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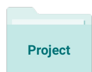
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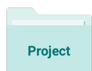
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Antioxidant activities of bambara groundnut (*Vigna subterranea*) protein hydrolysates and their membrane ultrafiltration fractions

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In this study, the bambara protein isolate (BPI) was digested with three proteases (alcalase, trypsin and pepsin), to produce bambara protein hydrolysates (BPHs). These hydrolysates were passed through ultrafiltration membranes to obtain peptide fractions of different sizes (<1, 1–3, 3–5 and 5–10 kDa). The hydrolysates and their peptide fractions were investigated for antioxidant activities. The membrane fractions showed that peptides with sizes <3 kDa had significantly ($p < 0.05$) reduced surface hydrophobicity when compared with peptides >3 kDa. This is in agreement with the result obtained for the ferric reducing power, metal chelating and hydroxyl radical scavenging activities where higher molecular weight peptides exhibited better activity ($p < 0.05$) when compared to low molecular weight peptide fractions. However, for all the hydrolysates, the low molecular weight peptides were more effective diphenyl-1-picrylhydrazyl (DPPH) radical scavengers but not superoxide radicals when compared to the bigger peptides. In comparison with glutathione (GSH), BPHs and their membrane fractions had better ($p < 0.05$) reducing power and ability to chelate metal ions except for the pepsin hydrolysate and its membrane fractions that did not show any metal chelating activity. However, the 5–10 kDa pepsin hydrolysate peptide fractions had greater (88%) hydroxyl scavenging activity than GSH, alcalase and trypsin hydrolysates (82%). These findings show the potential use of BPHs and their peptide fraction as antioxidants in reducing food spoilage or management of oxidative stress-related metabolic disorders.

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Introduction

Value addition to underutilized crops has become popular in recent times to maximize their potential use for human nutrition and health.[1] Bambara groundnut (*Vigna subterranea*), a scarcely studied crop of African origin, is the third most important legume seed after groundnut (*Arachis hypogea*) and cowpea (*vigna unguiculata*) in Africa.[2,3] The protein content of bambara may vary between 15 and 27%,[2–5] which is similar to that of cowpea[3,6,7] and slightly lower than the values reported for soya bean.[4,5,7] Bambara protein contains a high lysine content (6.5–6.8%) and a reasonable amount of methionine (1.8 g per 100 g) which is normally limiting in legumes.[5,8,9] Other important attributes of bambara include tolerance to drought and poor soil conditions, resilience in the face of extreme weather conditions such as hot temperatures and heavy rainfall and resistance to pests and diseases.[10] Despite these attributes, the use of bambara groundnut remains restricted to domestic food consumption.[3,4] However, with further research, bambara could be used for the manufacture of value-added products and its utilization may assist to solve the problem of food insecurity and poverty in developing countries.

In recent years, research has focused on the generation of bioactive peptides from food protein sources.[11,12] Bioactive peptides contain 2–20 amino acids per peptide as inactive sequences within large proteins. These peptides are released when the parent protein is hydrolysed by digestive enzymes (*in vitro* and *in vivo*), microbial enzymes or during food processing.[13] Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides without adversely affecting the nutritive value.[10,14] Peptides can be used in the formulation of functional foods and nutraceuticals to prevent damage related to oxidative stress in human disease conditions. Also, natural antioxidants are desirable because they can be used at higher concentrations without the toxic side effects associated with the use of their synthetic equivalents.[13,15] They also exhibit enhanced nutritional and func-

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tional properties in addition to their antioxidant activity.[1,16] To date, the antioxidant activities of enzymatic hydrolysates from plant food proteins including soy,[17,18] African yam bean,[19] canola protein,[1] hemp seed,[20] peanut protein[21] and chickpea protein hydrolysates,[15] have been widely investigated using many *in vitro* antioxidant evaluation systems. The antioxidant properties of these hydrolysates have been found to largely depend on the protease specificity, the degree of hydrolysis (DH) and the nature of the released peptides (molecular weight and amino acid composition).[1,18,19,22,23] Hence, enzymatically modified proteins could be used as natural antioxidants to protect the human body against oxidative damage and associated disease. These protein hydrolysates may also serve as natural sources of antioxidants in functional foods to maintain freshness and extend the shelf life.[1,19,22]

