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Systematic Purification of Peptides with In Vitro Antioxidant, Antihyperglycemic, Anti-Obesity, and Antidiabetic Potential Released from Sesame Byproduct Proteins

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Abstract

Sesame oil extraction byproduct (SOEB) contains a high percentage of protein (49.81 g/100 g), making it a suitable plant-based source for producing protein hydrolysates with nutraceutical potential. In this study, albumins, globulins, glutelins, and prolamins fractions were extracted and characterized from SOEB. These fractions were then enzymatically hydrolyzed with alcalase, yielding high soluble protein content (>90%) and hydrolysis degrees ranging from 34.66 to 45.10%. The hydrolysates were fractionated by molecular weight (<5 kDa, 3–5 kDa, 1–3 kDa, and <1 kDa). These fractions demonstrated potential for inhibiting the DPPH radical (25.19–95.79%) and the α-glucosidase enzyme (40.14–55.63%), particularly the fractions with molecular weight <1 kDa. We identified 28 peptides, with molecular weights between 332.20 and 1096.63 Da, which showed potent antioxidant activities (IC_{50} = 90.18 µg/mL), as well as inhibitory effects on key enzymes such as α-glucosidase (IC_{50} = 61.48 µg/mL), dipeptidyl peptidase IV (IC_{50} = 12.12 µg/mL), and pancreatic lipase (IC_{50} = 6.14 mg/mL). These results demonstrate the antioxidant, antihyperglycemic, antidiabetic, and anti-obesity potential of SOEB peptides, highlighting their use in the formulation of new functional foods or nutraceuticals.

Keywords: antioxidant activity; inhibition of α-glucosidase; inhibition of dipeptidyl peptidase IV; inhibition of pancreatic lipase; HPLC-DAD-MS/MS

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1. Introduction

Globally, the food industry faces a challenge in managing large quantities of byproducts or waste, which often generates environmental concerns and economic losses [1–3]. However, these wastes are increasingly considered as underexploited raw materials for obtaining and generating products or compounds of nutraceutical importance and are commonly referred to as byproducts [4,5]. This approach moves us closer to a circular

economy, emphasizing the transformation of agro-industrial waste into new value-added products [6–9]. An example of this is the work by Hoyos-Concha et al. [6], who used trout fishery byproducts in the formulation and stabilization of fish feed. Increasingly, the food industry is focusing on harnessing the potential of byproducts [5].

Among the byproducts of the food industry is the sesame oil extraction byproduct (*Sesamum indicum* L.), which is notable for its nutritional and nutraceutical value [10]; the byproduct has been primarily used for animal feed or discarded [11,12]. However, recent studies demonstrate the potential of sesame byproducts as a source of bioactive compounds [13]. For example, Quintero-Soto et al. [14] investigated the phenolic compound profile of extruded beverages based on sesame byproducts and found that the phytochemicals present in the beverages exhibited antioxidant and antidiabetic effects, even after simulated gastrointestinal digestion [15].

As for sesame proteins, they represent approximately 50% of the total weight of the byproduct [16], with globulins (67.30 g/100 g of protein) being the majority proteins, followed by albumins (8.60 g/100 g of protein), glutelins (6.90 g/100 g of protein), and prolamins (1.30 g/100 g of protein) [17]. These proteins can be used to prepare enzymatic hydrolysates with bioactive properties [18,19] and as raw material in the development of new functional ingredients and nutraceuticals [20,21]. These hydrolysates have great potential to address important health problems. In this regard, Awosika and Aluko [22] prepared peptide fractions of different molecular weights (<1 kDa, 1–3 kDa, 3–5 kDa, and 5–10 kDa) using various enzymes; these fractions demonstrated the capacity to inhibit metabolic enzymes such as pancreatic lipase, α-amylase, and α-glucosidase. Specific investigations into sesame-derived peptides have shown their anti-obesity effects through in silico analyses [23]. More recently, Chaipoot et al. [24] studied hydrolysates and peptide fractions of different molecular weights (<3 kDa, 3–10 kDa, and >10 kDa) generated from black sesame byproduct, demonstrating their antioxidant, antidiabetic, anti-obesity, and antihypertensive properties.

