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To cite this article: Atinuke O. Idowu, Akinsola A. Famuwagun, Tayo, N. Fagbemi & Rotimi E. Aluko (2021) Antioxidant and enzyme-inhibitory properties of sesame seed protein fractions and their isolate and hydrolyzate, International Journal of Food Properties, 24:1, 780-795, DOI: [10.1080/10942912.2021.1919704](https://doi.org/10.1080/10942912.2021.1919704)

To link to this article: <https://doi.org/10.1080/10942912.2021.1919704>



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Published online: 23 May 2021.



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Antioxidant and enzyme-inhibitory properties of sesame seed protein fractions and their isolate and hydrolyzate

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ABSTRACT

Sesame seed was defatted and subsequently fractionated into albumin, globulin, glutelin and prolamin. The defatted flour was also subjected to alkaline solubilization and acid precipitation to obtain protein isolate. The sesame seed protein isolate was hydrolyzed using a combination of pepsin and pancreatin to produce the protein hydrolyzate. The defatted flour, protein fractions, isolate and hydrolyzate were evaluated for their amino acid profiles as well as in vitro antioxidant and enzyme inhibitory activities. The results showed that the glutelin fraction had higher amounts of essential amino acid (38.1%) when compared with albumin (35.6%), globulin (31.3%), prolamin (37.1%), protein isolate (37.1%) and protein hydrolyzate (36.7%). The defatted flour exhibited the strongest DPPH radical scavenging activity while only the globulin, isolate and hydrolyzate exhibited superoxide radical scavenging activity. The defatted flour also had the strongest hydroxyl radical scavenging activity of 91.79%, although lower than the 95.93% for the control peptide (glutathione). The hydrolyzate had the strongest metal chelating activity (75.53%), while the isolate had the highest ferric antioxidant reducing power. The albumin was the most effective inhibitor of angiotensin converting enzyme and α -amylase with values of 30.04% and 29.44%, respectively. In contrast, renin activity was strongly inhibited (89.87%) by the isolate but acetylcholinesterase was weakly inhibited by the hydrolyzate (16.89%) and prolamin (16.29%). We conclude that the defatted flour and protein products are potential ingredients that could be incorporated into foods to extend shelf-life but also with potential bioactive properties.

ARTICLE HISTORY

Received 11 February 2021
Revised 13 April 2021
Accepted 16 April 2021

KEYWORDS

Sesame seed; protein fractions; protein isolate; protein hydrolyzate; antioxidant; angiotensin converting enzyme; RENIN; α -amylase; acetylcholinesterase

Introduction

Plant proteins are gaining increased attention in the research space lately because they are sources of natural proteins and bioactive peptides for the production of novel class of food products.^[1] Based on the amino acid composition, amino acid sequence and length of the peptides, plant proteins exhibit various health benefits, such as anticancer, antidiabetic, antidementia, antihypertensive and antioxidant activities.^[2] Sesame (*Sesamum indicum* L) is one of the most valuable oil seeds in the world with global production of 6.11 million in 2016.^[3] The sesame seed has 42%–54% oil and 22%–25% protein, depending on the varieties and agronomic conditions. The extraction of edible oil from sesame seed for commercial purpose leaves the seed cake as a by-product, with protein content of about 50%, which are mostly used to supplement animal feeds.^[4] However, the proteins could also form an

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important part of the human diet as ingredients for the formulation of novel foods. For example, the proteins could be used as antioxidants in foods to replace synthetic compounds or they could function as inhibitors of various physiologically relevant enzymes, which are relevant to maintaining human health.

Proteins can be isolated using a pH-shift method that involves alkaline solubilization followed by acid-induced precipitation.^[5] Plant protein fractions, such as prolamin, glutelin, globulin and albumin, can also be obtained by taking advantage of their differential solubility in different solutions. Therefore, the solubility of the extracted protein depends mainly on the choice of solvent used.^[6] In addition to whole proteins, enzymatic protein hydrolysis is a proven method to extract proteins and convert them to peptides with bioactive properties. The release of bioactive peptides in the form of enzymatic protein hydrolyzates could enhance the value-added utilization of natural proteins.^[7] Several research facts on sesame seeds have been made available that suggest that defatted meal could be used as a source of dietary supplements. For instance, the high content of sulfur-containing amino acid in sesame seed protein suggests its use as a good complement for legumes.^[8] The functional, physical, biochemical and immunological properties of sesame seed protein fractions, such as 2S,^[9] 7S,^[4] and 11S,^[10] have been studied. Several subunits of sesame seed proteins^[11] oil body proteins^[12] have also been explored by electrophoresis. Protein hydrolyzates produced from sesame seed proteins have been shown to exhibit various antioxidant^[8,13] and antihypertensive^[13,14] properties. However, there is a dearth of information on the antioxidant properties and enzyme inhibitory potentials of the various storage proteins of sesame seeds and the protein isolate. Therefore, the aim of this study was to provide a comparative evaluation of the antioxidant and enzyme inhibitory properties of sesame seed protein fractions, protein isolate and enzymatic protein hydrolyzate.

Materials and methods

Materials

Enzymes (rat intestinal acetone powder as a source of porcine pancreatic α -amylase, and rabbit lung ACE) and other reagents (acarbose, orlistat, and 4-nitrophenyl α -D-glucopyranoside) were of analytical grade and purchased from Sigma chemicals (St. Louis, MO, USA). Sesame seed was obtained from a local market in Kawo, Kaduna state, Nigeria. The seeds were milled into flour using a Smartgrind[®] coffee grinder (CBG 100SC model, Black & Decker, Baltimore, Maryland, USA). The flour was defatted with Soxhlet apparatus using n-hexane for 8 h. The defatted flour was oven-dried at 60°C and stored at 4°C.