A review of the available literature on studies carried out on bambara revealed that there is scanty information on the antioxidant properties of bambara protein hydrolysates. A previous study by Thammarat *et al.*,[10] on the functional and antioxidative properties of bambara, was limited to a hydrolysate produced using a single digestive protease. Further, these authors did not study the effect of peptide fractions. The use of different proteases is important in order to determine the enzyme that produces peptides with the best antioxidant activities. Also, by fractionating the hydrolysate, it is possible to identify the effect of the peptide size on antioxidant activities. Therefore, the main aim of this study was to evaluate the *in vitro* antioxidant potential of bambara protein hydrolysates obtained using three proteases. We also evaluated the effect of the peptide size on the measured antioxidant parameters.

Experimental section

Materials

Bambara groundnut seeds were obtained from Josini, KwaZulu-Natal province of South Africa. Alcalase 2.4 L, trypsin, pepsin, diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), glutathione (GSH) and other antioxidant reagents were purchased from Sigma (Sigma Chemicals, St Louis, MO, USA) while other analytical grade reagents and ultrafiltration membranes (1, 3, 5 and 10 kDa molecular weight cut-offs) were obtained from Fisher Scientific (Oakville, ON, Canada).

Preparation of bambara seed protein isolate (BPI)

Bambara flour was defatted with *n*-hexane in the ratio of 1 : 5 (flour : solvent) for 3 h using a magnetic stirrer at a speed of 198 rpm. The defatted flour was placed in a fume hood overnight to remove the remaining hexane. The fat content of the flour after defatting using a Soxhlet extractor was less than 0.01%. BPI was produced from the defatted bambara flour according to the method described in ref. 5 with slight modifications. Briefly, the bambara defatted flour was dispersed in deionized water (1 : 20, w/v), and the dispersion was adjusted to pH 10.0 with 2 M NaOH to solubilise the proteins. The resultant dispersion was stirred at 37 °C for 2 h followed by centrifugation (7000*g* at 4 °C) for 45 min. The residue was discarded and the supernatant was filtered with cheesecloth and adjusted to pH 5.0 with 2 M HCl to precipitate most of the proteins. Thereafter, the mixture was centrifuged (7000*g* at 4 °C) for 45 min. The resultant precipitate was re-dispersed in deionized water, adjusted to pH 7.0 with 2 M NaOH and freeze dried to obtain BPI powder. The protein content of the BPI was determined by the modified Lowry method.[24]

Preparation of bambara protein hydrolysates and peptide fractions

Hydrolysis of BPI was conducted using each of the following enzymes and reaction conditions as previously reported in ref. 14 with some modifications. Briefly, BPI (5%, w/v, protein basis) was suspended in deionized water in a reaction vessel equipped with a stirrer, heated to an appropriate temperature and adjusted to an appropriate pH value prior to addition of the proteolytic enzyme (alcalase 50 °C, pH 8.0; trypsin 37 °C, pH 8.0 ; pepsin 37 °C, pH 2.0). Each protease was added to the BPI slurry at an enzyme to substrate ratio (E/S) of 1 : 100, based on the BPI protein content. Digestion was performed for 4 h (pH maintained constant by addition of 1 M NaOH or 1 M HCl) after which the enzymes were inactivated by adjusting the reaction mixture to pH 4.0 with 2 M HCl followed by immersing the reaction vessel in a boiling water bath for 15 min at 90 °C. The undigested proteins were precipitated by centrifugation at 8000*g* for 60 min. A portion of the supernatant containing target peptides was freeze dried to obtain the BPH, while the remaining portion was passed through ultrafiltration membranes with molecular weight cut-offs (MWCO) of 1, 3, 5 and 10 kDa in an Amicon stirred ultrafiltration cell. The supernatant was first passed through the 1 kDa membrane and the retentate was passed through the 3 kDa membrane. The 3 kDa retentate was passed through a 5 kDa membrane whose retentate was then passed through a 10 kDa membrane. The permeate from each MWCO membrane (<1, 1–3, 3–5, and 5–10 kDa, respectively) was collected, lyophilized, and stored at 20 °C until needed for further analysis. The protein contents of the freeze dried bambara protein hydrolysates (BPH) and membrane fractions were also determined using the modified Lowry method.[24]

