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ORIGINAL PAPER

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CHARACTERIZATION OF SEED STORAGE PROTEIN OF *MORINGA OLEIFERA* AND ANALYSES OF THEIR BIOACTIVE PEPTIDE'S NUTRACEUTICAL PROPERTIES

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ABSTRACT

Background. Bioactive peptides derived from food proteins have been the subject of considerable interest in recent years. Therefore, the present study aimed to evaluate the nutraceutical properties (antioxidant, antihypertensive, and antidiabetic activities) of bioactive peptides encrypted in seed storage proteins of *Moringa oleifera* using *in vitro* gastrointestinal digestion.

Materials and methods. Seeds were ground and sieved. Next, the flour was defatted, washed, and dried. Seed storage proteins were obtained from moreinga seed flour using different solvents. Total proteins were extracted with sodium borato, centrifuged, and precipitated. Finally, hydrolysates were obtained with pepsin, trypsin, and chymotrypsin. The hydrolysates were fractionated by ultrafiltration with 10 and 3 kDa membranes.

Results. Based on the obtained results, total globulin hydrolysate possessed a high percentage of angiotensin-converting enzyme inhibition (79.51 ±6.89%) and α-amylase inhibition activity (61.19 ±2.93%). On the other hand, the fraction $F < 3$ kDa had good antioxidant activity (290.66 ±26.9 μM).

Conclusion. This study suggests that total globulin hydrolysates and $F < 3$ kDa had excellent in vitro nutraceutical potential.

Keywords: *Moringa oleifera*, storage proteins, enzymatic hydrolysis, bioactive peptides

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INTRODUCTION

Bioactive peptides have been investigated for several years, and have been related to the reduced risk of certain chronic diseases (Orona-Tamayo et al., 2018). The biofunctional properties of these peptides are associated with antioxidant effects (Bhandari et al., 2020), an antidiabetic effect related to α-amylase inhibition (Olusegun and Emmanuel, 2019) enzyme-inhibitory activities, antifungal and nematocidal properties among others. Also, protein hydrolysates of cowpea seeds have been evaluated for its antioxidant and antihypertensive potentials in vitro. However, there has been relative paucity of information on the α-amylase inhibitory activities of cowpea seed protein hydrolysates. Consequently, this study evaluated the α-amylase – inhibitory activities and antioxidant potentials of protein hydrolysates derived from cowpea seed proteins. Cowpea seeds were defatted using n--hexane and proteins were extracted from the resulting seed meal. The proteinases namely pepsin and trypsin were used for protein hydrolysis and the resulting hydrolysates were investigated for antioxidant properties (using hydrogen peroxide and ferric ions, and an antihypertensive function related to ACE inhibition (Aderinola et al., 2019). Peptides can be obtained by hydrolysis of proteins with the use of proteolytic enzymes (Abdel-Hamid et al., 2017). Several plants, microorganisms, and animal protein sources have been used to generate bioactive peptides (Bechaux et al., 2019). Recently, *Moringa oleifera* has been the subject of multiple investigations because it has been established to have the capacity to promote human health; many studies have focused on the nutraceutical capacity of moringa proteins, and their derived peptides obtained by enzymatic hydrolysis (Liang et al., 2020; Taiwo Aderinola et al., 2020). Based on the literature, hydrolysates of moringa seed proteins exhibit antidiabetic activity (Ekun et al., 2022) and angiotensin-converting enzyme inhibition (González--Garza et al., 2017). Furthermore, the physicochemical and functional properties of protein compositions of *Moringa oleifera* seed have been studied (Bassogog--Bakwo et al., 2022). Seed storage proteins have gained attention due to their capacity to accumulate in the developing seed. Storage proteins have encrypted amino acid sequences that release bioactive peptides when hydrolysed. But despite studies on the potentialities of *Moringa oleifera*, research on the bioactive properties of seed storage proteins with both anti-hypertensive and antidiabetic potential remains limited. Therefore, the aim of the present study was to evaluate the nutraceutical properties (antioxidant, antihypertensive, and antidiabetic activities) of bioactive peptides encrypted in seed storage proteins of *Moringa oleifera* using *in vitro* gastrointestinal digestion.