Preparation of sesame protein fractions

Sesame seed protein fractions were prepared from the defatted flour using the traditional Osborne procedure as described by^[15] with slight modification. The albumin fraction was first extracted from the defatted sesame seed flour by mixing with 10 volumes of ultrapure (Milli-Q[®], Millipore Corporation) water for 2 h under constant stirring. The mixture was centrifuged at 6500 \times g for 30 min and the resultant supernatant filtered using cheesecloth (Fisher Scientific, 6665–8) and saved. The residue was re-extracted with ultrapure water as described above and the supernatants pooled together and freeze-dried as the albumin (ALB) fraction. In order to obtain globulin (GLO), glutelin (GLU) and prolamin (PRO) fractions, the residue was sequentially re-suspended in 2% (w/v) NaCl, 0.1 M NaOH and 70% ethanol, respectively, followed by corresponding centrifugation and filtration steps to collect supernatants. The NaCl extract (GLO) was dialyzed against 20 volumes of deionized water at 4°C using 6–8 kDa molecular weight cutoff (MWCO) Spectra/Por1 cellulose membranes (Spectrum Labs, Rancho Dominguez, CA, USA). The dialysis was carried out over 3 days with four daily changes of water to remove the salt in the fraction. The dialysis protocol was similarly done for prolamin fraction (70% ethanol extract) in order to remove the ethanol. For the glutelin fraction, the

fraction was adjusted to pH 4.0 with 1 M HCl and centrifuged at $6500 \times g$ for 30 min to obtain a precipitate that was then re-suspended in five volumes of deionized water and adjusted to pH 7.0 with 1 M NaOH. All the extracted protein fractions were freeze-dried and stored at -20°C .

Preparation of sesame protein isolate and hydrolyzate

Sesame seed protein isolate (ISO) was prepared by isoelectric precipitation using the method of^[16] which was slightly modified as follows. A 10% (w/v) suspension of the defatted sesame seed flour in deionized water was adjusted to pH 9.5 with 1 M NaOH and then stirred continuously for 1 h at 50°C . The solution was then centrifuged at $4000 \times g$ for 10 min at 4°C . The supernatant was collected and adjusted to pH 4.9 using 1 M HCl and stirred for 20 min. The precipitate obtained after centrifugation was washed twice with distilled water to remove salt, re-dispersed in deionized water and adjusted to pH 7.0 prior to freeze-drying.

The ISO was dispersed in water (5%, w/v) and hydrolyzed with pepsin-pancreatin enzymes. The aqueous ISO mixture was heated to 37°C and adjusted to pH 2.0 using 1 M HCl followed by the addition of pepsin enzyme (4% w/v, ISO protein weight basis). After pepsin addition, the digestion was conducted for 2 h and the reaction mixture adjusted to pH 7.5 with 2 M NaOH. Then pancreatin enzyme (4% w/v, ISO protein weight basis) was added before incubating the mixture at 37°C for 4 h. The pancreatin reaction was terminated by heating to 95°C for 15 min to ensure total denaturation of residual enzymes. The digested mixture was centrifuged ($7000 \times g$ at 4°C) for 30 min and the resulting supernatant was freeze-dried to obtain sesame seed protein hydrolyzate (PH).

Amino acid composition

The amino acid profile of each sample was determined according to the established methods described by^[17] using a HPLC system after hydrolysis with 6 M HCl. The cysteine and methionine contents were determined after performic acid oxidation while the tryptophan content was determined after alkaline hydrolysis.

Determination of antioxidant properties

1,1-diphenylpicrylhydrazine (DPPH) radical scavenging activity (DRSA)

The DRSA of the samples was determined using the method described by^[17] with slight modifications for a 96-well flat bottom microplate. Samples were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 containing 1% (v/v) Triton-X to a final concentration of 0.0156–1.0 mg/mL. DPPH was dissolved in 95% methanol to a final concentration of 100 μM . A 100 μL aliquot of each sample was mixed with 100 μL of the DPPH radical solution in a 96-well plate and incubated at room temperature in the dark for 30 min. The buffer was used in the blank assay while reduced glutathione (GSH) served as the positive control. Absorbance was measured at 517 nm using a spectrophotometer and the percentage DRSA was determined using the following equation:

$$DRSA(\%) = \frac{(A1 - A2)}{A1} \times 100$$

where A1 and A2, are absorbance values of the blank and sample, respectively. The effective concentration that scavenged 50% of the free radicals (EC_{50}) was calculated for each sample by nonlinear regression from a plot of percentage DRSA versus sample concentration (mg/mL) using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

Superoxide radical scavenging activity (SRSA): The method described by^[18] was used to determine SRSA of the protein samples. Samples (1.0 mg/mL assay concentration) were each dissolved in 50 mM Tris-HCl buffer, pH 8.3 containing 1 mM EDTA followed by the transfer of 80 μL into a clear bottom microplate well while 80 μL of buffer was added to the blank well. This was followed by the addition of

40 μL 1.5 mM pyrogallol (dissolved in 10 mM HCl) into each well in the dark and the change in reaction rate was measured immediately at room temperature over a period 4 min (1 min interval) using a microplate reader at a wavelength of 420 nm. The SRSA was calculated using the following equation:

$$\text{SRSA} = \frac{\text{slope of blank for SRSA} - \text{slope of sample for SRSA}}{\text{slope of absorbance perminute of blank of SRSA}} \times 100$$

Hydroxyl radical scavenging activity (HRSA)

The HRSA of samples was determined using the method described by [19] with modifications. Samples prepared to assay concentrations of 1.0 mg protein/mL and 3 mM 1,10-phenanthroline were separately dissolved in 0.1 M phosphate buffer (pH 7.4) while 3 mM FeSO_4 and 0.01% (w/v) hydrogen peroxide were each separately prepared in distilled water. The mixture was kept at room temperature for 1 h and then centrifuged ($3500 \times g$) for 30 min. Fifty microliters of the samples or GSH standard were first added to a 96-well plate followed by 50 μL each of the 1, 10-phenanthroline and FeSO_4 . To initiate the Fenton reaction in the wells, 50 μL of hydrogen peroxide was added to the mixture and the covered plates incubated at 37°C for 1 h with constant shaking. The blank consisted of 50 μL phosphate buffers instead of the protein sample. Absorbance of the colored reaction mixtures was measured at 10 min intervals for 1 h in a microplate reader at a wavelength of 536 nm. The reaction rate ($\Delta A/\text{min}$) was then used to calculate the HRSA value as follows:

$$\text{HRSA} = \frac{\text{slope of blank for HRSA} - \text{slope of sample for HRSA}}{\text{slope of absorbance perminute of blank for HRSA}} \times 100$$

The slope is a measure of sample absorbance per minute.