Surface hydrophobicity (S_O)

S_o was determined using an aromatic hydrophobicity fluorescent probe (ANS) according to the method described in ref. 25 with some modifications. The BPH and ultrafiltration membrane permeates were serially diluted to a final concentration of 50–250 μg mL^{-1} in 0.01 M phosphate buffer (pH 7.0). A 20 μl ANS solution (8.0 mM in 0.01 M phosphate buffer, pH 7.0) was added to 4 mL of each sample dilution and the fluorescence intensity (FI) of the mixture was measured with a JASCO FP-6300 fluorescence spectrophotometer (JASCO, Tokyo, Japan) at excitation and emission wavelengths of 390 nm and 470 nm respectively. The initial slope of the FI

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versus sample concentration plot (calculated by linear regression analysis) was used as an index of S_o.

DPPH radical scavenging assay

The scavenging activity of BPHs and their membrane fractions against DPPH was determined as previously described[1] using a 96-well clear flat bottom plate. The peptide fractions were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100.

DPPH was dissolved in 95% methanol to a final concentration of 100 μM. Buffer was used as the blank while GSH served as the positive control. Appropriate dilutions of the samples (100 μL) were mixed with 100 μL of DPPH solution in the 96-well plate to a final assay concentration of 2.5 mg mL^{-1} and incubated at room temperature in the dark for 30 min. Thereafter, the absorbance of the sample (A_s) and control (A_c) were read at 517 nm. The scavenging activity of the peptide fractions was compared to that of the GSH (2.5 mg mL^{-1}). The percentage scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_b - A_s}{A_b} \times 100$$

where A_b and A_s are the absorbance of the blank and sample respectively.

Superoxide radical scavenging assay (SRSA)

The method described in ref. 1 was used to perform SRSA. Samples (1 mg mL^{-1} final concentration) were each dissolved in 50 mM Tris–HCl buffer, pH 8.3 containing 1 mM EDTA and 80 μL was transferred into a clear bottom microplate well. Subsequently, 80 μL of buffer was added to the blank well. This was followed by addition of 40 μL of 1.5 mM pyrogallol (dissolved in 10 mM HCl) into each well in the dark and the change in the reaction rate was measured immediately at room temperature over a period of 4 min at a wavelength of 420 nm. The superoxide scavenging activity was calculated using the following equation:

$$\text{Superoxide scavenging activity } (\%) = \frac{\Delta A/\text{min blank} - \Delta A/\text{min sample}}{\Delta A/\text{min blank}}$$

where ΔA is the change in absorbance.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was modified based on a method previously described.[19] The BPH, peptide fractions, GSH and 1,10-phenanthroline (3 mM) were each separately dissolved in 0.1 M sodium phosphate buffer (pH 7.4) while $FeSO_4$ (3 mM) and 0.01% hydrogen peroxide were each separately dissolved in distilled water. An aliquot (50 μL) of BPH, peptide fractions or GSH (equivalent to a final assay concentration of 1 mg mL^{-1}) or buffer (control) was first added to a clear, flat bottom 96-well plate followed by addition of 50 μL of 1, 10-phenanthroline and 50 μL of FeSO4. To initiate the reaction in the wells, 50 μL of hydrogen peroxide (H_2O_2) solution was added to the mixture, which was then covered and incubated at 37 °C for 1 h with constant shaking. Thereafter, the absorbance of the mixtures was measured at 536 nm every 10 min for a period of 1 h. The absorbance was also determined for a blank (does not contain peptides or H_2O_2) and a control (did not contain peptides)

$$\text{Hydroxyl radical scavenging activity } (\%) = \frac{\Delta A/\text{min blank} - \Delta A/\text{min sample}}{\Delta A/\text{min blank}}$$

where ΔA is the change in absorbance.