MATERIAL AND METHODS

Chemicals

Folin & Ciocalteu's Phenol Reagent ($C_7H_6O_5$, Item F9252-100 ML, Lot SHBK8810) 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) ($C_{18}H_{24}N_6O_6S_4$, Item A1888-1G, Lot SLBZ8095), (±)-6-Hidroxy-2,5+,7,8-tetramethylchromane--2-carboxylic acid(Trolox) ($C_{14}H_{18}O_4$, Item 238813, Lot101569377), 2-Mercaptoethanol ($HOCH_2CH_2SH$, Item M3148, Lot 40K088715), N-Hippuryl-His--Leu hydrate($C_{21}H_{27}N_5O_5 \times H_2O$, Item H1635--100MG, Lot 1002063839), bovine trypsin (Item T4799-5G, Lot SLBP8607V), and bovine chymotrypsin (Item C4129-250MG, Lot SLBV2540) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N,N,N′,N′-Tetramethylethylenediamine (TEMED) ($(CH_3)_2NCH_2CH_2N(CH_3)_2$, Item 1610801, Lot 210009769) was acquired from Bio Rad (Hercules, CA). Ammonium Persulfate ($(NH_4)_2S_2O_8$, Item 009342 A, Lot 64093530) was purchased from Bio Rad (Japan). Brilliant Blue R-250 ($C_{45}H_{44}N_3NaO_7S_2$. Item 1610400, Lot 210007110) was acquired from Bio Rad (Canada). Precision plus protein unstained standard (Item 1610363, Lot L001650 A) was purchased from Bio Rad (Hercules, CA). Methyl alcohol RA (CH_3OH, Item CTR 01220, Lot 91217), glacial acetic acid (CH_3COOH, Item CTR 138937, Lot 81589), and phosphoric acid (H_3PO_4, Item CTR 00696, Lot 61333) were acquired from CTR (Mexico). Laemmli (SDS-Sample) (Item 10570018-1, Lot 120928) was acquired from bioworld (Dublin, OH). Pepsin (Item 10108057001, Lot 36117720) from pig gastric mucosa was purchased from Roche (Germany).

Flour preparation from *Moringa oleifera* seeds

Moringa oleifera seeds were provided by the Centro de Agricultura Protegida from Facultad de Agronomía,

Universidad Autónoma de Nuevo León, México. The seeds were ground, and the obtained powder was sieved through meshes of 50–250 μm. The fine flour was defatted with a 1:3 (flour:hexane) ratio by continuous stirring for 1 h then filtrated to remove fat residues. The recovered flour was washed several times with water to remove polysaccharides and, finally, dried in an oven at 55°C for 3 h to be ground into a fine powder (150 μm).

Seed storage proteins extraction

The criteria for protein solubility were considered (Kumar et al., 2019), and the method of Osborne and Campbell (1898) was carried out (storage protein). Seed storage proteins were obtained from previously defatted moringa seed flour for sequential extraction. 10 g of flour were dissolved with 50 mL of distilled water and 100 μL of 0.1 M phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich St. Louis, MO, USA). The mixture was stirred continuously for 1 h at 4°C, then centrifuged at 10,000 rpm for 20 min at 4°C (Centrifuge 5804R, Eppendorf, Hamburg, Germany). The supernatant was recovered and labeled the albumin fraction, and the solid phase was used for the second extraction by adding to it 50 ml of buffer Tris--HCL 0.1 M pH 7.8 with 0.3 M NaCl. The mixture was stirred continuously for 1 h at 4°C, then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was recovered and labeled the globulin fraction, and the solid phase was used for glutelin extraction by adding to it 50 ml of 0.3 M sodium borate, 1% SDS, and 0.6% β-mercaptoethanol solution. The mixture was stirred continuously for 1 h at 4°C then centrifuged at 5,000 rpm for 20 min at 4°C. Finally, the supernatants were recovered and named the glutelin fraction. All recovered extracts were kept at –20°C.

