsin (Voet et al., 2016). Furthermore, it seems that in order to improve the degree of hydrolysis in relation to chymotrypsin proteolysis, a higher enzyme/substrate ratio is necessary.

Compared to other enzymes, papain is seldom used as a choice protease for hydrolysis of proteins for the purpose of producing bioactive peptides. It was used for this study because of its relative non-specificity, which in turn confers on the enzyme, the potential to yield many dissimilar peptides of varying aminoacyl compositions and sizes. Papain hydrolysates had a DH value of 18.89±2.495 at an E-S ratio of 1:50, which was the least of the DH values obtained in our study. This value is lower than 27.00 ± 0.50 % and 28.80 ± 0.57 % degrees of hydrolysis determined by Ulagesan and others (2018) papain hydrolysates of the giant snail, *Cryptozona bistrialis* at two different enzyme-substrate ratios of 1:100 and 2:100 respectively. The relatively lower DH of papain proteolysis could be that the protein is less susceptible to cleavage by papain, despite the enzyme being the least specific of the three hydrolytic enzymes employed.

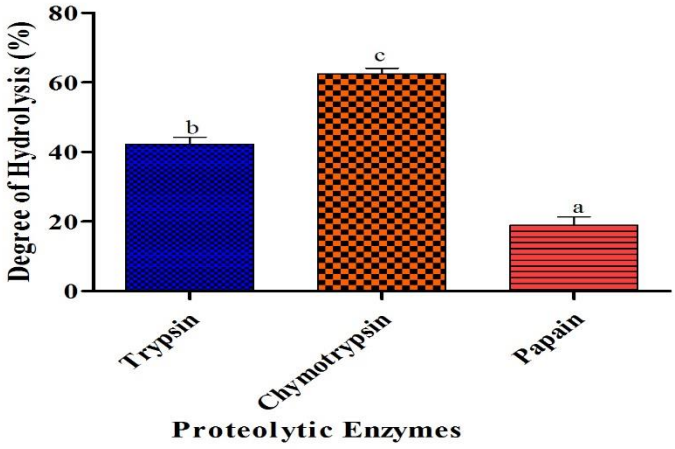

Figure 1. Degrees of *Albizia lebbeck* Seed Protein Hydrolysis by Proteolytic Enzymes

Each of the bars displayed represent the means ± standard error of the mean of three determinations made in triplicate. There is a substantial difference ($p<0.05$) between the bars that contain various letters. Bars with identical letters or symbols do not differ statistically ($p<0.05$).

α-Amylase Inhibitory Activity

The percentage inhibitory activities of *A. lebbeck* against α-amylase-catalyzed hydrolysis of starch to maltose, as well as their IC_{50} values are displayed in Figures 2 and 3 respectively. All hydrolysates exhibited a concentration-dependent inhibition of the enzyme. All hydrolysates attained maximum inhibition at the highest concentration of 2.0 mg/mL, with tryptic hydrolysates exhibiting a maximum α-amylase inhibition at 61.605±2.8.21%. Chymotrypsin and papain digests had achieved maximal inhibitions of 68.782±0.961 and 70.453±1.619% respectively, and these were not significantly different($p>0.05$) from each other.

However, these were significantly higher($p<0.05$) than that obtained by tryptic hydrolysates. Papain hydrolysate inhibited α-amylase to a 50% extent at a concentration of 1.405±0.170 mg/mL. This was significantly lower ($p<0.05$) than IC_{50} values of 2.026±0.133mg/mL and 1.994±0.061 mg/mL determined for tryptic and chymotrypsin digests respectively. The hydrolase, α-amylase cleaves α-(1-4) glycosidic bonds in several α-linked polysaccharides such as starch and glycogen, producing a mixture of glucose, maltose and isomaltose (Rodwell *et al.,* 2015). It is found as a component of both pancreatic juice and saliva of mammals, and in many seeds as well as in fungi (Voet *et al.,* 2016).