Given the considerable protein content of sesame byproducts and the wide variety of biological activities of their hydrolysates, sesame byproduct proteins are a good alternative for the systematic purification of peptides with important biological activities [24]. This approach not only adds value to an otherwise underutilized byproduct but also contributes to the development of compounds with health-promoting properties, aligning with the trend toward valorizing food industry byproducts for various applications [1,2,25]. Therefore, the objective of the present investigation was to perform a systematic purification of peptides from sesame byproduct proteins with in vitro antioxidant, antihyperglycemic, antidiabetic, and anti-obesity properties.

2. Materials and Methods

2.1. Materials

Beige varita variety sesame seeds grown from September to December 2022 in the town of El Saladito, Elota, Sinaloa, Mexico (geographic coordinates latitude: 23.8833, length: −106.85) were used. The seeds were cleaned and stored in airtight containers at 4 °C until use. The byproduct of sesame oil extraction (press cake) was obtained by Quintero-Soto et al. [14] as described below. First, the sesame seeds were passed through a mechanical press twice to remove as much oil as possible. Next, the press cake was collected and let dry (40 °C) until reaching a water activity of 0.5 to prevent microbial growth. Finally, the flour obtained was passed through sieve #100. The flour obtained was named as a byproduct of sesame oil extraction.

2.2. Protein Extraction and Quantification

Albumins, globulins, glutelins, and prolamins were extracted from the sesame byproduct as described by Quintero-Soto et al. [26] with some modifications (Figure 1). Four distinct solutions were prepared: albumin solution [sterile deionized water (pH 8.0), 10 mM $CaCl_2$, 10 mM $MgCl_2$, 1 mM PMSF], globulin solution [0.1 M Tris-HCl (pH 8.0), 10% NaCl, 1 mM PMSF, 10 mM EDTA], prolamin solution [70% Etanol, 1 mM PMSF, 10 mM EDTA), and glutelin solution [0.1 M NaOH, 1 mM PMSF, 10 mM). First, 50 g of the sample was weighed and defatted with 500 mL of hexane at 500 rpm for 4 h. This was followed by centrifugation (10,000× g/15 min), and the supernatant was discarded. The residue-1, obtained from the previous centrifugation, was extracted with albumin solution under constant agitation (500 rpm/4 h/20 °C) and centrifuged (10,000× g/15 min) to obtain the albumin fraction (supernatant 1). Residue-2, from the preceding centrifugation step, was extracted with globulin solution under constant agitation (500 rpm/16 h/20 °C) and centrifuged (10,000× g/15 min) to yield the globulin fraction (supernatant 2). Residue-3, obtained from the prior centrifugation, was extracted with prolamin solution under constant agitation (500 rpm/16 h/at 20 °C) and centrifuged (10,000× g/15 min) to collect the prolamin fraction (supernatant 3). Finally, residue-4, from the previous centrifugation, was extracted with glutelin solution under constant agitation (500 rpm/4 h/20 °C) and centrifuged (10,000× g/15 min) to acquire the glutelin fraction (supernatant 4). Supernatants 1, 2, 3, and 4 were then dialyzed against 1 L of water for 3 days using an 8 kDa cellulose membrane (88245, Thermo Fisher Scientific, Waltham, MA, USA). The dialysate of supernatant 1 was centrifuged (10,000× g/15 min), and the resulting supernatant was lyophilized to obtain the albumins. The dialysates of supernatants 2, 3, and 4 were centrifuged (10,000× g/15 min), and the resulting pellets were lyophilized to obtain the globulins, prolamins, and glutelins, respectively.