Total protein fraction preparation

The total protein fraction preparation was carried out based on the method of Poms et al. (2004) with modification. A total protein (TP) fraction was prepared, and 10 g of flour were dissolved in 50 ml of 0.3 M sodium borate. The mixture was stirred continuously for 1 h at 4°C, then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was recovered, and its pH was adjusted to pH isoelectric 4.5 for protein precipitation. A second centrifugation was executed under the same conditions, the supernatant was removed, and the precipitate was recovered as the total protein fraction and stored at –20°C.

Protein quantification and SDS-PAGE analyses

The protein concentration was determined using the Lowry method (Lowry et al., 1951), and a standard curve prepared with a stock solution of 2 mg mL^{-1} of bovine serum albumin (BSA). A volume of 100 μL of each sample was used (each sample preparation of protein precipitate was prepared by diluting 10 mg in 1 mL of distilled water) and 1 mL of Lowry reagent (Sigma-Aldrich) was added. The mixture was incubated for 10 min in dark conditions after which 100 μL of Folin-Ciocalteau reagent (Sigma-Aldrich) were added to each sample. The samples were left for 45 min in dark conditions, and absorbance was then determined at 700 nm. For SDS-PAGE a total of 100 μg of protein sample in tricine buffer were loaded under reduction conditions (2-mercaptoethanol) and non-reduction conditions. Electrophoresis was run at 80 V for 20 min for the first step to concentrate the sample in the gel well followed by 120 V for 80 min to resolve proteins according to their molecular weight.

In vitro enzymatic hydrolysis

Total hydrolysate (TH) was obtained with three proteolytic enzymes: porcine pepsin and bovine trypsin and chymotrypsin (Sigma-Aldrich). Pepsin was used to simulate the gastric phase. Trypsin and chymotrypsin were used in protein digestion (Verhoeckx et al., 2015) or specific components thereof. Many such models exist and include, for example, those used to study digestion and fermentation in the small and large intestine, to investigate absorption (e.g. ussing chamber, epithelial cell systems. The treatments were prepared in a 1:5 (w/w) ratio enzyme: a substrate simulating gastrointestinal digestion by sequential digestion with pepsin and trypsin-chymotrypsin for 5 h of hydrolysis. The procedure employed was that described by González Garza et al. (2017) with modifications. Around 200 mg of the globulin fraction were dissolved in 20 mL of distilled water, the pH of the mixture was adjusted to 2.0 with HCl 10%, and then 10 mg of pepsin were added. The solution was stirred continuously at 37°C for 2.5 h and pH variations were monitored. Once the pepsin digestion time was finished, the pH of the reaction was

adjusted to 7.8 with KOH 2 M, and 10 mg of trypsin and 10 mg of chymotrypsin were added. The mixture was stirred continuously at 37°C and pH variations were monitored for another 2.5 h. The reaction was stopped by heating the mixture in a water bath at 80°C for 5 min and then the mixture was centrifuged at 6,000 rpm for 10 min. The supernatant was recovered and stored at –20°C for subsequent analysis.

Peptide fractions preparation

Peptide fractions were prepared by ultra-filtration using 10 kDa and 3 kDa membranes (Amicon Ultrasystem 15 MWCO; Millipore, Billerica, MA, USA). A volume of 15 mL of around 2 mg mL^{-1} of the total globulin hydrolysate fraction was centrifuged at 5,000 rpm for 15 min through the 10 kDa membrane; the filtered sample was fractioned by a second ultra-filtration using the 3 kDa membrane. Both peptide fractions (samples filtered with 10 kDa and 3 kDa membranes labeled '<10' and '<3', respectively) were collected and stored at –20°C for subsequent analysis.

Ultrafiltration of the peptide fractions

The globulin hydrolysates were fractionated by ultrafiltration tubes with membranes of 10 and 3 kDa, respectively. Approximately 15 mL of the initial concentration of 2 mg mL^{-1} of hydrolysate was ultrafiltrated, obtaining a concentration of 1.2 mg mL^{-1} of protein after passing through the 10 kDa membrane and a concentration of 0.8 mg ml^{-1} after passing through the 3 kDa membrane. This could indicate that a major part of the peptide was retained in the 10 kDa membrane. These fractions were used to evaluate the nutraceutical properties.