Ferric reducing antioxidant power (FRAP)

The FRAP of the protein samples was determined using the modified method of [20]. FRAP working reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20 mM FeCl_3 in the ratio of 55:1:1, respectively, to obtain a straw-colored solution, and the temperature of the mixture raised to 37°C . Protein samples were dissolved in distilled water to assay concentration of 1 mg/mL. Into a clear 96-well microplate, 40 μL of samples and 200 μL of FRAP reagents were added and absorbance read at 593 nm. Iron II sulfate hepta hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) at 0.025–0.25 mM was used as standard. Iron reducing activity of the samples was determined from the standard curve and the results expressed as Fe^{2+} (mM).

Metal chelation ability

The metal (iron) chelating ability (MCA) of the samples was determined according to the modified method of [18]. Protein samples/standards were prepared in distilled water to give 1 mg/mL assay concentration. A 1 mL aliquot of the sample solution or blank (distilled water) was mixed with 50 μL of 2 mM FeCl_2 and 1.85 mL double-distilled water in a reaction tube. This was followed by the addition of 100 μL of 5 mM Ferrozine. The mixture was vortexed thoroughly and incubated at room temperature for 10 min. After incubation, a 200 μL aliquot of the reaction mixture was transferred into a 96-well plate and absorbance values of both the blank (A_b) and samples (A_s) were measured at 562 nm using a microplate reader. The metal chelating activity was calculated as follows:

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

where A_b is the absorbance of the blank and A_s is the sample absorbance.

ACE inhibition assay

Inhibition of *in vitro* ACE activity by the protein samples was measured using the method described by [21]. Briefly, 1 mL of 0.5 mM N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG: dissolved in 50 mM Tris-HCl buffer containing 0.3 M NaCl, pH 7.5, and kept at 37°C) was mixed with 20 µL of ACE (20 mU assay activity) and 200 µL of the samples (0.2 mg/mL assay concentration) dissolved in the Tris-HCl buffer. The rate of decrease in absorbance at 345 nm was recorded for 2 min at room temperature. Tris-HCl buffer (no sample) was used as the assay blank. ACE activity was expressed as the change in the rate of reaction ($\Delta A/\text{min}$) and inhibitory activity was calculated using the equation below:

$$\text{ACE inhibition} = \frac{\Delta A/\text{min}(\text{blank}) - \Delta A/\text{min}(\text{sample})}{\Delta A/\text{min}(\text{blank})} \times 100$$

where $\Delta A/\text{min}(\text{blank})$ and $\Delta A/\text{min}(\text{sample})$ are ACE activities in the absence and presence of samples, respectively.

Renin-inhibitory activity

The ability of the sesame seed samples to inhibit *in vitro* activity of human recombinant renin was determined by fluorescence spectrometry using the renin inhibitor screening assay kit according to the previously described method of [21]. The total assay volume of 190 µL included 19 µL of 10 mg/mL sample, which had earlier been dissolved in 50 mM Tris-HCl buffer containing 100 mM NaCl (pH 8.0), 10 µM Arg-Glu (EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys (Dabcyl)-Arg (renin substrate dissolved in dimethyl sulfoxide), and human recombinant renin. Tris-HCl buffer was used instead of the protein solution in the blank experiment while each sample well, contained an assay sample final concentration of 1 mg/mL. The 96-well plate containing the reaction mixtures was mixed and prewarmed to 37°C for 15 min to attain thermal equilibrium before monitoring the periodic increases in fluorescence intensity (FI) using a fluorometric microplate reader (Spectra MAX Gemini, Molecular Devices, Sunnyvale, CA, USA) with excitation and emission wavelengths set at 340 and 490 nm, respectively. The percentage inhibition was calculated as follows:

$$\text{Renin inhibition}(\%) = \frac{(FI.\text{min}^{-1}(\text{blank}) - (FI.\text{min}^{-1}(\text{sample})))}{FI.\text{min}^{-1}(\text{blank})} \times 100$$

where $FI.\text{min}^{-1}(\text{blank})$ and $FI.\text{min}^{-1}(\text{sample})$ are the fluorescent intensity in the absence and presence of peptides, respectively

Acetylcholinesterase (AChE) inhibition assay

The AChE inhibition assay was performed as previously described by [22] with slight modifications as follows. Briefly, the reaction was carried out at room temperature using 0.1 M sodium phosphate buffer (pH 7.5) to prepare samples (1 mg/mL) and an assay mixture volume of 200 µL in a 96-well microplate. A 20 µL aliquot of sample (or galantamine as standard at a concentration of 1 mg/mL) was added to the microplate wells that already contained 130 µL of the buffer. Then, 20 µL of DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid) and 10 µL of acetylthiocholine were added to each well followed by 20 µL of AChE (0.5 U/mL assay concentration) to initiate the reaction. The mixture was incubated for 15 min at room temperature. The absorbance and reaction kinetics were read for 2 min at a wavelength of 412 nm using a microplate reader. In the uninhibited wells, 20 µL of the buffer was used to replace the sample. In the background wells, 20 µL of the buffer was used to replace AChE while 40 µL of buffer was used to replace both AChE and sample in the blank wells.