The inhibition of α-amylase activity slows down glucose release into the bloodstream, and hence this represents one of the therapeutic options in the treatment of diabetes mellitus (Katzung *et al.,* 2012). Tryptic digests of *A. lebbeck* seed protein digests had higher IC_{50} values (2.026±0.133mg/mL) than 0.591±0.025mg/ml, 0.234 mg/mL and 0.223±0.009 mg/ml obtained for tryptic hydrolysates of the trio of *M. oleifera* seed protein (Olusola *et al.,* 2018) *Citrullus lanatus* proteins (Arise *et al.,* 2016[b]) and Cowpea seed protein hydrolysates (Olusola and Ekun 2019[b]) respectively, translating to lesser activity than those of earlier studies. However, chymotrypsin hydrolysates of *A. lebbeck* inhibited the enzyme better (68.782±0.961%) than unfractionated chymotrypsin digests of *M. oleifera* seed protein (<60% inhibitory activity) (Ekun *et al.,* 2022). Papain hydrolysates had the best α-amylase inhibitory activities in this study, and it was higher than the activities of unfractionated *M. oleifera* seed protein digests which attained lesser α-amylase inhibitory activity at all study concentrations (Ekun *et al.,* 2023).

Albizia lebbeck seed proteins are rich in aromatic aminoacyl residues (Zia-Ul-Haq *et al.,* 2013) and this provides several cleavage sites for chymotrypsin hydrolysis (Voet *et al.,* 2016). In the same vein, it had been suggested in previous studies that peptides containing aromatic and/or cationic aminoacyl residues have potent α-amylase inhibitory properties (Arise *et al.,* 2016[b]). This could explain the inhibitory activities of tryptic and chymotryptic digests of *A. lebbeck* proteins. Papain, being relatively nonspecific when compared to the other two enzymes, may have produced several inhibitory peptides of varying peptide compositions and sizes, causing higher inhibition than peptides from the trypsin and chymotrypsin digestion (Ekun *et al.,* 2023). It is therefore suggested that in further studies, peptides to be designed against α-amylase should contain aromatic and/or positively charged aminoacyl residues.

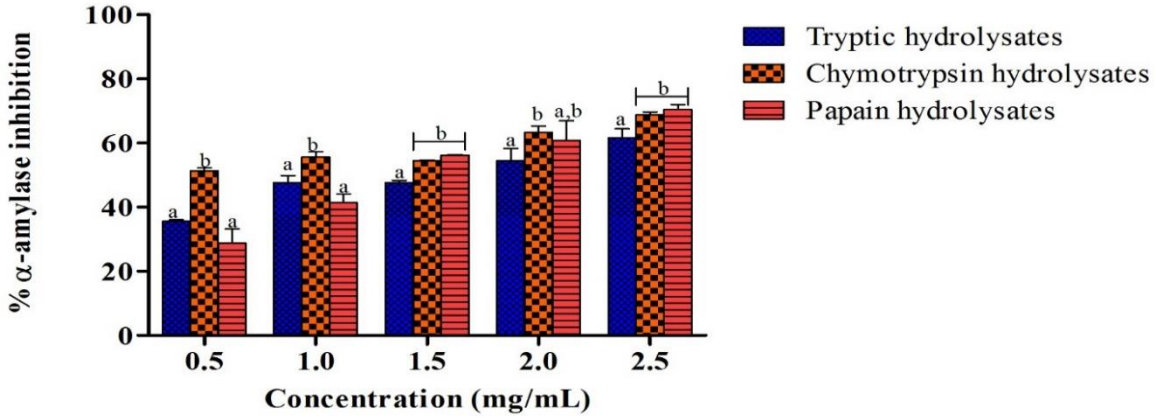

Figure 2. Alpha-amylase Inhibitory Activities of Albizia lebbeck Seed Protein Hydrolysates

Bars represent the means ± standard error of the mean of the three determinations made in triplicate. Bars with different letters differ from one another statistically significantly ($p<0.05$). Bars with identical letters or symbols do not differ statistically ($p<0.05$).