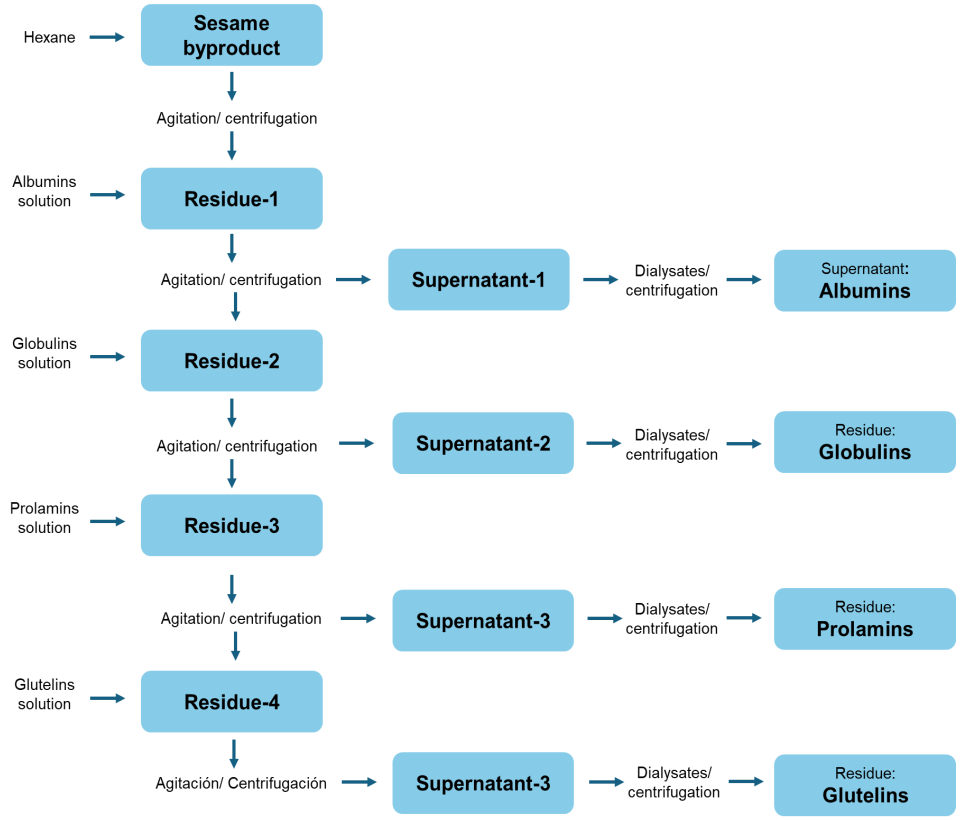


Figure 1. General description of the protein extraction process.

2.3. Preparation of Protein Hydrolysates of Albumins, Globulins, Prolamins, and Glutelins and Determination of the Degree of Hydrolysis

Prior to hydrolysis, the proteins were washed with ethyl acetate to remove any phenolic compounds that might interfere with the biological activity readings. Protein hydrolysates from the protein fractions were prepared following the methodology of Félix-Medina et al. [27]. Five grams of protein were suspended in 50 mL of water (pH 8.0) and hydrolyzed with Alcalase 1.5 U (P4860, Sigma Aldrich®, St. Louis, MO, USA) at constant temperature and pH (50 °C/pH 8.0/90 min). Throughout the hydrolysis process, the samples were kept under constant stirring at 300 rpm. Hydrolysis was stopped by increasing the temperature (80 °C/20 min). Finally, the samples were allowed to cool to 25 °C and then centrifuged (5000× *g*/20 min). The recovered supernatant was considered the protein hydrolysate.

The degree of hydrolysis (DH) of the samples was determined as reported by Xu et al. [28], using the following formula:

$$DH(\%) = [(B \times Nb) \div MP] \times (1 \div \alpha) \times (1 \div h_{tot}) \times 100 \quad (1)$$

$$\alpha = \left(10^{pH-pK}\right) \div \left(1 + 10^{pH-pK}\right) \quad (2)$$

where B is the volume of NaOH consumed to maintain constant pH during hydrolysis (mL), Nb is the normality of NaOH MP is the protein content (g), h_{tot} is the total number of peptide bonds in the substrate (7.22 mmol/g protein), and α is the degree of dissociation of the α-NH2 groups, estimated from the pH and pK values.

2.4. Preparation of Peptide Fractions

Peptide fractions were prepared using molecular weight cut-off (MWCO) tubes of 1 kDa (MCP001C46, Cytiva®, Marlborough, MA, USA), 3 kDa (VS2091, Vivaspin®, SartoriusR, Gottingen, Germany), and 5 kDa (VS2011, Vivaspin®, SartoriusR, Gottingen, Germany). The protein hydrolysates from albumins, globulins, prolamins, and glutelins were loaded into 5 kDa MWCO tubes and centrifuged (4000× *g*/40 min). The retentate-1 (>5 kDa) was stored, and the filtrate-1 was re-centrifuged in 3 kDa MWCO tubes (4000× *g*/40 min). Retentate-2 (3–5 kDa) was stored, and filtrate-2 was centrifuged in 1 kDa MWCO tubes (4000× *g*/40 min). Retentate-3 (1–3 kDa) and filtrate-3 (<1 kDa) were then stored [27]. The four resulting fractions (>5 kDa, 3–5 kDa, 1–3 kDa, and <1 kDa) were adjusted to a concentration of 100 µg/mL. The content of peptide fractions was determined using the Bradford® colorimetric method, with bovine serum albumin as the standard. Their antioxidant and antihyperglycemic activities were determined as described in Sections 2.6.1 and 2.6.2, respectively.