Metal ion chelation activity

The metal chelating activity was measured using a slightly modified version of a previous method.[19] The BPH and peptide solutions or GSH (final concentration of 1 mg mL^{-1}) were each combined with 0.05 mL of $FeCl_2$ (2 mM) and 1.85 mL distilled water in a reaction tube. Thereafter, 0.1 mL of 5 mM FerroZine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt] solution was added and mixed thoroughly. The mixture was allowed to stand at room temperature for 10 min. Thereafter, a 200 μL aliquot of the reaction mixture was pipetted into a clear bottom 96-well plate. The control experiment contained all the reaction mixtures except that distilled water was used to replace the peptide sample. The absorbance of sample (A_s) and control (A_c) was measured using a spectrophotometer at 562 nm and the metal chelating activity of the sample was compared with that of GSH. The percentage chelating effect (%) was calculated using the following equation:

$$\text{Metal chelating effect } (\%) = \frac{A_b - A_s}{A_b} \times 100$$

where A_b and A_s are the absorbance of the blank and sample respectively.

Ferric reducing power assay

Ferric reducing power was measured according to a method reported in ref. 22 with slight modifications. The peptide sample (250 μL) or GSH was prepared in 0.2 M sodium phosphate buffer (pH 6.6), and mixed with 250 μL of buffer and 250 μL of 1% potassium ferricyanide solution dissolved in distilled water. The final peptide or the GSH concentration in the assay mixture was 1 mg mL^{-1} while the control reaction contained buffer and ferricyanide only. Each resulting mixture was heated at 50 °C and incubated for 20 min, followed by addition of 250 μL 10% aqueous trichloroacetic acid. Subsequently, a 250 μL aliquot of the reaction mixture was combined with 50 μL of 0.1% aqueous ferric chloride solution and 200 μL of distilled water was added. The mixture was allowed to stand at room temperature for 10 min and then centrifuged at 1000*g* for 10 min. The absorbance of the supernatant was measured at 700 nm.

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Statistical analysis

Data were collected as means of 3 separate determinations and subjected to one way analysis of variance using Statistical Analysis System Software (SAS version 9.2, SAS institute, Cary, NC, USA). Significant differences between the mean values were determined by using the Duncan's multiple range tests and accepted at $p < 0.05$.

Results and discussion

Surface hydrophobicity (S_o) of BPHs and their membrane fractions

The hydrophobic properties of peptides of food protein-derived hydrolysates may play important roles in their bioactivities.[13,19] The highest DPPH radical scavenging activity found in the <1 kDa peptide fraction of the African yam bean protein hydrolysate was linked to its high hydrophobicity.[19] Fig. 1 shows that S_o was directly related to the peptide size because the values increased significantly ($p < 0.05$) with the increase in peptide size from <1 to 5–10 kDa. Given that hydrophobic groups are buried inside the core of the folded structure of native protein molecules, the subsequent exposure of some of these groups after partial hydrolysis may have contributed to the increase of S_o.[14,25] On the other hand, as enzyme hydrolysis progresses, the hydrophobic patches are disrupted and S_o decreases with increased enzymatic hydrolysis or reduced peptide size. A similar increase in S_o values with the increase in peptide size has been reported for the rape seed protein hydrolysate.[14] Further, the results obtained show that the S_o of the BPH obtained for the trypsin hydrolysate (1421) is higher than the S_o obtained for alcalase and pepsin (800) hydrolysates. The results suggest that the trypsin hydrolysate and its membrane fractions contained higher molecular weight peptides than the other hydrolysates. A high hydrophobicity similar to the trypsin hydrolysate has been reported for the hemp protein hydrolysate according to ref. 20 Trypsin is a highly specific protease that hydrolyses arginine or lysine-containing peptide bonds and will produce big-sized peptides. In contrast, alcalase and pepsin are more effective random-acting proteases that will produce smaller peptides with lower S_o.

