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Research article

Blood pressure and sugar regulating potentials of *Anarcadium occidentale* nut globulin and albumin hydrolysates

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ABSTRACT

Several novel functional peptides have been successfully extracted from plant storage proteins. This study investigated the degree of hydrolysis, peptide yield, amino acid constituents, angiotensin converting enzyme (ACE), alpha amylase inhibitory and *in vitro* antioxidant activities of cashew (*Anarcardium occidentale*) nut proteins (CNP) hydrolysates (CNPHs). Cashew nut proteins (albumin and globulin) were hydrolysed using pancreatin, Alcalase and trypsin. The peptide yield and degree of hydrolysis (DH) of CNP by pancreatin (75.69 ± 0.84%; 37.39 ± 0.31) was significantly higher than those by Alcalase (61.67 ± 0.55%; 23.87 ± 0.23) and trypsin (43.33 ± 0.45%; 11 ± 0.15). The inhibition of ACE by albumin and globulin hydrolysates was concentration dependent. At 1.2 mg/mL, ACE-inhibitory activity of pancreatic cashew nut globulin (CNGH) hydrolysate (51.65 ± 1.2%) was significantly higher than those of Alcalase (34.603 ± 0.65%) and tryptic (29.92 ± 0.73%) CNGHs. Cashew nut albumin hydrolysate (CNAH) demonstrated concentration-dependent alpha-amylase inhibition (IC_{50} 0.17 ± 0.02–0.41 ± 0.021 mg/mL). The order of inhibition was tryptic > Alcalase > pancreatic CNAHs. The pancreatic hydrolysates of both albumin and globulin fractions displayed the highest DPPH antioxidant activity, while pancreatic CNAH was the most potent superoxide anion scavenger. These findings therefore posit that cashew nut globulin and albumin hydrolysates are laden with useful bioactive peptides that may be further explored for regulation of blood pressure and sugar in hypertensive and diabetic *in vivo* models.

1. Introduction

Bioactive peptides are produced in the cell as prepropeptides that are subsequently transformed into functional products. They play physiological roles such as blood pressure and sugar regulation in the body asides their nutritional value (Vermeirssen et al., 2004).

Cashew nut (*Anarcadium occidentale*) is a member of Anacardiaceae family, it is commonly cultivated in the tropical regions (Ogunwolu et al., 2010). Like other nuts, cashew is rich in protein with about 21% of the dry nut being protein. Proteins in seeds can been developed into valuable nutraceutics. Food-based peptides are attracting attention owning to their antioxidants, α-amylase and renin-angiotensin system inhibitory activities, hence they are of considerable food and economic importance in managing diabetes and hypertension (WHO, 2019; Saadi et al., 2015; Arise et al., 2019a; Malomo et al., 2020).

Hypertension is blood pressure exceeding 140/90 mmHg, it is the leading cause of deaths among non-communicable diseases globally. About 918 million adults were hypertensive worldwide in the year 2000, while this figure has risen to 1.13 billion adults in 2019, low and middle income countries accounted for most of these cases (Burnier and Egan, 2019; WHO, 2019; Mills et al., 2020). Reports from World Health Organisation revealed that there are at least 10 million hypertension associated death globally every year (Kitt et al., 2019). Targeting of ACE for inhibition can help to regulate blood pressure by rennin-angiotensin system (RAS). Renin produces angiotensin I which is transformed to angiotensin II (an excellent vasoconstrictor) by ACE. Therefore, inhibition of ACE will help to prevent the degradation of bradykinin, a compound responsible for dilating blood vessels and consequently lowers blood pressure (Udenigwe et al., 2009).

Diabetes results from disorder of glucose metabolism; it often leads to elevated blood glucose concentration (hyperglycemia). Inability to regulate this elevated glucose level can affect several organs and is a risk factor for hypertension, blindness, kidney failure etc. Diabetes can be as a result of insufficient insulin (type I) or due to insulin resistance (type II)

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or due to pregnancy in some women (Arise et al., 2016; Bihari et al., 2011; Papitha et al., 2018; Pereira et al., 2019). People with fasting blood glucose above 126 mg/dL (7.0 mmol/L) are said to be diabetic. About 463 million people are diabetic worldwide in 2020, with global health expenditure of $727 billion. Based on the present prevalence rate of 9.3%, about 700 million are projected to be diabetic by 2045. High income countries and urban dwellers accounted for most of the diabetes cases (Saeedi et al., 2019; IDF, 2020). α-Amylase breaks down starch into smaller molecules. Inhibiting α-amylase is an effective strategy for lowering of postprandial hyperglycemia in diabetes management, because it helps in delaying the digestion of starch thereby preventing elevated level of glucose in the blood stream (Wu et al., 2018). Acarbose, voglibose and miglitol are commercial diabetes drugs that work based on this principle.

Both hypertension and diabetes are of serious public health concerns in both developed and developing countries. Synthetic drugs used for managing these diseases are expensive; they also have their side effects due to long term usage (Mohammed et al., 2015). In some people, treatment is usually inadequate or not yielding the desired positive result. More so, diabetes is a risk factor for hypertension and vice versa, hence it is necessary to look for a multi-potent treatment regimen from cheaper, safer, efficacious and accessible natural sources that can help to manage both diseases. More so, oxidative stress aids the development and advancement of hypertension and diabetes due to the depletion of antioxidants (Arise et al., 2014; Barrows et al., 2019; Oguntibeju, 2019). Therefore, this research was designed to investigate *in vitro* antioxidant, ACE and alpha-amylase inhibitory activities of cashew nut globulin and albumin hydrolysates obtained via Alcalase, trypsin and pancreatin.