2.5. Determination of Total Protein Content, Protein Fractions, and Soluble Protein

The total protein content, protein fractions, and soluble protein of the samples were determined following methodology 960.52 (micro-Kjeldahl) of the AOAC [29]. A conversion factor of 5.83 indicated for sesame seeds was applied.

2.6. Evaluation of Biological Activities

2.6.1. Determination of Antioxidant Activity

Antioxidant activity was determined using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) colorimetric method [30]. Briefly, 100 µL of the sample was mixed with 100 µL of DPPH (D9132, Sigma Aldrich®, St. Louis, MO, USA) solution (0.1 mmol/L in ethanol). After 30 min of incubation at 37 °C, the absorbance was measured at 510 nm. Results are expressed as percentage of inhibition (%) and as the IC_{50} (Median Inhibitory Concentration).

2.6.2. Determination of Antihyperglycemic Activity

For antihyperglycemic activity, the inhibition of the α-glucosidase enzyme was determined as described by Mojica et al. [31] with modifications. Briefly, 100 µL of an α-glucosidase (G5003, Sigma Aldrich®, St. Louis, MO, USA) solution (1 U/mL) and 50 µL of the sample were mixed and incubated (10 min/25 °C). Subsequently, 50 µL of p-nitrophenyl-α-D-glucopyranoside (5 mmol/L) was added to the mixture and incubated again (5 min/25 °C). Finally, the absorbance of the samples was read at 405 nm. Results were expressed as a percentage of inhibition (%) and the IC_{50}.

2.6.3. Determination of Antidiabetic Activity

The inhibition of the dipeptidyl peptidase IV enzyme was determined using the MAK203 kit (Sigma-Aldrich, St. Louis, MO, USA). This assay quantifies the ability of the dipeptidyl peptidase IV enzyme to cleave the Gly-Pro dipeptide from the amino terminus of the non-fluorescent substrate, Gly-Pro-7-amido-4-methylcoumarin hydrobromide (H-Gly-Pro-7-AMC), in the presence of an inhibitor. Each unit of the dipeptidyl peptidase IV that hydrolyzes the H-Gly-Pro-7-AMC substrate generates 1 nmol of the fluorescent product, 7-amino-4-methylcoumarin (AMC), which can be monitored fluorometrically. Therefore, a lower fluorescence signal indicates a greater capacity of the inhibitor to inactivate the dipeptidyl peptidase IV enzyme. Results were expressed as the IC_{50}.

2.6.4. Determination of Anti-Obesity Activity

Anti-obesity activity was determined by the inhibition of the pancreatic lipase enzyme as described by Awosika and Aluko [22]. Twenty-five microliters of the samples was mixed with 225 µL of 0.5 mM 4-methylumbelliferone and incubated (15 min/37 °C). Subsequently, 25 µL of pancreatic lipase enzyme (L3126, Sigma Aldrich®, St. Louis, MO, USA) solution (4 U/mL) was added and incubated again (37 °C/1 h). Finally, absorbance was measured at 400 nm, and results were expressed as the IC_{50}.