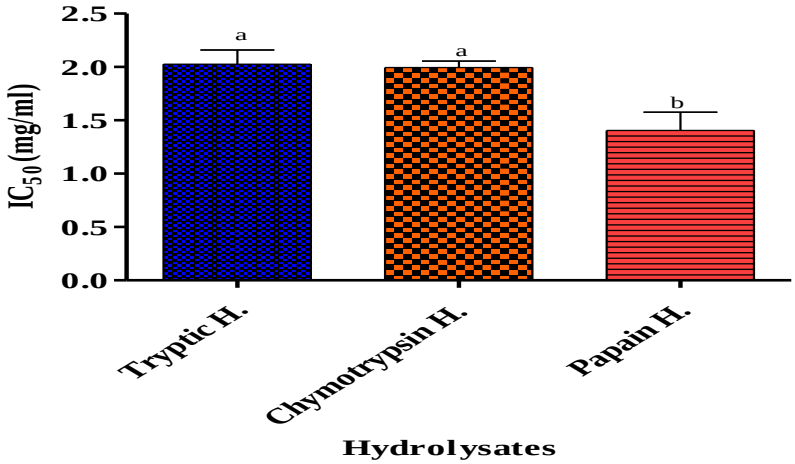

Figure 3. Fifty Percent Inhibitory Concentration (IC50) Values of α-Amylase Inhibitory Activities of Albizia lebbeck Seed Protein Hydrolysates

Each of the bars displayed represent the means ± standard error of the mean of three determinations made in triplicate. There is a substantial difference ($p<0.05$) between the bars that contain various letters. Bars with identical letters or symbols do not differ statistically ($p<0.05$).

α-Glucosidase Inhibitory Activity

The effects of *A. lebbeck* seed protein digests on the α-glucosidase-catalyzed hydrolysis of p-nitrophenyl glucopyranoside (p-NPG) to 2,4-dintrophenol are illustrated in Figure 4, and their IC_{50} values are displayed in Figure 5. The protein digests displayed dissimilar inhibitory patterns as concentration increased. Only tryptic hydrolysates exhibited an inhibitory extent above 50% and it was significantly ($p<0.05$) higher than the activities of other hydrolysates. In addition, tryptic hydrolysates displayed a maximum inhibitory capacity

of 55.354±0.808% at a concentration of 1.0 mg/mL and decreased afterwards with increasing concentration; whereas chymotrypsin digests exhibited increasing inhibitory activity, reaching its activity peak of 45.455±5.051% at a concentration of 1.5 mg/mL. Papain hydrolysates displayed a concentration-dependent inhibition of α-glucosidase activity, achieving maximal activity of 34.343±2.020% at the highest concentration (2.5mL). It seems likely that, at higher concentrations of these hydrolysates, there were peptides of longer sizes which could act antagonistically to smaller peptides, thus causing a reduction in inhibitory activity with increasing hydrolysate concentration (Malomo and Aluko, 2016).

The enzyme α-glucosidase (EC 3.2.1.20) is resident on the brush border membranes of intestinal mucosal cells, where they function to degrade small and medium-sized oligosaccharides into glucose units. The activity of the enzyme is essential to glucose release into the bloodstream and its subsequent absorption by cells (Voet *et al.,* 2016). As a result, the enzyme has been a key pharmacological target of hypoglycemic drugs, for the glycemic control in diabetes mellitus management (Ekun *et al.,* 2022). The inhibition of α-glucosidase by tryptic digests of *A. lebbeck* seed proteins was higher than the tryptic digests of *Luffa cylindrica* proteins (Arise *et al.,* 2019) but lower than that of *M. oleifera* seed protein hydrolysates (Ekun *et al.,* 2019[b]). The difference in activities could most likely be attributed to nature of the plant as well as conditions for hydrolysis. In addition, earlier studies reported that peptides possessing certain amino acid units such as serine, lysine or arginine and proline at specific positions have significant α-glucosidase-inhibitory effects (Ibrahim *et al.,* 2018). This could indicate that trypsin digestion of the protein yielded peptides with cationic residues which in turn elicited potent α-glucosidase-inhibitory activities, compared to other hydrolysates.