DPPH radical scavenging activities

The DPPH radical is a stable free radical that shows maximum absorbance at 517 nm in methanol and is used to evaluate the antioxidant activity of natural compounds.[13] The DPPH scavenging activity indicates the electron-donating ability of antioxidant compounds, which then converts the radical into a more stable species.[15] The ability of BPH and its membrane fractions to scavenge the DPPH radical are shown in Fig. 2. It is interesting to note that pepsin hydrolysate fractions had the highest DDPH scavenging activity (67–72%) when compared to GSH which was used as the standard. All the peptide fractions had better DPPH scavenging activities compared to their hydrolysates. Also, the peptide size was inversely related to the DPPH radical scavenging activity. A similar trend was also reported for African yam beans, canola protein hydrolysates and hemp peptides.[1,19,22] The scavenging activity of the BPHs and their fractions revealed that the peptides were able to effectively scavenge the DPPH radical compared to GSH. The DPPH scavenging properties of low molecular weight peptides could make them useful ingredients to prevent oxidative deterioration of foods.

Superoxide radical scavenging activities (SRSA)

Numerous biological reactions such as the oxidation of haemoglobin and normal catalytic function of a number of metabolic enzymes generate superoxide radicals (O_2^-), which is a highly

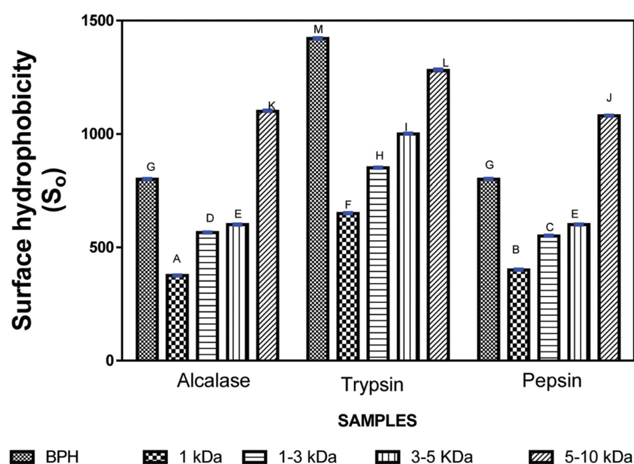

Fig. 1 Surface hydrophobicity of enzymatic bambara protein hydrolysates and membrane ultrafiltration fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

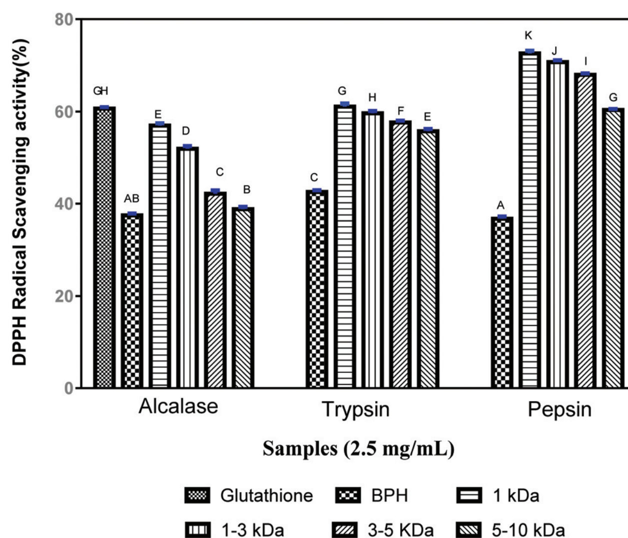

Fig. 2 DPPH radical scavenging activities of bambara protein hydrolysates and membrane fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