Inhibition of α -amylase activity

Inhibition of porcine α -amylase activity was determined using the dinitrosalicylic acid described by [23]. One hundred microliters (100 μ L) of samples (500 μ g/mL assay concentration) or standard (acarbose; 1 μ g/mL assay concentration) prepared in 0.02 M phosphate buffer, pH 6.9 containing 0.006 M NaCl was added to 100 μ L of α -amylase. The mixtures were preincubated at 25°C for 10 min and 200 μ L of 1% (w/v) starch (prepared in 0.02 M phosphate buffer, pH 6.9 containing 0.006 M NaCl) added. The reaction mixtures were incubated at room temperature for 10 min and then enzyme activity terminated by adding 1 mL of dinitrosalicylic acid followed by incubation in a boiling water bath for 5 min. The reaction mixtures were cooled to room temperature, diluted 1:5 with distilled water, 200 μ L transferred to the 96-well microplate and the absorbance measured at 540 nm using microplate reader. The percentage inhibition of enzyme activity was calculated as:

$$\text{Alpha - amylase inhibition} = \frac{(\text{Absorbance of control} - (\text{Absorbance of sample} - \text{Absorbance of sample blank}))}{\text{Absorbance of control}} \times 100$$

Statistical analysis

Analyses were performed in triplicates and the results subjected to analysis of variance using SPSS version 18.0. The statistical significant differences ($p < .05$) between mean values were determined using the Duncan Multiple Range Test.

Results and discussion

Amino acid composition

The amino acid compositions (g/100 g) of defatted sesame flour, albumin, globulin, glutelin, prolamin, protein isolate and protein hydrolyzate are shown in Table 1. The results indicate the samples as having Glu, Asp and Arg in the highest amounts of 18.44%–24.89%, 6.63%–11.08% and 9.06%–14.95%, respectively, when compared with Val (3.95%–5.84%), Ile (2.66–4.55%) and Trp (0.93–1.48%). The potential of Glu and Asp to donate electrons as free radical scavengers (strong antioxidant) and the ability of Arg to operate as NO-precursor (vasodilator) make them potential tissue protective agents against oxidative stress [24] and hypertension [25]. In addition, Asp and Glu play important roles in metal ion chelation and hydroxyl radical scavenging activities because they contain carboxyl and amine groups in their side chains. [26,27] The globulin fraction had the highest Glu and Arg but had the least Asp content, which indicates weak potential as an antioxidant or cardioprotective protein when compared to the other protein fractions. There were variations between the amino acid composition of the precursor defatted sesame seed defatted flour (DF), protein fractions and protein hydrolyzates. For instance, albumin and glutelin fractions were richer in threonine, proline and tyrosine when compared to the amount in DF. In contrast, cysteine and methionine were present in greater amounts in the DF when compared to albumin and glutelin fractions. The prolamin fraction exhibited higher quantities of leucine, isoleucine, proline and valine than the other fractions, which is consistent with the highly hydrophobic character and solubility only in an alcoholic solution. With the exception of prolamin and protein isolates, the protein hydrolyzate had higher contents of hydrophobic and branched chain amino acids; specifically, the hydrolyzate exhibited the highest amount of alanine, tyrosine and phenylalanine. The values (3.24%–4.66%) obtained in this study for proline, an important amino acid, which has been reported to contribute to ACE-inhibitory activity of peptides were comparable to the 3.27%–6.25% reported for cashew nut and fluted pumpkin protein hydrolyzates, [28] but higher than the 1.33%–2.24% for *Phaseolus lunatus* protein hydrolyzates. [29] With the exception of lysine, some of the values reported for the amino acid composition of the samples were

Table 1. Percentage amino acid composition of sesame seed defatted flour, storage proteins, protein isolate and hydrolyzate.

Sample ID	Defatted flour	Albumin	Globulin	Glutelin	Prolamin	Protein isolate	Protein hydrolyzate	FAO/WHO
Asp+Asn	8.63	11.08	6.63	9.26	8.99	8.64	9.38	1.40
Thr	3.37	3.78	2.26	3.76	3.46	3.33	3.57	3.40
Ser	4.96	5.13	4.61	4.87	5.06	4.65	4.89	
Glu+Gln	20.11	20.73	24.89	18.44	19.29	20.05	21.05	
Pro	3.93	4.22	3.24	4.26	4.66	3.75	3.85	
Gly	4.82	5.02	3.87	4.68	5.52	4.53	4.67	
Ala	5.88	5.87	5.50	5.95	5.53	5.88	6.16	3.50
Cys	2.18	2.16	4.19	1.16	2.08	1.75	1.57	
Val	5.00	4.95	3.95	5.55	5.84	5.40	5.58	3.50
Met	2.88	2.32	4.35	2.29	2.89	2.61	1.99	
Ile	3.84	3.49	2.66	4.47	4.55	4.27	4.33	
Leu	6.82	6.13	5.74	7.30	7.51	7.08	7.04	6.60
Tyr	3.29	3.38	3.80	3.52	2.95	3.49	3.67	1.10
Phe	4.50	3.87	3.74	4.95	4.30	4.66	4.76	6.30
His	3.01	3.00	2.33	3.01	2.84	2.98	2.84	1.90
Lys	2.99	4.89	2.16	2.50	2.67	2.61	2.52	5.80
Arg	12.54	9.06	14.95	12.54	10.86	12.91	11.02	
Trp	1.25	0.93	1.13	1.48	1.00	1.43	1.10	
EAA	36.95	36.74	32.12	38.83	38.00	37.86	37.41	
AAA	9.04	8.18	8.67	9.95	8.25	9.58	9.53	
HAA	39.57	37.31	38.30	40.94	41.31	40.31	40.06	
SCAA	5.06	4.48	8.54	3.45	4.97	4.36	3.56	
BCAA	15.67	14.56	12.34	17.32	17.90	16.74	16.96	

Hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine and cysteine; aromatic amino acids (AAA) = phenylalanine, tryptophan and tyrosine; positively charged amino acids (PCAA) = arginine, histidine, lysine; negatively charged amino acids (NCAA) = aspartic + asparagine, glutamic + glutamine, threonine, serine; essential amino acids (EAA) = histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; sulfur containing amino acids (SCAA) = methionine, cysteine; branched chain amino acids (BCAA) = leucine, isoleucine, valine

higher when compared to the recommended allowance by WHO/FAO for some of the amino acids. results obtained further confirm work by earlier authors [13] that sesame protein hydrolyzates are rich sources of amino acids, such as the hydrophobic (HAA) and aromatic (AAA) amino acids which contributes to the antioxidant and renin-angiotensin system (RAS) inhibition activities.^[1] This is important because the RAS is the main target of antihypertensive agents, which indicates potential use of these proteins as cardioprotective factors. Similarly, the albumin fraction contained higher amounts of lysine and threonine when compared to other protein fractions, isolate and the hydrolyzate, while the globulin fraction was richer in methionine, tyrosine and arginine, than the rest of the samples. The difference in the amounts of amino acid among the protein fractions was supported by previous work on the amino acid composition of coconut protein fractions.^[30] The presence of glycine in the protein molecule also plays a critical role in improving the antioxidant properties because it can easily neutralize free radicals due to its ability to donate protons.^[31] Hydrophobic amino acids, such as proline and methionine, are believed to contribute to free radical scavenging activities because they facilitate interaction with hydrophobic-free radicals.^[32] Aromatic amino acids, such as histidine and tryptophan, are known as antiradicals because of the presence of special side groups.^[33] Also, the high content (>30%) of total essential amino acids suggests that the protein products have good nutritional values.

Antioxidant properties

DPPH radical scavenging activity (DRSA)

The DRSA is a measure of the ability of an antioxidant compound to freely donate electrons to free radical species, which results in the formation of more stable and less toxic species.^[34] The DRSA EC₅₀ values of sesame seed samples are shown in Figure 1. Overall, the DF had the strongest DRSA as evident in the significantly ($p < .05$) lower EC₅₀ value when compared to the other samples. The results suggest that the DF contained molecules that interacted with and readily donated hydrogen atoms to

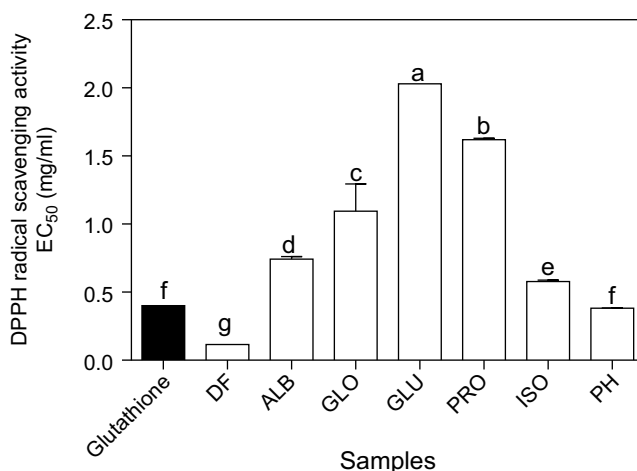


Figure 1. DPPH radical scavenging activities of sesame seed proteins.

the DPPH radical among the sesame seed samples. There was no significant difference ($p > .05$) in the DRSA of the protein hydrolyzate (PH) and standard (glutathione), which indicates strong hydrogen atom-donating potency of the peptides released during enzymatic hydrolysis of sesame seed proteins. The glutelin (GLU) was the least potent and exhibited significantly ($p < .05$) highest DRSA EC₅₀. Among the protein fractions, albumin (ALB) demonstrated stronger DRSA while glutelin (GLU) was the weakest. Although prolamin (PRO) and GLU fractions had the highest amounts of hydrophobic amino acids, the weaker DRSA indicates that the position of the hydrophobic amino acids on the protein sequence and the pattern of folding of the protein fractions may be more important as previously suggested.^[35,36] The results are similar to a previous report showing stronger DRSA of *Moringa* seed ALB when compared to the GLO.^[37] However, the *Moringa* seed flour and protein isolate had weaker DRSA, which is in contrast to the results obtained in the present work. The differences in activities may be due to variations in the level and type of antioxidant molecules in the sesame seed and *Moringa* seed flours.

Superoxide (SRSA) and hydroxyl (HRSA) radical scavenging activities

Superoxide and hydroxyl radicals are compounds produced through several biological reactions. The superoxide radical is a potential precursor to other highly reactive and toxic reactive species, such as the hydroxyl radical and peroxides.^[38] The SRSA of the samples is shown in Figure 2. The antioxidant standard (glutathione) had significantly ($p < .05$) greater SRSA when compared to the sesame seed samples. Among the protein fractions, only the globulin showed positive superoxide activities, while albumin, glutelin and prolamin, including defatted flour, acted as pro-oxidants. The value obtained for the globulin fraction (14.78%) is lower when compared with the 16.87% reported for the coconut globulin fraction.^[30] When all the sesame seed samples were compared, the protein isolate showed greater SRSA, while there was no significant ($p > .05$) difference in the values for the globulin and protein hydrolyzate. The results suggest that the isoelectric precipitated protein may be used as a good superoxide radical scavenger to prevent the formation of highly lethal radicals.

The hydroxyl radical is a wild oxygen species, which is capable of destroying almost all the important macromolecules in the living cells such as DNA, protein, nucleic acid and amino acids. Scavenging this free radical is a good approach to enable the body to maintain a balance between free radicals and antioxidants. When this equilibrium is distorted, it can lead to oxidative stress, which if prolonged leads to negative health outcomes, such as aging, cardiovascular diseases and certain types of cancer, which are hydroxyl radical mediated.^[39] The percentage HRSA of the samples is shown in Figure 3. Glutelin and protein isolate showed negative values, suggesting that they are potential pro-

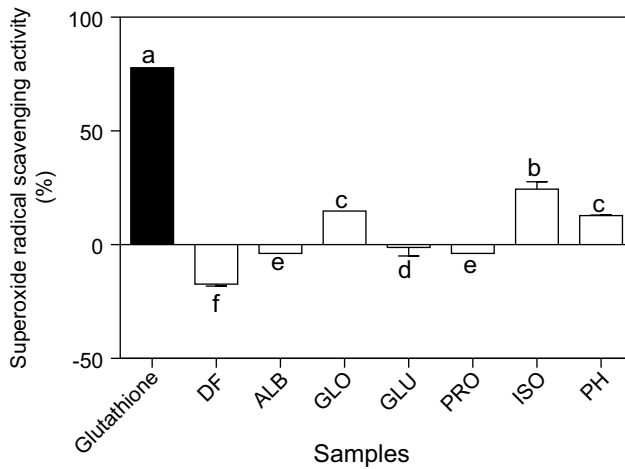


Figure 2. Superoxide radical scavenging activities of sesame seed proteins.