Biofunctionality property evaluations of peptides. The subsequent analyses were carried out for the globulin fraction, total globulin hydrolysate, $F < 10$, and $F > 3$. Three repetitions were performed for each sample. The assays were carried out at concentrations of 0.5, 1, 1.5, and 2 mg/mL of proteins.

α-Amylase inhibition activity

The antidiabetic property was analysed based on the procedure described by (Nair et al., 2013). α-Amylase of human saliva was used as a substrate. 1 mL of human saliva was obtained and centrifuged (10,000 rpm, 25°C, 10 min, Minispin®, Eppendorf AG, Germany), and the supernatant was dissolved in 2 mL buffer NaH_2PO_4 at pH 6.8. 12 g of potassium sodium tartrate and 8 mL of NaOH 2 M were added in a baker. The solution was mixed and heated at 70°C. 0.4374 g of 3,5-dinitrosalicylic acid (DNS) and 20 mL of distilled water were added in another baker. The DNS solution was mixed until homogeneous. This solution was also mixed and heated at 70°C until homogeneous. Then 100 µL of the human enzyme and 100 µL of 1% starch were added to each sample and incubated at 37°C for 30 min. Subsequently, 100 µL of DNS solution were added and heated at 90°C for 5 min. The samples were cooled and diluted with 900 µL of deionized water. Finally, the samples' absorbance was read at 540 nm. The activity and inhibition percentages of α-amylase (%IAA) were quantified using Equation (1) and Equation (2), respectively.

$$\text{α-amylase activity, \%} = \frac{\text{ESI} - \text{CIE}}{\text{ES} - \text{CES}} \times 100 \quad (1)$$

$$\text{α-amylase inhibition, \%} = 100 - \text{α-amylase activity} \quad (2)$$

where:

ESI – the enzyme-substrate-inhibitor with the total hydrolysate and the peptide fractions as the inhibitor,
ES – the enzyme-substrate,
CIE – the control-inhibitor-enzyme,
CES – the control-enzyme-substrate.

Assays of angiotensin-converting enzyme

The antihypertensive property was evaluated by ACE-I activity according to the method described by Hayakari et al. (1978). The angiotensin-converting enzyme (ACE) was extracted according to Abdulazeez-Mansurah et al. (2013) with some modifications. Rabbit lungs were obtained, frozen, and ground using the buffers of KH_2PO_4 (0.1 M) pH 8.3 and Trizma-HCl buffer. His-His-Leu (HHL) tripeptide was used as a substrate. Absorbance was determined at 382 nm in the supernatant obtained after centrifugation at 10,000 g for 10 min (Minispin®, Eppendorf AG, Germany). The percentages of ECA activity and inhibition of ECA (%IECA) were quantified using Equation (3) and Equation (4), respectively.

$$\text{ECA activity, \%} = \frac{\text{ESM} - \text{BEM}}{\text{ES} - \text{BES}} \times 100 \quad (3)$$

$$\text{ECA inhibition, \%} = 100 - \text{ECA activity} \quad (4)$$

where:

BEM – the absorbance of the blank sample enzyme,
ESM – the absorbance of the sample substrate enzyme,
BES – the absorbance of the blank substrate enzyme,
ES – the absorbance of the enzyme sample.

Antioxidant activity by ABTS

Antioxidant activity was measured using a modified method (Re et al., 1999). Solutions of 7 mM 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS; Sigma-Aldrich) and 2.45 mM potassium persulfate were prepared and mixed. Afterwards, the solutions were left mixing in the dark for 12 h. This reagent was dissolved in absolute ethanol to an absorbance of 0.7 at 734 nm. A standard curve was prepared at different concentrations of Trolox: 100, 200, 400, 600, 800, 1,000, and 1,200 µM. Reactions consisted of 10 µL of each sample or standard and 1 mL of the ABTS reagent; absorbance was determined after 6 min at 734 nm. The antiradical activity ABTS was expressed as Trolox equivalents (ET; µM ET mg^{-1} of protein).