2.7. Identification and Purification of Peptides

The identity of peptides present in the peptide fractions with the best antioxidant and antihyperglycemic potential was determined according to the method reported by Quintero-Soto et al. [26] with some modifications. Fifty microliters of the sample was separated using an HPLC-DAD (1100 Series, Agilent Technologies, Santa Clara, CA, USA) coupled to a mass detector (1100 Series LC/MSD Trap, Agilent Technologies, Santa Clara, CA, USA). A Luna C18 column (150 × 4.6 mm) was used with mobile phases consisting of water–formic acid (1%) (A) and acetonitrile (B). The flow rate was set to 0.2 mL/min, and a gradient of 95–60% A was applied for 50 min, followed by 60–100% A for another 10 min. Mass spectra were acquired using the Agilent ChemStation for LC/3D software (Rev. A. 09. 01 [1206] Agilent Technologies, Santa Clara, CA, USA) and analyzed with Proteome Discoverer 1.2 software. The SEQUEST search engine was used in automatic mode, referencing the sesame proteome (UniProt: UP000504604). Identified peptides were further analyzed on the BIOPEP-UWM platform [https://biochemia.uwm.edu.pl/en/biopep-uwm-2/ (accessed on 20 January 2025)] to investigate potential biological activities. First, the 'Bioactive peptides' tool was selected, followed by the 'Analysis' option. Next, the 'Profiles of potential biological activity' option was chosen, followed by 'For your sequence', where a box appears to place the peptide sequence to be analyzed. Finally, the 'Report' option is selected, which displays a box with the predicted biological activities for the peptide [32]. The physicochemical properties of the peptides were analyzed using the PepDraw platform [https://www.pepdraw.com/ (accessed on 10 March 2025)], where the peptide sequence is inserted and the 'Draw Peptide' option is selected; this option generates the structure

and physicochemical properties (isoelectric point, hydrophobicity, and net charge) of the peptide. The identified peptides were collected as they eluted from HPLC-DAD until a concentration of 1 mg/mL for each peptide was achieved. The content of the peptide was determined using the Bradford® colorimetric method, with bovine serum albumin as the standard. The purified peptides were stored at −20 °C until further use.

2.8. Statistical Analysis

Data were analyzed by one-way ANOVA, and Tukey's test ($p < 0.05$) was used for comparisons of means. Analyses were performed using STATGRAPHIC plus 5.1 software (Statistical Graphics Corp., The Plains, VA, USA).

3. Results and Discussion

3.1. Characterization of Hydrolysates and Peptide Fractions

3.1.1. Protein Content and Degree of Hydrolysis

The growing interest in plant proteins is largely driven by their sustainability, economic viability, and health benefits. In this study, the sesame oil extraction byproduct (SOEB) was found to have a protein content of 49.81 g/100 g of flour. With nearly 50% of SOEB being protein, it stands as an excellent raw material for protein extraction and the preparation of peptide hydrolysates. These values are consistent with those reported by Chatterjee et al. [33] (55.60 g/100 g), Reis de Souza et al. [16] (53.50 g/100 g), and Noptana et al. [13] (42.62 g/100 g) for sesame byproducts.

We found values of 10.05, 30.53, 7.5, and 2.08 g/100 g of flour for albumins, globulins, glutelins, and prolamins fractions, respectively (Table 1). These fractions accounted for 20.17% (albumins), 60.53% (globulins), 15.05% (glutelins), and 4.17% (prolamins) of the total protein. Koysuren et al. [17] reported a higher proportion of globulins (67.30%), followed by albumins (8.60%), glutelins (6.90%), and prolamins (1.30%), which is a similar trend to what was observed in our study. Differences in protein content can be attributed to variations in sesame variety and protein extraction techniques.

Table 1. Protein content, soluble protein, and degree of hydrolysis.

Feature	Sample
Protein fractions (g/100 g of flour)	
Albumin	10.05 ± 0.50^{b}
Globulin	30.53 ± 1.85^{a}
Glutelin	7.5 ± 0.50^{c}
Prolamin	2.08 ± 0.28^{d}
Soluble protein (g/100 g of hydrolyzed)	
Albumin hydrolysate	93.54 ± 4.01^{a}
Globulin hydrolysate	96.25 ± 0.93^{a}
Glutelin hydrolysate	92.86 ± 1.40^{a}
Prolamin hydrolysate	91.61 ± 2.64^{a}
Degree of hydrolysis (%)	
Albumin hydrolysate	40.00 ± 2.00^{b}
Globulin hydrolysate	45.10 ± 3.05^{a}
Glutelin hydrolysate	36.33 ± 1.15^{bc}
Prolamin hydrolysate	34.66 ± 3.51^{c}

Results are the mean ± standard deviation of three biological replicates. Means with different letters for the same characteristic indicate a significant difference (Tukey, $p < 0.05$).

The extracted protein fractions were subsequently used to prepare protein hydrolysates, which exhibited a soluble protein content exceeding 90% (Table 1). These values are higher than those reported by Koysuren et al. [17] (3.9 to 36.42%) and Noptana

et al. [13] (45%), and comparable to those reported by Chatterjee et al. [33] (95%) for sesame proteins hydrolyzed with the same enzyme used in this study.