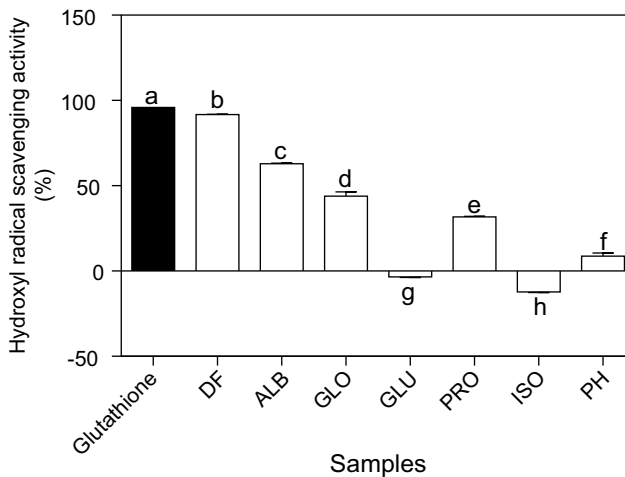


Figure 3. Hydroxyl radical scavenging activities of sesame seed protein.

oxidant within the assay system. The sesame defatted flour was a better hydroxyl radical scavenger when compared with the other protein fractions and the protein hydrolyzate. Among the protein fraction, albumin (62.85%) had significantly ($p < .05$) greater HRSA than globulin (43.89%) and prolamin (31.83%) fractions. Earlier work on coconut protein fractions reported 8.83%, 16.67% and 68.67% hydroxyl radical scavenging activities for albumin, globulin and prolamin fractions, respectively.^[30] Amza et al.^[40] also reported 47.30%, 46.50% and 58.80% hydroxyl radical scavenging activities for the albumin, globulin and glutelin fractions, respectively, obtained from Gingerbread plum seed proteins. Variations in structural conformation and amino acid composition may be responsible for the differences between the HRSA of the sesame seed, coconut and plum seed proteins. The results suggest that the defatted sesame flour and albumin fraction may be used in food systems as defensive agents against hydroxyl radicals, which has been implicated as one of the causes of many cardiovascular diseases.

Metal chelation and ferric reducing antioxidant power (FRAP)

There are reports of active participation of metallic ions such as ferrous in the generation of several free radicals, especially in the oxidative deterioration of unsaturated fatty acids. The involvement of ferrous ions in the Haber–Weiss reaction leads to the generation of superoxide radicals, which is a precursor to many toxic-free radicals. Reaction of ferrous ions with H_2O_2 through Fenton reaction generates the highly reactive hydroxyl radicals that precede many oxidation-induced disorders.^[18,41] Therefore, using an antioxidant compound to chelate the ferrous metallic ion is a good approach to prevent lipid peroxidation in foods and safeguard the body against degenerative diseases. The metal chelation activities of the sesame seed products are shown in Figure 4. The prolamin fraction had the least (13.33%) metal chelating activity when compared to the other samples. There was no significant ($p > .05$) difference in the metal chelating activities of defatted sesame flour, albumin, globulin fractions and protein isolate. Similarly, there was no significant difference ($p > .05$) in the chelating activities of glutelin fraction and glutathione. The protein hydrolyzate showed a stronger ability to chelate metals than the other samples, which may be due to the presence of several carboxylic groups produced when peptide bonds are broken during enzymatic hydrolysis of the native protein.^[26] Earlier workers [26] have recognized the important roles of carboxylic and basic amino acids in the chelation of metal ions. The albumin and glutelin fractions in this study chelated 20.85% and 16.63%, respectively, of ferric ions, and the values were higher when compared with albumin (5.13%) and glutelin (3.16%) fractions of coconut proteins.^[30] The metal chelating activity of the globulin fraction (18.94%) in this study is greater than the 4.12% reported for *Moringa* seed globulin fraction.^[37] The overall results indicate that pepsin-pancreatin hydrolyzate of sesame protein has a good potential for use as ferric ion chelator in food systems.

Oxidation in living systems has evolved through a multifaceted free radical mechanism, which has necessitated the use of different approaches to halt the effect of free radicals. FRAP is another approach to overcome the reactive metallic ions causing free radicals. This assay involves measuring the rate of color change as a result of the conversion of ferricyanide complex to ferrous ions, when the Fe^{3+} encounters an antioxidant compound in a reducing system.^[42] Therefore, an antioxidant compound with positive ferric reducing properties may be a potential compound to reduce the amount of the highly reactive ferric ions that participate in oxidation reactions. As shown in Figure 5, there was no significant difference ($p > .05$) in the FRAP of albumin fraction and protein hydrolyzate. Also, albumin protein fraction was a better reducing agent among the protein fractions while globulin was the least. Except the protein isolate, the glutathione had greater reducing ability than the other samples. The high FRAP contents of protein isolate agreed with the trend of superoxide radical scavenging activities

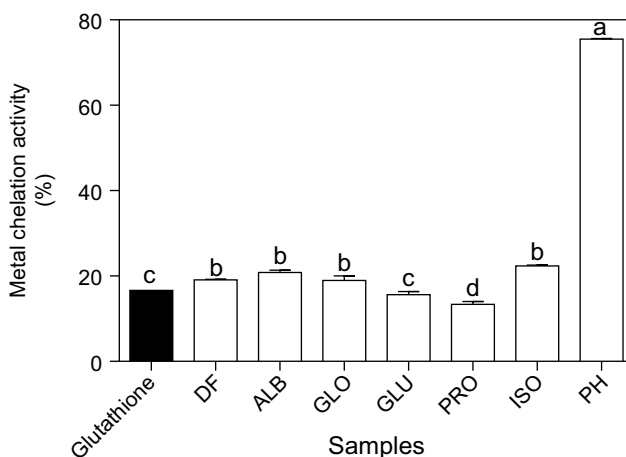


Figure 4. Metal chelating activities of sesame seed proteins.