Statistical analysis

The biofunctional properties (ABTS, %IAA, and %IECA; y_i) of bioactive peptides were measured from moringa, total protein, total hydrolysate, $F < 10$, and $F < 3$, and incorporated into the model as factor τ_i. These parameters in the statistical model were evaluated to determinate differences between the biofunctional properties (ABTS, %IAA, and %IECA; y_i) of bioactive peptides. The statistical model ($y_i = \mu + \tau_i + \varepsilon_{ijk}$) incorporated the experimental error (ε_{ijk}). The significance level used for all statistical analyses was 5%, and when H_0 was rejected (p-value < 0.05), the Tukey statistical test was performed to compare the means between treatments and interaction effects. Minitab® (version 17.3.0, 2014) was used for data analysis. All experiments were carried out in triplicate. The half maximal inhibitory concentrations IC_{50} were calculated employing non-linear regression and expressed as a mean and its 95% confidence interval. They were determined by regression analysis of α-amylase inhibition %/ECA inhibition % versus protein fraction concentration (µg $µL^{-1}$).

RESULTS AND DISCUSSION

Protein quantification and SDS-PAGE analyses

The SDS-PAGE seed storage protein profiles of *Moringa oleifera* are shown in Figure 1. All the fractions contain fragments of high molecular weight with the exception of the albumin fraction, where fragments of low molecular weights 10–20 kDa were found (Fig. 1). Under non-reducing conditions the polypeptides could be grouped into three classes according to their molecular weight, as shown in Figure 1A. The first class, of approximately 50 kDa, was present in the total protein, globulin, and glutelin fractions; the second, of around 30 kDa, can be seen at high resolution in the globulin fraction (Fig. 1A line 3); and the third was detected at 6–25 kDa. Interestingly, under reducing conditions with 0.6% β-mercaptoethanol, it was observed that polypeptides of ~50 kDa were reduced to polypeptides of around ~30–35 KDa and ~20–25 kDa as observed in the total protein, globulin, and glutelin fractions (Fig. 1B). Similar results have been reported by Cattan et al. (2022), who reported group bands at ~35 and ~12 kDa with seed and leaf protein from *Moringa oleifera*. They also reported a ~50 kDa band under reducing conditions, which was not detected in this study. The results are consistent with Taiwo Aderinola et al. (2020), who reported the disintegration of the ~50 kDa group band and the appearance of a band of low molecular weight after β-mercaptoethanol treatment.

Evaluation of the biofunctional properties of peptides. The process for producing bioactive peptides is based on *in vitro* hydrolysis with proteolytic enzymes (González Garza et al., 2017). Here, the globulin fraction was considered to evaluate the biofunctional properties due to its abundant protein concentrations relative to the albumin and glutelin fractions. In addition, it is widely reported that the globulins of, for example, chia (Hernández-Pérez et al., 2020) and soybean and amaranth (Marcone and Kakuda, 1999) have excellent biofunctional properties.