2. Materials and methods

2.1. Materials

Anarcadium occidentale (Cashew) nuts were purchased at the new market in Ilorin, Kwara State, Nigeria and authenticated at the Department of Plant Biology, University of Ilorin (Voucher number: UILH/001/612). Bovine serum albumin, HCl, NaCl, NaOH, sodium phosphate monobasic, sodium phosphate dibasic, hydrolytic proteases: pancreatin, trypsin, Alcalase, ACE, N-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Trichloroacetic acid (TCA), ferric chloride, ethanol, ascorbic acid, potassium ferricyanide and ferrous sulphate were purchased from Sigma-Aldrich, Germany. Kits used for bioassay were bought from Randox Laboratories Ltd., Co. Antrim, UK. All other reagents were of standard grades.

2.2. Methods

2.2.1. Isolation, fractionation, purification and characterization of cashew nut proteins

The cashew nuts were cut into pieces, dried, dehulled, pulverized, defatted and grounded into flour according to the method of Malomo et al. (2020). It was sieved and stored in a plastic container at –20 °C. Its protein component was extracted using the method of Arise et al. (2019). The freeze-dried cashew nut protein isolate (CNPI) was refrigerated until when needed. The protein fractions of *Anarcardium occidentale* nut was fractionated, purified and characterized according to the method of Liu et al. (2018). The defatted cashew nut flour was dissolved in deionised water (1:10, w/v) and stirred for 2 h at room temperature. The suspension was then centrifuged at 6000 g for 30 minutes, thereafter the supernatant was collected as the albumin extract. The pellet was extracted with 1 M NaCl (1:10, w/v) for 2 hours and centrifuged to give the globulin fraction. Water and oil holding capacity; emulsifying and foaming properties as described by Liu et al. (2018) were used to characterize the fractions.

2.2.2. Preparation of CNPHs

The CNPI obtained was hydrolysed with three commercial proteases, Alcalase, at 60 °C, pH 8.0; pancreatin, at 50 °C, pH 8.0 and trypsin at 45 °C, pH 7.5 for 240 min using the method described by Arise et al. (2016). Bradford method (1976) was used to determine the protein content.

2.2.3. Determination of the degree of hydrolysis

The DH was determined by using the method described by Arise et al. (2016). The protein content of the supernatant was determined using Braford method (1976).

2.2.4. Determination of percentage peptide yield

The percentage peptide yields of CNPHs were determined by using the method of Girgih et al. (2011) by calculating the ratio of peptide weight of lyophilized CNPH to the protein weight of non-hydrolyzed CNPI.

2.2.5. Amino acid analysis of cashew nut protein hydrolysates (CNPH)

The various amino acids in the cashew nut albumin and globulin hydrolysates (CNAH and CNGH respectively) were determined using S433 Amino Acid Analyzer (SYKEM, Germany). Pancreatic CNAH (PCNAH) and CNGH (PCNGH), Alcalase CNAH (ACNAH) and CNGH (ACNGH); tryptic CNAH (TCNAH) and CNGH (TCNGH) were lyophilized and then hydrolyzed for a day at 110 °C with 6N HCl (Blackburn, 1978). Thereafter, the samples were stored in sodium citrate buffer (pH 2.2) at 4 °C until when needed.

2.2.6. Determination of ACE-inhibitory activity

The ability of CNPH to inhibit ACE was determined by the method by Udenigwe et al. (2009) using FAPGG as substrate.

2.2.7. Determination of α-amylase inhibitory activity

The method of Oboh et al. (2011) was used to assay for α-amylase inhibition. Acarbose was used as the control.

2.2.8. Determination of DPPH radical scavenging activity of CNAHs and CNGHs

The ability of CNAHs and CNGHs to scavenge DPPH radicals was determined by using the assay method of Shimada et al. (1992).

2.2.9. Determination of ferric reducing power of CNAHs and CNGHs

The ability of CNAHs or CNGHs to reduce Fe^{3+} to Fe^{2+} was determined using the method described by Arise et al. (2016).

2.2.10. Determination of superoxide anion scavenging activity of CNAHs and CNGHs

The method of Alashi et al. (2014) was used to determine the ability of CNAHs and CNGHs to scavenge superoxide radical.

2.2.11. Statistical analysis

Analyses were done in triplicates. Data were means of triplicates ± standard deviation (SD), and were subjected to ANOVA and Tukey's multiple range tests using GraphPad Prism version 6.0 (San Diego, USA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Degree of hydrolysis, percentage peptide yield and amino acid composition

The degree of CNP hydrolysis by pancreatin (37.39 ± 0.31) was significantly higher ($p < 0.05$) than by Alcalase (23.87 ± 0.23) and trypsin (11 ± 0.15). The percentage peptide yield of pancreatic hydrolysis (75.69 ± 0.84) was also significantly higher ($p < 0.05$) than tryptic (43.33 ± 0.45) and Alcalase hydrolysis (61.67 ± 0.55) (Table 1). Pancreatic, Alcalase and tryptic CNGHs are all rich in arginine,

Table 1. Degree of hydrolysis and peptide yield of CNPI.

	Degree of hydrolysis (%)	Peptide Yield (%)
Alcalase	23.87 ± 0.23[a]	61.67 ± 0.55[a]
Trypsin	11.00 ± 0.15[b]	43.33 ± 0.45[b]
Pancreatin	37.39 ± 0.31[c]	75.69 ± 0.84[c]

Values are expressed as means ± SD. Values with different letters down the column are significantly different ($p < 0.05$).

Table 2. Amino acid composition of CNGHs (g/100g sample).