Regarding the degree of hydrolysis, the values are presented in Table 1. A maximum degree of hydrolysis of 45.10% was achieved for the globulin hydrolysate within 90 min. Conversely, the prolamin hydrolysate showed the lowest value at 34.66%. These degree hydrolysis values are higher than those reported by Noptana et al. [13], who observed a degree of hydrolysis of 13% after 12 h.

Chatterjee et al. [33] reported degree of hydrolysis values of 65% at 90 min, which are higher than those observed in the present study. The variations in the degree of hydrolysis are correlated with the soluble protein content. In this study, a positive correlation was observed between the soluble protein content and the degree of hydrolysis of the hydrolysates ($r = 0.561$; $p < 0.05$). This finding aligns with observations from other authors, where lower soluble protein content typically leads to lower degree hydrolysis values, and vice versa [13,33].

3.1.2. Antioxidant Activity of Hydrolysates and Peptide Fractions

Table 2 presents the DPPH assay results for the hydrolysates and peptide fractions, all evaluated at a concentration of 1 mg/mL. This assay relies on the transfer of protons from the peptides to the DPPH radical. Inhibition percentages of the radical ranged from 25.19% for the prolamin hydrolysate (PROH) to 95.79% for the albumin fraction <1 kDa (ALBF < 1 kDa). Among the complete hydrolysates, the albumin hydrolysate (ALBH) showed the highest inhibition percentage (43.43%), indicating the greatest antioxidant activity. It was followed by the glutelin hydrolysate (GLUH) with 40.17%, globulin hydrolysate (GLOBH) with 37.10%, and finally, PROH with 25.19%.

Table 2. Antioxidant and antihyperglycemic activity of hydrolysates and peptide fractions.

Sample (1 mg/mL)	DPPH (% Inhibition)	α-Glucosidase (% Inhibition)
	Albumin	
ALBH	43.43 ± 1.99 ^{e}	55.63 ± 3.62 ^{e}
ALBF > 5 kDa	62.48 ± 3.83 ^{d}	61.25 ± 2.51 ^{d}
ALBF 3–5 kDa	74.43 ± 2.51 ^{c}	68.42 v 1.93 ^{c}
ALBF 1–3 kDa	83.16 ± 2.52 ^{b}	74.98 ± 1.07 ^{b}
ALBF < 1 kDa	95.79 ± 2.32 ^{a}	85.47 ± 1.67 ^{a}
	Globulin	
GLOBH	37.10 ± 2.31 ^{e}	47.05 ± 1.56 ^{e}
GLOF > 5 kDa	51.68 ± 1.81 ^{d}	53.10 ± 1.49 ^{d}
GLOF 3–5 kDa	65.71 ± 2.65 ^{c}	62.05 ± 0.81 ^{c}
AGLOF 1–3 kDa	76.66 ± 2.52 ^{b}	74.16 ± 0.88 ^{b}
GLOF < 1 kDa	83.93 ± 2.17 ^{a}	79.31 ± 0.95 ^{a}
	Glutelin	
GLUH	40.17 ± 1.33 ^{e}	42.79 ± 2.10 ^{d}
GLUF > 5 kDa	58.72 ± 4.28 ^{d}	54.14 ± 2.39 ^{c}
GLUF 3–5 kDa	65.84 ± 1.45 ^{c}	57.36 ± 1.55 ^{c}
GLUF 1–3 kDa	76.87 ± 1.39 ^{b}	64.14 ± 1.32 ^{b}
GLUF < 1 kDa	86.92 ± 0.83 ^{a}	74.93 ± 2.47 ^{a}
	Prolamin	
PROH	25.19 ± 1.68 ^{e}	40.14 ± 1.92 ^{e}
PROF > 5 kDa	39.77 ± 2.56 ^{d}	47.48 ± 1.45 ^{d}
PROF 3–5 kDa	47.27 ± 0.46 ^{c}	50.96 ± 0.96 ^{c}
PROF 1–3 kDa	55.85 ± 3.09 ^{b}	59.10 ± 0.09 ^{b}
PROF < 1 kDa	71.40 ± 0.57 ^{a}	65.21 ± 1.57 ^{a}

The percentage inhibition values for DPPH and α-glucosidase correspond to the evaluation of samples with a concentration of 1 mg/mL. ALBH = Albumin hydrolysate, GLOH = globulin hydrolysate, GLUH = glutelin hydrolysate, PROH = prolamin hydrolysate, ALBF = albumin fraction, GLOF = globulin fraction, GLUF = glutelin fraction, PROF = prolamin fraction. The results are the mean ± standard deviation of three biological replicates and are expressed as dry weight. Means with different letters in the same characteristic indicate a significant difference (Tukey, $p < 0.05$) for the same type of protein (albumin, globulin, glutelin, or prolamin).