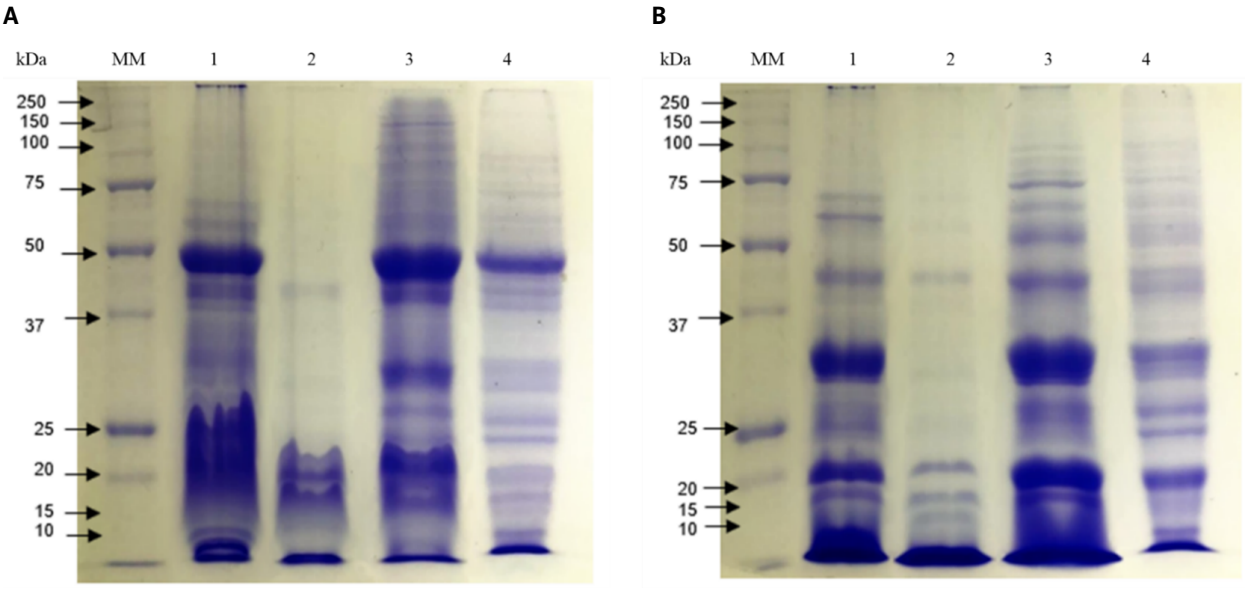

Fig. 1. SDS-PAGE seed storage protein profiles of *Moringa oleifera* extract in polyacrylamide gel 12%: **A** – non-reducing conditions, **B** – reducing conditions with β-mercaptoethanol, MM – Precision Plus Protein™ 250 kDa, 1 – total protein isolate at pI, 2 – albumin fraction, 3 – globulin fraction, 4 – glutelin fraction

α-Amylase inhibition activity

The total globulin hydrolysate had higher inhibition activity of α-amylase than the globulin fraction. The %IAA for the $F < 3$ fraction was 387.9% higher than that of the total globulin hydrolysate (Table 1). The highest α-amylase inhibition activity (61.19 ±2.93%) was found in the total globulin hydrolysate. The α-amylase-inhibitory activity in total hydrolysate was lower than previously reported for hydrolysates of moringa oleifera seed flour with pepsin (77.59 ±0.17%) (Olusola et al., 2018). Nevertheless, protein hydrolysate with alcalase and tryptic obtained from the seed of *Luffa cylindrica* (36.36 ±0.71% and 27.96 ±0.06% respectively; Arise et al., 2019) demonstrated a lower α-amylase inhibition than total hydrolysate and the $F < 10$ fraction. The percentage α-amylase inhibitory activity of the total globulin hydrolysate at different concentrations is illustrated in Figure 2. The total globulin hydrolysate inhibited α-amylase by 50% at concentrations of 1.4 g mL^{-1}.

This concentration is consistent with that reported by Ekun et al. (2022) with hydrolysate of Moringa oleifera seed (1.312 mg mL^{-1}). However, the half maximal inhibitory concentration was higher than

Table 1. Biofunctional properties of bioactive peptides of moringa seeds

Treatment	Biofunctional traits		
	%IAA	%IECA	ABTS, μM
Globulin fraction	45.20 ±0.62^{b}	75.70 ±9.98^{ab}	266.37 ±21.0^{a}
Total globulin hydrolysate	61.19 ±2.93^{a}	79.51 ±6.89^{a}	229.22 ±22.68^{a}
$F < 10$	48.00 ±1.15^{b}	68.38 ±9.18^{ab}	163.55 ±23.3^{b}
$F < 3$	12.54 ±0.45^{c}	55.50 ±8.22^{b}	290.66 ±26.9^{a}

ABTS – 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), Trolox equivalent/mg protein, %IAA – percentage of α-amylase inhibition, IECA – percentage of inhibition of angiotensin-converting enzyme.

$^{a–b}$Means ($n = 3$; $\alpha < 0.05$) within the same column (biofunctional evaluation) for seed flours per treatment method with different lowercase superscripts differ significantly when the p-value of (τ_i) < 0.05.