Amino acid	PCNGH	ACNGH	TCNGH
Isoleucine	6.13	5.83	4.29
Leucine	5.73	6.53	5.59
Lysine	4.13	3.83	3.18
Cysteine	6.41	1.82	1.60
Methionine	0.40	0.20	0.33
Tyrosine	5.22	4.54	3.12
Phenylalanine	1.65	1.10	0.30
Threonine	5.10	5.63	4.37
Tryptophan	4.95	3.98	2.47
Valine	5.64	4.01	2.78
Histidine	0.96	0.34	0.22
Arginine	8.68	7.92	7.67
Aspartic acid + asparagines	11.89	12.17	11.14
Glutamic acid + glutamine	9.92	9.43	10.23
Serine	4.54	5.34	8.42
Proline	3.74	4.02	3.62
Glycine	4.14	4.13	9.95
Alanine	2.10	2.11	2.12

isoleucine, leucine, threonine, aspartic acid and glutamic acid with values ranging from 4.29 to 12.17 g/100g, while the amount of methionine, phenylalanine and histidine in PCNGH, ACNGH and TCNGH is extremely low with values ranging between 0.22 and 1.65 g/100g. The amount (g/100g) of cysteine and valine in PCNPH is higher than those of ACNPH and TCNPH, while the amount of serine and glycine in TCNPH is higher than those of ACNPH and PCNPH (Table 2). Pancreatic, Alcalase and tryptic CNAHs are very rich in arginine, glycine, aspartic and glutamic acid with values ranging from 4.77 to 21.53 g/100g. The amount (g/100) of methionine, tyrosine, phenyalanine, threonine, tryptophan, valine, isoleucine, leucine, lysine, cysteine, histidine and arginine in PCNAH are higher than those in ACNAH and TCNAH; while the amount of aspartic acid and serine in ACNAH are higher than those in PCNAH and TCNAH. Also, the amount (g/100g) of glutamic acid, serine, glycine and alanine in TCNAH are higher than those in PCNAH and ACNAH (Table 3). The amount (g/100g) of isoleucine, leucine, tyrosine, threonine, aspartic acid in CNGHs are higher than those in CNAHs; while the amount of methionine, phenylalanine, histidine, arginine, glutamic acid in CNAH are higher than those in CNGH. The amount of cysteinein PCNGH is higher than that of PCNAH (Tables 2 and 3).

Table 3. Amino acid composition of CNAHs (g/100g sample).

Amino acid	PCNAH	ACNAH	TCNAH
Isoleucine	2.68	2.60	2.37
Leucine	3.10	2.98	2.95
Lysine	3.69	3.50	3.00
Cysteine	3.00	1.98	1.91
Methionine	2.01	1.84	1.79
Tyrosine	2.99	2.71	2.64
Phenylalanine	3.96	3.11	2.67
Threonine	2.92	1.99	1.61
Tryptophan	3.63	2.44	2.10
Valine	5.01	4.12	3.84
Histidine	3.01	2.42	2.00
Arginine	9.87	9.11	8.96
Aspartic acid + asparagines	6.73	6.75	5.89
Glutamic acid + glutamine	20.01	20.88	21.53
Serine	4.31	5.04	6.85
Proline	3.00	3.95	2.94
Glycine	4.91	4.77	5.35
Alanine	2.31	2.40	2.61

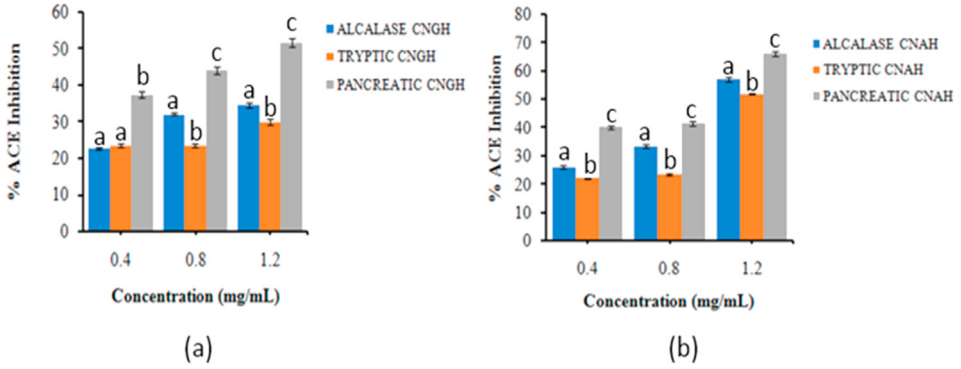

Figure 1. ACE-inhibitory activity of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

3.2. ACE-inhibitory activity of Anarcardium occidentale nut globulin and albumin hydrolysates

Cashew nut globulin and albumin hydrolysates at different concentrations of ACNGHs, TCNGHs, PCNGHs, ACNAHs, TCNAHs and PCNAHs significantly inhibited ($p < 0.05$) ACE in a dose dependent manner (Figure 1). At 1.2 mg/mL inhibition of ACE by pancreatic CNGH (51.65 ± 1.2%) was significantly higher than those of Alcalase (34.603 ± 0.65%) and tryptic (29.92 ± 0.73%) CNGHs. The order of ACE inhibition at 0.8 mg/mL of CNGHs was pancreatric > Alcalase > tryptic. However, at 0.4 mg/mL there was no significant difference ($p > 0.05$) in the ACE inhibitory activity between Alcalase and tryptic CNGHs (Figure 1a). At 1.20 mg/mL, ACE inhibition by pancreatic CNAH (66.03 ± 0.82%) was significantly higher than tryptic (51.82 ± 0.37%) and Alcalase (57.03 ± 0.85%) CNAHs (Figure 1b). However, the value obtained for pancreatic hydrolysate of the albumin fraction was higher than pancreatic hydrolysate of globulin fraction of cashew nut protein. The IC_{50} of pancreatic CNGH was significantly lower ($p < 0.05$) than Alcalase and tryptic CNGHs (Figure 2a). The IC_{50}values were 0.68,1.68and 0.35mg/mLfor Alcalase, tryptic and pancreatic CNAHs respectively (Figure 2b).