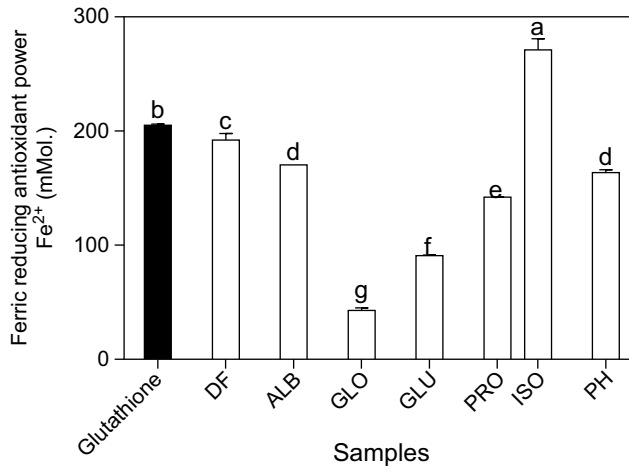


Figure 5. Ferric reducing antioxidant power of sesame seed proteins.

as shown in Figure 2. The protein isolate had the highest ferric reducing ability among the samples and this suggests that the isoelectric pH-precipitated protein can be used as a reducing agent in food systems.

Enzyme inhibitory activities

Angiotensin and renin inhibitory activities

The renin-angiotensin system (RAS) is a critical regulator of artery blood pressure, which is a risk factor for hypertension. Control of blood pressure by RAS is achieved by the inhibition of the angiotensin converting enzyme. Angiotensin-Converting Enzyme (ACE) converts angiotensin I to angiotensin II that eventually lead to the release of aldosterone from the adrenal cortex, resulting in increased blood pressure via sodium retention. Hence, inhibiting the angiotensin converting and renin enzymes is a good approach to manage hypertension and other cardiovascular diseases.^[13,43,44] As indicated in Figure 6, albumin fraction (30.03%) exhibited significantly ($p < .05$) greater ACE-inhibitory activity than the other samples, a pattern which was in line with the trend of some amino

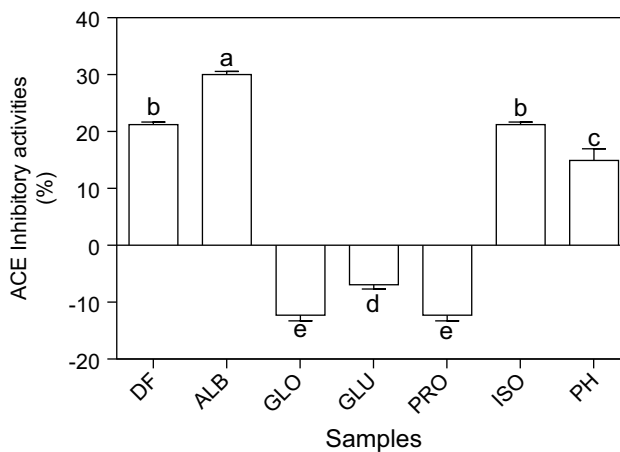


Figure 6. ACE inhibitory activities of sesame seed proteins.

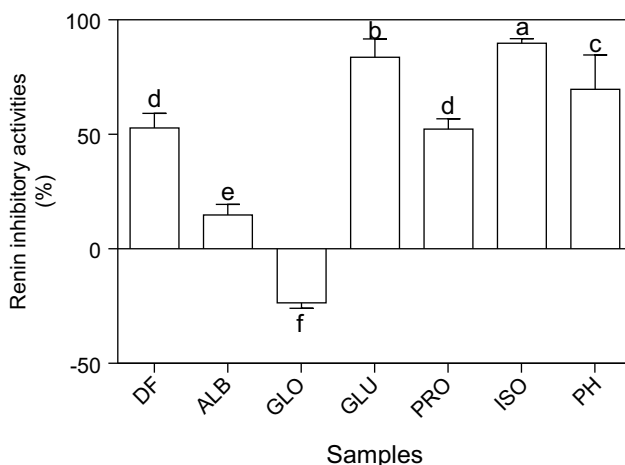


Figure 7. Renin enzyme inhibitory activities of sesame seed proteins *a-amylase enzyme inhibitory activities*.

acids, such as aspartic amino acid, serine and lysine (Table 1). The defatted flour (21.21%) and protein isolate (21.23%) also exhibited appreciable ACE-inhibitory activities, which were not significantly ($p < .05$) different from each other. However, globulin, glutelin and prolamin fractions showed no inhibitory activities against ACE (negative values). The protein hydrolyzate inhibited 14.90% of the ACE enzyme, which is lower when compared with about 70% reported for wonderful cola protein hydrolyzate.^[45] This result showed that the water-soluble protein fraction of sesame seed could find application as a potential ACE inhibitor in living systems.

On the other hand, since renin is the first enzyme in the RAS pathway, high levels or activity may constitute a risk factor for hypertension development. Therefore, compounds that inhibit renin activity are potential antihypertensive agents.^[39] Among the protein fractions, glutelin (83.72%) demonstrated greatest renin inhibition while albumin fraction (14.86%) exhibited the least renin inhibition (Figure 7). The protein hydrolyzate had renin inhibitory activity of 69.70%, which is lower than the 89.86% obtained for the protein isolate. There was no significant difference ($p > .05$) in the renin inhibitory activities of defatted flour (52.75%) and prolamin (52.36%). Only the globulin fraction of sesame protein showed negative inhibitory value against renin enzyme. The result showed that the isoelectric pH-precipitated protein has good potential as a renin inhibitor since it has the highest value.