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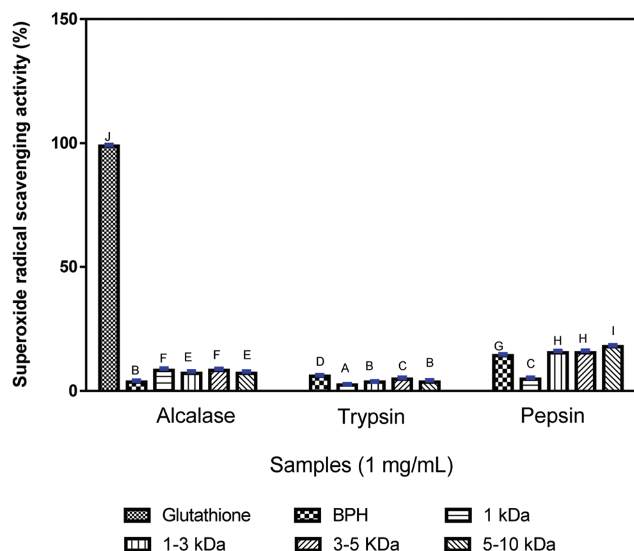

Fig. 3 Superoxide radical scavenging activities of bambara protein hydrolysates and membrane fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potential precursors of highly reactive species such as the hydroxyl radical and therefore the study of the scavenging activity of this radical is important.[15] Bambara protein hydrolysates and their membrane-separated fractions showed different SRSAs based on the different proteases used at 1 mg ml^{-1} final concentration (Fig. 3). The GSH was significantly ($p < 0.05$) a more effective superoxide radical scavenger than the BPHs and peptide fractions. Generally, all hydrolysates had lower superoxide activities (<20%). However, the pepsin hydrolysate and its membrane fractions (except for <1 kDa) had the highest scavenging activity when compared to alcalase and trypsin hydrolysates or their corresponding membrane fractions. Similar higher superoxide activity was reported for the canola pepsin hydrolysate compared to its alcalase and trypsin hydrolysates.[1] The results obtained in this study are lower when compared to the values reported for Africa yam bean peptides.[19] The bambara protein hydrolysate and its peptide fractions were not as effective as GSH in superoxide radical scavenging. Therefore, depending on the degree of bioavailability, the peptides may provide some level of protection to cells against the toxic effect of superoxide free radicals.

Hydroxyl radical scavenging activities

The reactive oxygen radicals are unstable and react readily with other groups or substances in the body resulting in cell damage and hence diseases in humans.[23] Among these reactive oxygen radicals the hydroxyl radical is the most reactive and can be formed from a superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron. The hydroxyl radical severely damages adjacent biomolecules such as proteins, nucleic acids and almost any other biological molecules. This damage may lead to aging as well as development of chronic diseases such as cancer, diabetes and neurodegeneration.[19] Therefore, scavenging of the hydroxyl radical is important for protection against various metabolic disorders that are due to hydroxyl radical activities. Bambara protein hydrolysates and its fractions showed different hydroxyl scavenging activities which was dependent on the type of protease used. Trypsin and pepsin hydrolysates had similar hydroxyl radical scavenging, which is also favourable compared to GSH (standard). Moreover, the 5–10 kDa pepsin hydrolysate fractions had a slightly higher percentage (88%) of hydroxyl radical scavenging activity than GSH (82%). This high activity of the trypsin hydrolysate may be due to its high surface hydrophobicity as shown in Fig. 1. A number of research studies have linked high hydrophobicity to high hydroxyl scavenging activity. For example, Pownall *et al.*[13] reported a strong hydroxyl scavenging activity for highly hydrophobic pea protein hydrolysates. In addition, the peptide molecular weight was positively related to hydroxyl radical scavenging. The hydroxyl radical activity (Fig. 4) obtained in this study is higher than the values reported for African yam bean alcalase hydrolysates but lower than that of chickpea alcalase protein hydrolysates.[15,19] The inhibition of the hydroxyl radical exhibited by the pepsin hydrolysate and trypsin hydrolysate (82%) is closer to that of a peptide isolated from hoki (81%).[26] According to the present findings, the bambara protein hydrolysate might be useful in providing bioactive peptides with good hydroxyl radical scavenging activity for the formulation of oxidative stress-reducing foods.