3.3. Alpha-amylase inhibitory activity of Anarcardium occidentale nut globulin and albumin hydrolysates

Alpha-amylase inhibition by *Anarcardium occidentale* nut globulin and albumin hydrolysates displayed a dose-dependent pattern. Pancreatic CNGH had the least α-amylase inhibition at all concentrations investigated. There was no significant difference ($p > 0.05$) between alpha-amylase inhibition by Alcalase and tryptic CNGHs at the all concentrations investigated. The percentage of α-amylase inhibited by Alcalase and tryptic CNGHs was significantly higher ($p < 0.05$) than acarbose (control) at 0.5–1.0 mg/mL hydrolysate concentrations; but there was no significant difference ($p > 0.05$) between Alcalase and tryptic CNGHs when compared with acarbose (control) at 1.5 mg/mL. The percentage of α-amylase inhibited by acarbose was significantly higher ($p < 0.05$) than all CNGHs at 2 mg/mL (Figure 3a). The tryptic CNAH was the most effective in inhibiting α-amylase at 1–2 mg/mL. At 2 mg/mL, inhibitory activity was 61.1 ± 1.14% for tryptic CNAH, 54.5 ± 0.71% for Alcalase CNAH and 38.1 ± 1.41% for pancreatic CNAH. Inhibition by tryptic CNAH was significantly higher compared to the control (acarbose) at all concentrations investigated in this study (Figure 3b). The IC_{50} value of tryptic CNGH (0.17 ± 0.010) was significantly lower ($p < 0.05$) than acarbose (0.25 ± 0.020), Alcalase CNGH (0.29 ± 0.010) and pancreatic CNGH (0.39 ± 0.010). Also, tryptic CNAH had the least IC_{50} value followed by acarbose (0.25 ± 0.020), Alcalase CNAH (0.29 ± 0.014 mg/ml) and pancreatic CNAH (0.41 ± 0.021 mg/ml) (Figure 4).

3.4. Antioxidant study

3.4.1. DPPH radical-scavenging activity of Anarcardium occidentale nut globulin and albumin hydrolysates

Pancreatic hydrolysate was the best scavenger of DPPH radicals in both the *Anarcardium occidentale* nut globulin and albumin hydrolysates investigated. Pancreatic CNGH exhibited 71.53 ± 0.35% at 2.0 mg/mL, while Alcalase and tryptics CNGHs displayed 47.00 ± 0.73% and 32.95 ± 0.77% respectively (Figure 5a). The order of radical-scavenging power of the globulin and albumin hydrolysates at 2.0 mg/mL was pancreatic > Alcalase > tryptic hydrolysates (Figure 5b). However, ascorbate (control) displayed DPPH radical scavenging activity than pancreatic, Alcalase and tryptic CNGHs and CNAHs. In the globulin hydrolysate, the EC_{50} values of pancreatic CNGH was significantly lower than Alcalase and tryptic CNGHs, while there was no significant difference ($p > 0.05$) between the EC_{50} of Alcalase and tryptic CNGHs (Figure 6a). The EC_{50} of Alcalase CNAH (1.1 ± 0.15 mg/mL) was significantly lower ($p < 0.05$) than tryptic (2.5 ± 0.14 mg/mL) and pancreatic (2.15 ± 0.15 mg/mL) CNAHs

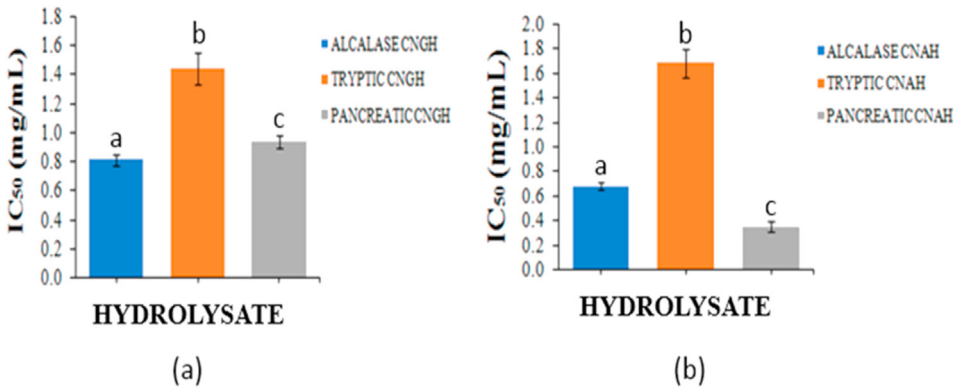

Figure 2. ACE-inhibitory concentration (IC_{50}) values of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

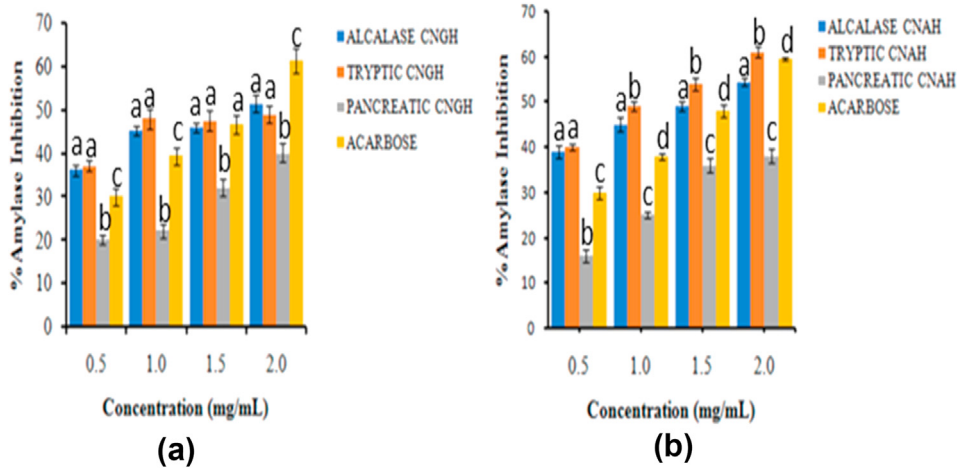

Figure 3. Percentage α-amylase inhibitory activity of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