Acetylcholinesterase enzyme inhibitory activities

Acetylcholinesterase (AChE) is an enzyme that catalyzes and promotes the breakdown of acetylcholine, which is the main neurotransmitter in the body. During neurotransmission process, acetylcholine (ACh) is released from the presynaptic neuron into the synaptic cleft and attaches to AChE receptors on the postsynaptic membrane, relaying the signal from the nerve. AChE, also located on the postsynaptic membrane, ends the signal transmission by hydrolyzing ACh. The released choline is taken up again by the presynaptic neuron and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase.^[46] An inhibitor prevents AChE from breaking down acetylcholine into choline and acetate, thereby increasing both the level and duration of neurotransmission in the central nervous system. The potential of sesame proteins to inhibit the acetylcholinesterase is shown in Figure 8. The result showed that all the samples (6.59–16.88%) exhibited greater inhibitory activities when compared with the standard galantamine (5.38%), which may be due to the strong synergistic effect of the several amino acids in the sesame seed sample. There was no significant difference ($p > .05$) in the inhibitory activities of albumin, glutelin and the protein isolate. Similarly, no significant difference ($p > .05$) was observed in the AChE-inhibitory activities of prolamin fraction

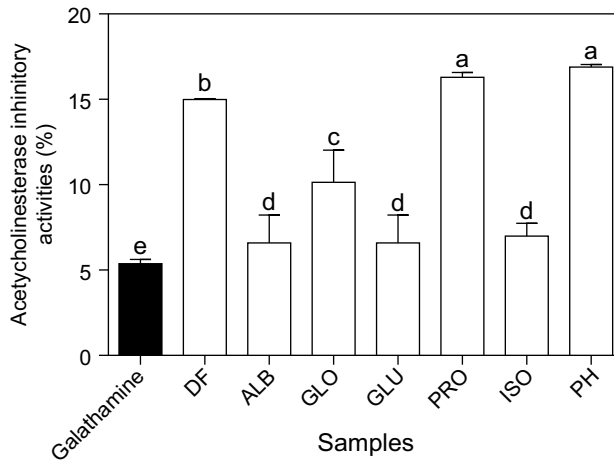


Figure 8. Acetylcholinesterase inhibitory activities of sesame seed proteins.

(16.29%) and protein hydrolyzate (16.88%). The overall results indicate that the pepsin-pancreatin hydrolyzate and prolamin may be good acetylcholinesterase inhibitors and could have potential use in the management of neurodegenerative diseases.

The α -amylase enzyme is one of the targets for reducing glucose levels in the body because it is involved in blood glucose elevation in noninsulin mediated diabetic mellitus and obesity hyperglycemia.^[47] It is one of the key enzymes that is involved in starch digestion to release oligosaccharides, which are further digested to glucose, the absorbable form. Inhibiting α -amylase is, therefore known as one of the ways to manage type 2 diabetes. As indicated in Figure 9, the commercial α -amylase inhibitor (acarbose) exhibited stronger inhibitory activities when compared with the sesame seed proteins. The α -amylase inhibitory activities of defatted flour and glutelin were not significantly ($p > .05$) different from one another. The results showed that albumin protein fraction exhibited greater inhibitions than the other samples, while the least inhibitory activities was observed for the globulin fraction. The high albumin inhibitory activity against α -amylase enzyme may be related with the trends of some amino acids, such as high levels of serine, threonine and aspartic acid.

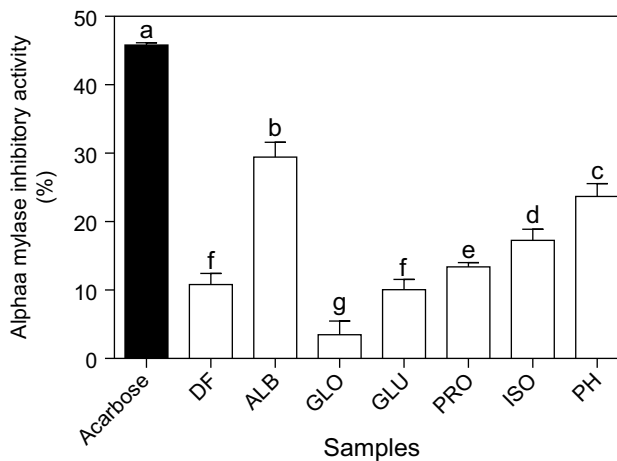


Figure 9. Alpha amylase enzyme inhibitory activities of sesame seed proteins.

The protein hydrolyzate inhibited 23.66% of the alpha amylase enzyme, which was lower when compared with earlier reports on cowpea pepsin (75.00%) and trypsin (80.45%) protein hydrolyzates.^[48] The differences in the inhibitory activities may be due to variations in the plant materials (sesame vs cowpea) and enzymes (pepsin, trypsin vs pepsin/pancreatin). Also, rice bran protein hydrolyzate was reported to inhibit 24.98% α -amylase enzyme^[47] which was similar to the value reported for sesame seed protein hydrolyzate in this study. The albumin fraction of sesame seed is, therefore, recommended for use to inhibit α -amylase enzyme activity.

Conclusion

The study compared the antioxidant properties and enzyme inhibitory activities of sesame seed proteins, that is, the defatted flour, fractions, isoelectric pH-precipitated protein and pepsin/pancreatin hydrolyzate. The results showed that the samples contained optimal amounts of essential amino acids, which indicate that they are good sources of nutrition. The protein fractions exhibited different responses to the scavenging of free radicals, chelation and reduction of ferric ions, which may be attributable to differences in their solubility and content of amino acids. The albumin fraction exhibited the highest ACE and α -amylase inhibitory activities while the isoelectric pH-precipitated protein exhibited highest inhibition against renin enzyme. The protein hydrolyzate demonstrated better inhibition of AChE but with a similar value as the prolamin fraction. The results from this study indicate that sesame seed albumin fraction, protein isolate and hydrolyzate have potential as ingredients in the management of free radicals induced disorders and in the inhibition of various enzymes of importance to hypertension, neurodegeneration and hyperglycemia. *In vivo* studies using animal experiments will be necessary to validate these observations.

Acknowledgments

The authors wish to acknowledge the Mountain Top University for granting the main researcher a research leave and support for her upkeep in the course of this research work and the Natural Sciences and Engineering Research Council of Canada (NSERC), funding reference number RGPIN 2018-06019. Cette recherche a été financée par le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), numéro de référence RGPIN 2018-06019.

Funding

This work was supported by the Natural Sciences and Engineering Research Council of Canada [RGPIN 2018-06019].

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Declaration of interest

The authors declare they have no conflict of interest.

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