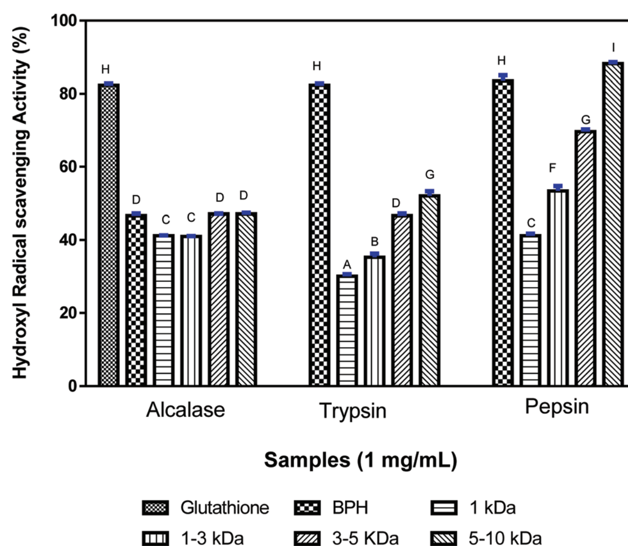

Fig. 4 Hydroxyl radical scavenging activities of bambara protein hydrolysates and membrane fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

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Metal chelating activity

Involvement of transition metal ions in many *in vivo* oxidation reactions has been recognized. The ferrous ion (Fe^{2+}) can catalyse the Haber–Weiss reaction and induce the superoxide anion to form the more hazardous hydroxyl radical. This hydroxyl radical reacts rapidly with the adjacent biomolecules and can induce severe cellular damage. The ferrous ion is one of the products formed during the Fenton reaction, where hydrogen superoxide produces hydroxyl radicals.[13,16] It has been reported that scavenging of hydroxyl radicals by antioxidants was effective mainly through metal ion chelation. Since compounds that interfere with the catalytic activity of metal ions could impair the peroxidative process, measurement of the chelating ability is important for evaluating the antioxidant potential of a compound.[13] Fig. 5 shows the Fe^{2+} chelating effects of reduced GSH, BPHs and their membrane fractions. The alcalase hydrolysate and its membrane fractions exhibited a higher chelating ability than the GSH (standard) while pepsin and its membrane fractions did not display any metal chelating activity. Similar results have been observed for the lower molecular weight thermolysin pea protein hydrolysate which did not display any metal chelating activity.[13] Interestingly, 5–10 kDa trypsin fractions showed the highest percentage of metal chelation (90%), which could be due to the high hydrophobicity of trypsin hydrolysates as shown in Fig. 1. In addition, for trypsin hydrolysates, the metal ion chelating activities increased with the molecular weight, which may be due to an additive effect from constituent peptides. However, for alcalase the trend was different because the 1–3 kDa fractions had the highest metal chelation percentage (86%) when compared to other peptide fractions. The results obtained in this study are similar to those obtained for African yam beans and hemp seeds.[19,22] The observed ion chelating properties of the BPHs and their membrane fractions (alcalase and trypsin) may be beneficial towards the protection of cellular components against metal cation-dependent oxidative damage.

Ferric reducing power activity (FRAP)

The ability of natural antioxidants to donate electrons or hydrogen can be evaluated by using the ferric reducing antioxidant power (FRAP) assay. Some reports have indicated that there is a direct correlation between the reducing power of protein hydrolysate fractions and antioxidant activities.[13,27] The reducing power of BPHs and their membrane fractions are shown in Fig. 6. An increase in absorbance indicates a better reducing power of the test sample. The BPHs and their membrane fractions exhibited higher absorbance values of 0.035–0.07 compared to GSHs which had the lowest absorbance value of 0.034. This implied that BPHs and their membrane fractions had the highest reducing power when compared to GSHs. Among the BPHs and their fractions, the unfractionated hydrolysates had higher reducing power when compared to their membrane fractions. The trypsin hydrolysate had the highest absorbance (0.077) among the hydrolysates. In addition, the ferric reducing power of the BPH fractions increased with an increase in the molecular size of the peptide. This may be due to the additive effect from the constituent peptide. However, the synergistic effects of the fractions may have been instrumental in providing the high activity exhibited by the unfractionated hydrolysate. The results may also be an indication of additive effects of the active groups within the long chain peptide containing more reducing groups than in the short chain peptides. This same trend was observed for the S_o, which increased as the molecular weight increased, furthermore, the same trend observed