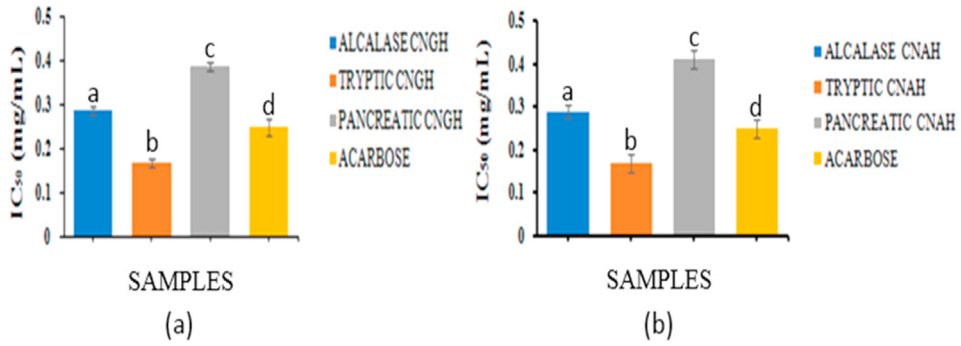

Figure 4. Fifty percent (50%) α-amylase inhibitory concentration (IC_{50}) values of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

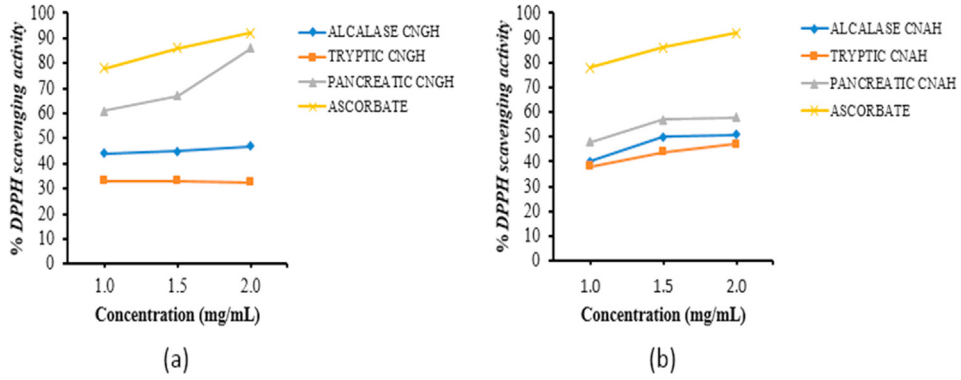

Figure 5. DPPH radical-scavenging activity of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

(Figure 6b). However, the EC_{50} of ascorbic acid (0.6 ± 0.14 mg/mL) was significantly lower ($p < 0.05$) than Alcalase, tryptic and pancreatic CNGHs ad CNAHs.

3.4.2. Superoxide radical scavenging activity of Anarcardium occidentale nut globulin and albumin hydrolysates

The ability of both the globulin and albumin hydrolysate to scavenge superoxide radical was dose dependent (Figure 7). The percentage inhibition of Alcalase, pancreatin and trypsin CNGHs were 58.23%, 35.89% and 21.2% respectively at 0.8 mg/ml hydrolysate concentration (Figure 7a). The order of superoxide radical scavenging inhibition by the albumin hydrolysates was pancreatic > tryptic > Alcalase CNAHs (Figure 7b). However, the percentage of O_2^- inhibited by ascorbic acid (control) was significantly higher the hydrolysates of globulin and albumin fractions.

3.4.3. Ferric reducing power activity of Anarcardium occidentale nut globulin and albumin hydrolysates

At 0.2–0.4 mg/mL of globulin hydrolysates, there was no significant difference ($p > 0.05$) in the ferric reducing potential of Alcalse and tryptic CNGHs. However there was a significant increase ($p < 0.05$) in the ferric reducing power of pancreatic hydrolysate than Alcalase and tryptic hydrolysates. At 0.8 mg/mL, ferric reducing potential of pancreatic CNGH (1.29 ± 0.02 mM) was significantly higher ($p < 0.05$) than tryptic (0.92 ± 0.025 mM) and Alcalase (0.91 ± 0.023 mM) CNGHs (Figure 8a). For the albumin hydrolysates, at 0.2–0.4 mg/mL there was no significant

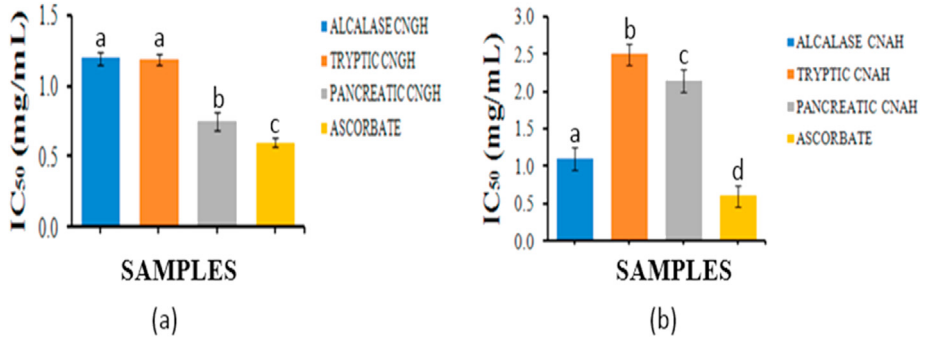

Figure 6. DPPH EC_{50} values of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