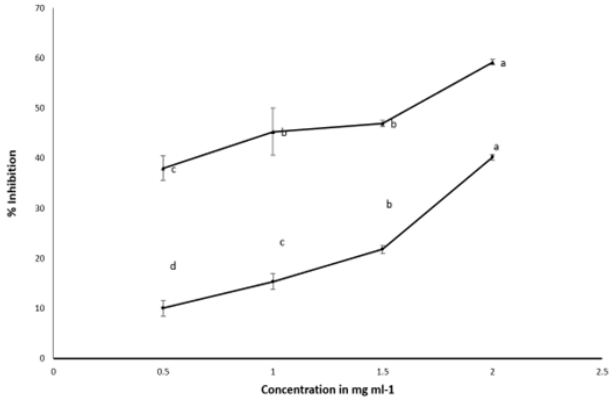

Fig. 2. Percent inhibition of α-amylase (triangle) and angiotensin-converting enzyme (circle) of total globulin hydrolysate at different concentrations. [a–b]Means (n = 3) – the concentrations with different lowercase superscripts differ significantly when the p-value of (τ_i) < 0.05

previously reported for hydrolysates of *Moringa oleifera* seed flour with trypsin fraction > 10 kDa, 5 h (0.664 mg mL^{-1}; González Garza et al., 2017), and trypsin, 8 h (0.591 mg mL^{-1}; Olusola et al., 2018).

Assays of angiotensin-converting enzyme

The highest %IECA was obtained for total globulin hydrolysate and the lowest for the F < 3 fraction (Table 1). The %IECA for the F < 3 fraction was 36% lower than that of the globulin fraction. When compared with other seeds, ACE inhibitory activity was lower than the values reported for *Lens culinaris* 89.74 ±0.56% (Park et al., 2019). A direct comparison of the results with those for other seeds (Park et al., 2019), such as *Helianthus annuus* (36.03 ±0.93%) and *Arachis hypogaea* (21.14 ±1.79), reveals that all results represent higher ACE inhibition activity than has been described for them. The percentage ACE inhibition activity of the total globulin hydrolysate at different concentrations is shown in Table 1. The total hydrolysate inhibited ACE by 50% at concentrations of 2.70 g mL^{-1}. This concentration is higher than reported for hydrolysates of *Moringa oleifera* seed flour with pepsin and trypsin, 2.5 h (0.883 mg mL^{-1}; González Garza et al., 2017) and *Amaranthus hypochondriacus* seeds with alcalase, 5 h (0.6 mg mL^{-1}; Quiroga et al., 2017). However, the IC_{50} of the hydrolyzed moringa seeds was lower than that of *Perilla frutescens* seeds with flavourzyme, 4 h (5.124 mg mL^{-1}; Meena et al., 2020; Park and Yoon, 2019).

Antioxidant activity by ABTS

The results of antioxidant activity assays show that the globulin fraction, total globulin hydrolysate, and their fractions had antioxidant activity. These data demonstrate that peptide size has a significant impact on the scavenging reaction. The F < 10 fraction had the lowest antioxidant capacity (163.55 ±23.3), which was 63.2% lower than that of the globulin fraction. Our results are consistent with those of Cotabarren et al. (2019), who in their study of chia protein hydrolysates treated with papain, demonstrated that peptides with molecular weight of <15 kDa have a potent radical scavenging effect by the ABTS method. Comparing antioxidant activity with the total hydrolysates from moringa seed protein with 5,260 μM Trolox equivalent/mg protein (González Garza et al., 2017), all the fractions had lower antioxidant activity.

CONCLUSIONS

This study concludes that peptide fractions obtained using pepsin and trypsin-chymotrypsin for 5 h digests of *M. oleifera* seed proteins elicited antidiabetic and antihypertensive potential. In addition, %IAA and %IECA were higher for the hydrolyzed globulin fraction and lower for the globulin fraction. Peptide fractions <3 kDa possess antioxidant activity superior to fractions <10 kDa. Total globulin hydrolysate had the best biofunctional properties. Nowadays, moringa is important in the prevention of different chronic degenerative diseases. In future studies, it would be interesting to investigate the effects of total globulin hydrolysate in food.

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