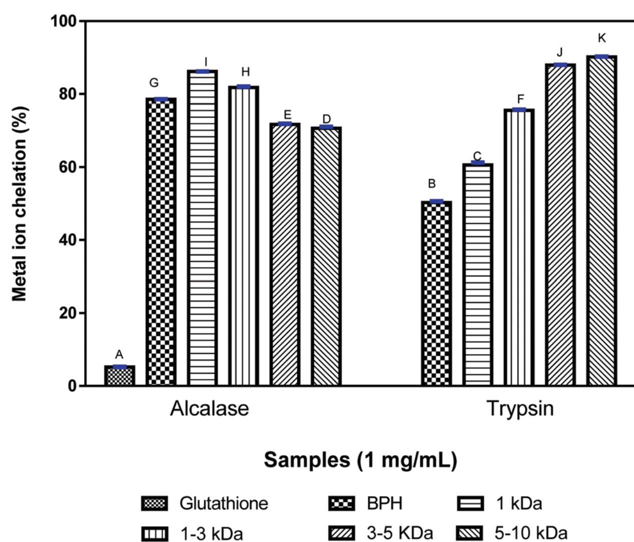

Fig. 5 Metal chelating effects of bambara protein hydrolysates and membrane fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

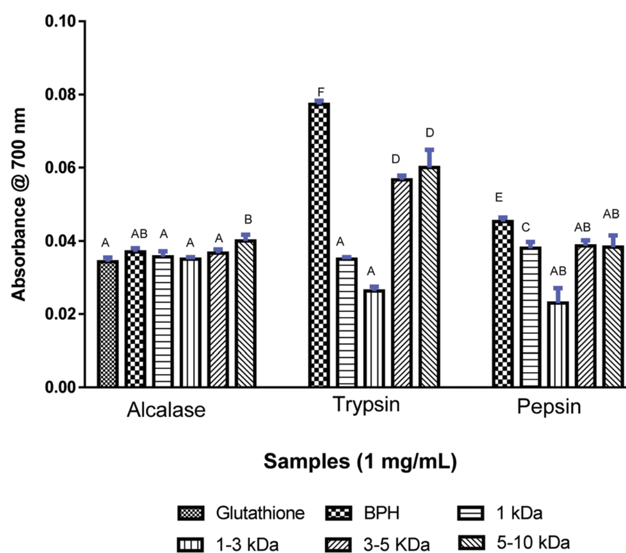

Fig. 6 Ferric reducing power of bambara protein hydrolysates and membrane fractions (mean ± standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)).

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for FRAP was observed for the metal chelation of BPHs and their membrane fractions, which had the 5–10 kDa fraction as the most effective fraction. In contrast, Ajibola *et al.*[19] reported a decrease in reducing activities with an increase in peptide size for the alcalase hydrolysate of African yam bean. However, the present results are similar to the reducing power values observed for the hemp seed hydrolysate.[22]

Conclusions

Protein hydrolysates derived from bambara groundnut possess antioxidant properties against a variety of physiologically relevant free radicals studied *in vitro*. High surface hydrophobicity and the molecular size of the peptide seem to be important for scavenging of hydroxyl radicals, ferric reducing power and metal chelation. The activity increased with an increase in peptide size except for DPPH which had higher activity with a smaller molecular size peptide. The activity of <1 kDa for pepsin was higher than that of GSH. Similarly, the peptides had better ferric reducing and metal chelating activities when compared to GSH except for the pepsin hydrolysate and its fractions that did not exhibit any metal chelating activity. The bambara protein hydrolysate and its membrane fractions may be suitable ingredients for the formulation of functional foods and nutraceuticals that can be used to prevent or manage oxidative stress.

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