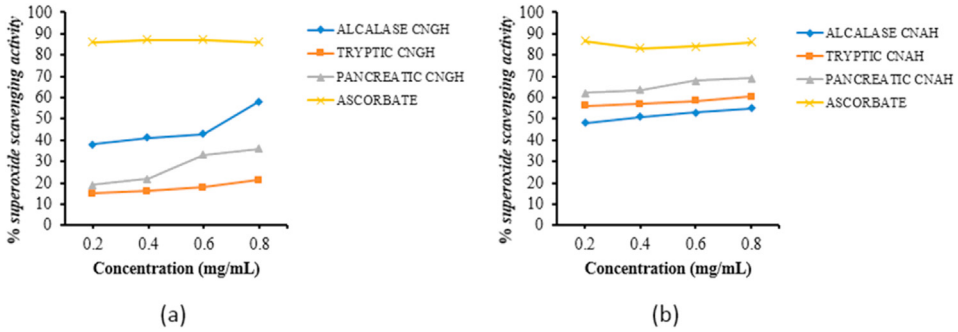

Figure 7. Superoxide radical-scavenging activity of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

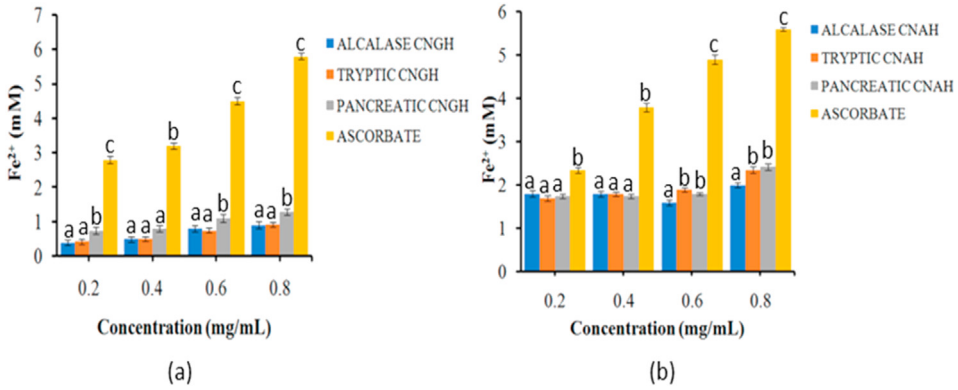

Figure 8. Ferric reducing power of *Anarcardium occidentale* nut (a) globulin and (b) albumin hydrolysates.

difference ($p > 0.05$) in ferric reducing potential of Alcalse, trypic and pancreatic CNAHs. However at. 0.8 mg/mL, pancreatic CNAH exhibited the highest ferric reducing power (2.425 ± 0.21%), followed by tryptic CNAH (2.355 ± 0.73%) and Alcalase CNAH (2.00 ± 0.77%) (Figure 8b). Ascorbate (control) significantly exhibited better ferric reducing power than both the globulin and albumin hydrolysates at all concentrations investigated in this study (Figure 8).

4. Discussion

4.1. Degree of hydrolysis, percentage peptide yield and amino acid composition

The DH of CNP by endopeptidase (Alcalase) and digestive enzymes (trypsin and pancreatin) is directly proportional to their peptide yield. The values obtained for DH showed that pancreatin was the most efficient in the hydrolysis of globulin fractions of *Anarcardium occidentale* nut protein isolate. Variation in degree of hydrolysis was probably due to protease specificity; higher degree of hydrolysis by pancreatin may be due to the fact that it contains aromatic and cationic amino acids contents in the cashew nut protein for which pancreatin has broad specificity (Malomo et al., 2020). The low DH obtained for tryptic CNPH might be due to the limited amount of peptide linkages present in cashew nut for which trypsin have high specificity. The degree of hydrolysis of pancreatic and Alcalase CNPHs obtained in this study is higher than that of Liu et al. (2018) for cashew nut. The DH value for *Anarcardium occidentale* Alcalase hydrolysates (23.87 ± 0.23) is close to what was obtained by Yust et al. (2003) for Alcalase hydrolysates of chickpea legumin (DH of 27%).

Knowledge of the amino acid composition and the quantity in which they are present give insight into the bioactivities of the hydrolysate. The amount of threonine, leucine, isoleucine, valine and lysine obtained in this study surpasses FAO/WHO recommended essential amino acids in both adult and children. This implies that CNAHs and CNGHs may be useful as cheap and quality sources of protein for formulating infants and children meal. The high amount of arginine, isoleucine, leucine,

threonine, aspartic acid and glutamic acid obtained from CNGHs was also reported by Liu et al. (2018). The high level of aspartic and glutamic acid obtained from CNAHs and CNGHs suggests that the hydrolysates exhibited strong antioxidant potentials because negatively charged amino acids scavenge free radicals by donating electrons. Also, the high hydrophobic and branched chain amino acids in CNAHs and CNGHs have the potential of improving their solubility in lipids and hence their antioxidant status. The high content of arginine in CNAHs and CNGHs offers the potential of preventing and reducing high blood pressure as arginine is used for synthesizing nitric oxide which is a good vasodilators (Malomo et al., 2020). The high ratio of arginine to lysine in CNGH confers it the advantage of preventing hypercholesterolemia and cardiovascular diseases (Liu et al., 2018). The amount (g/100g) of serine, aspartic acid, glycine, valine, isoleucine and lysine obtained in this study for CNAHs and CNGHs agrees with the work of Malomo et al. (2020).