The extent of α-glucosidase inhibition by chymotrypsin hydrolysates obtained in this study was comparable to 38.723 ± 1.508% determined for unfractionated chymotrypsin digests of *M. oleifera* seed protein hydrolysates (Ekun *et al.,* 2022). However, the decrease in activity as concentration increased could be due to antagonistic interactions that may have occurred between small sized peptides and high molecular weight peptides in solution, limiting their inhibitory activities in the process (Malomo and Aluko, 2016). In comparison to the usage of other enzymes, there is a dearth of research data on the usage of papain as a frequently used proteinase for the preparation of bioactive protein hydrolysates and peptide fractions. α-Glucosidase inhibition by papain digests of *A. lebbeck* seed proteins was also not different from 30.555±0.825 obtained for unfractionated papain digests of *M. oleifera* in earlier studies (Ekun *et al.,* 2023). Papain has a broad specificity as a protease (Voet *et al.,* 2016) and this may have facilitated the cleavage of the protein into numerous peptides of differing molecular weights (Ekun *et al.,* 2023). This may have in turn caused reactions among peptides which are antagonistic in nature, leading to limited inhibitory impacts on α-glucosidase at the majority of the concentrations of the hydrolysates in our study (Malomo and Aluko, 2016).

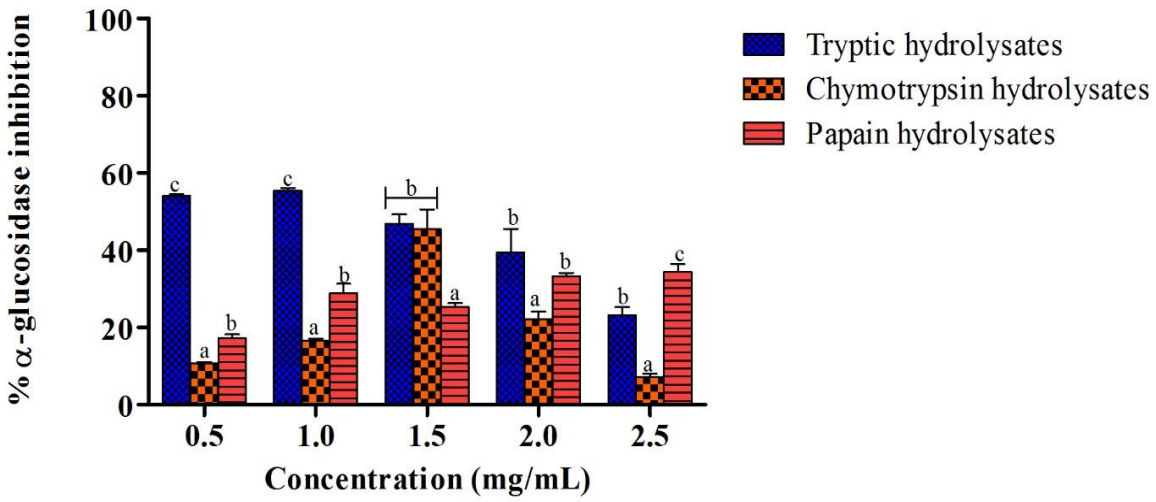

Figure 5. Fifty Percent Inhibitory Concentration (IC50) Values of α-Glucosidase Inhibitory Activities of Albizia lebbeck Seed Protein Hydrolysates

Each bar displayed represent the means ± standard error of the mean of three determinations made in triplicate. There is a substantial difference ($p<0.05$) between the bars that bear different letters. Bars with the same letters do not differ statistically ($p<0.05$).

Conclusion

The proteolysis of *Albizia lebbeck* seed proteins produced hydrolysates which demonstrated differing inhibitory capabilities against the carbohydrate-hydrolyzing enzymes. The protein was more susceptible to tryptic cleavage than the other enzymes. Hydrolysates obtained from papain digestion exhibited the best amylase-inhibitory activity whereas trypsin digestion produced hydrolysates with the best inhibitory effects against α-glucosidase in this study. The fractionation and further characterization of these hydrolysates are suggested, so as to further identify the nature of the bioactive peptides responsible for these effects. In general, peptide digests of *A. lebbeck* seed proteins show promise as potential sources of antidiabetic peptides.

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