4.2. ACE inhibition

Alcalase and pancreatic CNGHs showed remarkable dose-dependent inhibitory effects against ACE activity, while dose-dependent inhibition by trypsin CNGH was rather weak. Inhibition of ACE has been reported to help lower blood pressure, because this inhibition protects bradykinin which is responsible for dilating blood vessels from degradation (Udenigwe et al., 2009). The remarkable ACE inhibitory activity observed for pancreatic CNAHs and CNGHs might be due to their high rate of hydrolysis and peptide yield compared to Alcalase and tryptic CNAHs and CNGHs. Also, the high amounts of hydrophobic amino present in pancreatic CNGH lend credence to its ability to inhibit ACE (Arise et al., 2019b). The inhibition of ACE by CNGHs and CNAHs is higher than the value reported by Mirdhayati et al. (2016) for *Capra aegagrus* and Bougatef et al. (2008) for *Sardinella aurita*, but is identical to those reported by Shu et al. (2019) for CNPH, they reported that inhibition of ACE by CNPH can be enhanced when further purified. However, the inhibition of ACE by Alcalase CNPH reported by Malomo et al. (2020) is higher than the one observed in this study for CNAHs and CNGHs, this might be due to differences in protein fractions and enzyme-substrate (ES) ratio used, degree of hydrolysis, peptide yield, length of peptides released and other experimental conditions. The IC_{50}values observed for all the albumin and globulin hydrolysates are similar to 0.18 mg/mL documented for corn gluten (Kim et al., 2004); 0.18 mg/mL for chickpea legumin (Yust et al., 2003); 0.64 mg/mL for mung bean protein (Li et al., 2005); 1.377–1.757 mg/mL obtained for water melon (*C. latanus*) seeds (Arise et al., 2016). Conversely, IC_{50} values documented so far for animal protein hydrolysates are usually higher than that of plant sources 8–11.2 μg/mL for milk (Gobbetti et al., 2000) and 20–74.4 μg/mL for egg white (Miguel et al., 2007).

4.3. Alpha-amylase inhibitory activity of Anarcardium occidentale nut globulin and albumin hydrolysates

Inhibition of α-amylase (a key enzyme responsible for breaking starch into smaller molecules) is essential in drug design for managing diabetics (Gropper and Smith, 2013). Our values for Alcalase and tryptic CNPHs (CNGHs and CNAHs) indicate impressive inhibitory effects against α-amylase activity that is significantly higher than that of acarbose (the reference drug). It has been reported that Alcalase have potentials of hydrolysing branched and cationic amino acid which usually inhibit amylase thus preventing the hydrolysis of starch (Arise et al., 2019b). Tryptic CNAH displayed the highest α-amylase inhibition, this ascersion is supported by its low IC_{50} (0.17 ± 0.001 mg/mL). The variation in the inhibitory activities shows that the nature of peptide affects the extent of inhibition of α-amylase. Uraipong and Zhao (2016) reported high α-amylase inhibitory activity of rice bran Alcalase hydrolysate which is similar to what was obtained in this study. Hydrolysates of barley proteins have also been reported to inhibit α-amylase (Connolly et al., 2014). Arise et al. (2016) reported that tryptic and Alcalase watermelon protein hydrolysates inhibited alpha amylase activity. The observed high level of inhibition of alpha-amylase by tryptic CNAH may be due to the high specificity of the enzyme and the release of a greater amount of smaller peptide upon hydrolysis which block the enzyme at diverse active sites. There is similarity in the high potentail of both the globulin and albumin hydrolysates to inhibit α-amylase. Thus, tryptic CNGHs and CNAHs may regulate elevated postprandial glucose level by slowing down the rate at which alpha amylase breaks down starch. This is of great importance in the management of diabetes where low insulin levels prevent the fast clearance of glucose from the blood. This is the first report on the ability of *A. occidentale* nut globulin and albumin hydrolysates to inhibit α-amylase activity. The similar pattern obtained in the alpha amylase IC_{50} values of both the globulin and albumin hydrolysates might imply that the bioactive amino acids responsible for inhibiting α-amylase might be soluble in both aqueous and salt solutions employed in the fractionation process.

4.4. Antioxidant studies

4.4.1. DPPH radical scavenging activity

The donation of hydrogen to a free radical transforms it to a non-toxic one, thus helping to prevent oxidation that can damage tissues (Arise et al., 2016). The differences in the mopping up of DPHH radical by globulin fractions might be due to the role of the amino acids in CNGH. Pancreatin CNGH from this study is better at scavenging radical than marine rotifer (46–50% at 1 mg/mL) and hen egg white (25.7%) hydrolysed with trypsin, pepsin and chymotrypsin (Byun et al., 2009; Rao et al., 2012). Peptide size, amino acid compositions, specificity of the enzymes, duration of hydrolysis and other conditions might be responsible for the differences in the potential of the hydrolysate to mop up free radicals. The high amount of tyrosine and tryptophan in pancreatin CNGH could be responsible for its high radical-scavenging power over Alcalase and tryptic hydrolysates. The result obtained in this study is similar to the pattern of result obtained by Adiamo et al. (2016), where it was observed that pancreatin hydrolysates exhibited higher scavenging activity (81.02% at 2.5 mg/mL). The higher antioxidative activity of Alcalase hydrolysate over tryptic hydrolysate is also similar to what was reported by Wu et al. (2003) for *S. austriasicus* and Ghanbari et al. (2015) for sea cucumber. This implies that *Anarcardium occidentale* nut protein might contain strong proton donating peptides that reacted and terminated the activities of unsteady DPPH radicals by converting them to stable products. High antioxidant potential of Alcalase hydrolysates compared to pepsin and flavourzyme hydrolysates from marine sources have been reported (Sun et al., 2011). The higher activity of Alcalase hydrolysate might be due to its endopeptidase nature. Alcalase breaks peptide linkages in a polypeptide chain from the interior, thereby producing small and average-sized oligopeptides with antioxidant activity (Adler-Nissen, 1986). However, the antioxidant potential of the hydrolysates in this study is less than of ascorbic acid (control); studies have shown lower molecular weight peptides have high antioxidant activities, hence ultrafiltration and other purification procedures might be helpful in enhancing the antioxidant potentials of the hydrolysates (Malomo et al., 2020). The amount of a compound that inhibit 50% of the oxidant (EC_{50} value) is frequently used in evaluating antioxidant efficiencies of drugs, low EC_{50} signifies better radical savenging potentials (Salleh et al., 2015). The low EC_{50} values of CNAHs and CNGHs shows that they are excellent free radical scavengers. EC_{50} of Alcalase CNAHs and CNGHs obtained in this study is lower than cobia skin and *Arca subcrenata* Alcalase hydrolysates, but is similar to what was obtained for watermelon seed hydrolsates (Song et al., 2008; Razali et al., 2015; Arise et al., 2016).

4.4.2. Superoxide ion scavenging activity

Superoxide ions are usually produced *in vivo* via auto oxidation or enzyme activity (Arise et al., 2016). Unique peptide sequence and composition of the amino acid are key determining factors for determining antioxidant efficiency of bioactive peptides. The notably higher O^{2-}scavenging capacity of pancreatic CNPH compared to other CNPHs indicates that pancreatic CNPH contained bioactive peptides with hydrophobic residues that are capable of preventing the formation of O_2^-. Pancreatin is an endo- and exo- peptidase with specificity for hydrophobic and acidic amino acid residues (Silvestre et al., 2012). Acidic residues donate hydrogen ion thereby neutralizing O_2^- by converting them to harmless water molecules. Also, the availability of hydrophobic residues at peptides terminal facilitates dismutation of free radicals (Sarmadi and Ismail, 2010). The amount O_2^- scavenged is proportional to the hydrolysate concentration. Moure et al. (2006) reported that soy protein hydrolysates showed superoxide anion radical scavenging capacities ranging between 24.7% and 85.6% and were increased with increasing concentration, while that of wheat germ protein hydrolysates (0–0.60 g/L) was from 0% to 75.40% (Cheng et al., 2006). The lower IC_{50} value of pancreatic CNAHs and CNGHs further validate their higher O_2^- radical scavenging power over tryptic and Alcalase CNAHs and CNGHs. The effective lowering of the chain of oxidation induced by O_2^- shows that globulin and albumin hydrolysates of *Annarcardium occidentale* natural excellent antioxidantss.

4.4.3. Ferric reducing power activity

Cashew nuts globulin and albumin hydrolysates displayed a dose dependent ferric reducing potential. The ferric reducing potential in this study is lower than that of ascorbic acid. Nazeer and Kulandai (2012) also reported increased reducing power with increasing concentrations of muscle and skin protein hydrolysate obtained from giant kingfish, also their antioxidant potential were lower than that of the reference. This is not different from the observation of Zhu et al. (2006) for wheat germ protein hydrolysate. This study showed that pancreatic CNAH and CNGH possess the highest Fe^{3+} reducing power at high concentrations, high degree of hydrolysis by pancreatin may be responsible for this. Vastag et al. (2011) has earlier reported that ferric-reducing power is proportional degree of hydrolysis, because smaller peptides often display higher reducing potential. The trend of the reducing power of the hydrolysate is well corroborated in the documentation of pancreatic palm kernel cake protein hydrolysate exhibiting higher reducing power compared to the peptic palm kernel cake protein hydrolysate (Zarei et al., 2014). Pancreatic kariya protein hydrolysate was also found to possess more reducing power than the peptic hydrolysate (Famuwagun and Gbadamosi, 2016). The result of this study shows better reducing potential than 0.69 at 2.0 mg/mL for alfafa leaf (Xie et al., 2008) and 0.63 for kariya seeds at 1.0 mg/mL (Famuwagun and Gbadamosi, 2016). Juntachote and Berghofer (2005) reported that the ease of electron transfer to the free radical by the hydrolysate translates to higher reducing potential. Therefore, CNAHs and CNGHs probably contain molecules which may act as electron donors with potent ability to transform free radicals to stable products.

4.5. Limitations

This present work is an *in vitro* study on antihypertensive and antidiabetic potential of CNPHs, studies involving animal models and human clinial trials might provide more holistic insight into the potential of CNPHs'.

5. Conclusion

This study showed that cashew nut protein hydrolysates (CNPHs) contain several essential amino acids in quantities that surpassed FAO/WHO recommendation. Hydrolysates of cashew nut globulin and albumin fractions obtained using trypsin, Alcalase and pancreatin exerted ACE and alpha amylase inhibitory and free radical mopping roles. Tryptic CNAH exerted higher α-amylase inhibitory activity than the reference drug. Pancreatic hydrolysates displayed higher ACE inhibitory activity than Alcalase and tryptic hydrolysates. These hydrolysates typify germane sources of novel blood pressure and sugar regulating biopeptides for enhanced wellness and as alternative sources of essential amino acids in formulated infant diet.

Declarations

Author contribution statement

Rotimi Olusanya Arise: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Oluwaseun Oluwatosin Taofeek, Kehinde Babaita: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Raphael Idowu Adeoye: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Omorefosa Osemwegie: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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