Norouzi et al. [11] evaluated the antioxidant activity using the DPPH assay in sesame at different hydrolysis times (1 to 4 h), finding values ranging from 75% to 93% using the same enzyme as in this study. When comparing the concentrations used, we observed that these authors conducted their evaluation at higher concentrations (20 mg/mL) than those used in our study. Therefore, the results obtained in this research are superior to those reported by them. Values similar to those observed in this study were reported by Félix-Medina et al. [27] in corn protein hydrolysates (26.0% to 30.7% at 1 mg/mL).

In the analyzed peptide fractions, we observed that as the size of the fractions decreased, antioxidant activity increased across all four types of protein fractions. This behavior has been previously noted by Lu et al. [34] (for fractions < 3 kDa, 3–5 kDa, 5–8 kDa, 8–10 kDa, and >10 kDa) and Noptana et al. [13] (for fractions < 1 kDa, 1–10 kDa, 10–100 kDa, and >100 kDa) in peptide fractions of varying molecular weights. This effect is because, as the peptides are hydrolyzed and their molecular weight decreases, the charged amino acid residues are exposed to the surface, facilitating interactions between the peptides and free radicals [34].

3.1.3. Antihyperglycemic Activity of Hydrolysates and Peptide Fractions

Peptides that inhibit the α-glucosidase enzyme work by delaying the hydrolysis of non-absorbable complex carbohydrates into simpler carbohydrates. Benjamin et al. [35] suggest that these peptides act as competitive inhibitors of the enzyme, while other research indicates that α-glucosidase inhibition results from multiple interactions with the enzyme [36]. Awosika and Aluko [22] determined the inhibition of α-glucosidase by sesame protein hydrolysates, finding values of 8% at a concentration of 5 mg/mL. They also evaluated α-glucosidase inhibition by peptide fractions of different molecular weights (<1 kDa, 1–3 kDa, 3–5 kDa, and 5–10 kDa), observing an increase in inhibition percentage (19%, 17%, 15%, and 10%, respectively) as the molecular weight of the fraction decreased. Qiao et al. [36] evaluated α-glucosidase inhibition by protein hydrolysates from *Paeonia ostii* byproduct, finding values around 20% at a concentration of 5 mg/mL.

In this study, the hydrolysates and peptide fractions were evaluated at a concentration of 1 mg/mL, which is lower than the concentrations reported by Awosika and Aluko [22] and Qiao et al. [36]. Despite this, our samples demonstrated a greater inhibitory effect on the enzyme (α-glucosidase) (Table 2). The complete hydrolysates (ALBH, GLOBH, GLUH, and PROH) showed inhibition values ranging from 40.14% for PROH to 55.63% for ALBH. Félix-Medina et al. [27] observed similar behavior in corn, where the prolamin hydrolysate (zeins) exhibited the lowest inhibition values. Across all analyzed peptide fractions, the percentage of α-glucosidase enzyme inhibition was higher than in the complete hydrolysates (Table 2), demonstrating the potential of sesame protein hydrolysates as a source of natural antihyperglycemic compounds. For the next stage, the peptide fractions with molecular weight less than 1 kDa from each protein type (ALBF < 1 kDa, GLOBF < 1 kDa, GLUF < 1 kDa, and PROF < 1 kDa) were selected due to their highest antioxidant activity and α-glucosidase inhibition values.

3.2. Characterization of Purified Peptides

3.2.1. Peptide Profile

Figure 2 displays the chromatographic separation of the <1 kDa fractions, revealing 28 well-defined chromatographic peaks corresponding to the peptides listed in Table 3. In total, 28 peptides were identified, showing a wide variation in isoelectric point (pI), with most values falling below neutral pH (pH 7), ranging from 2.83 to 11.11. The hydrophobicity values varied from 6.23 to 27.24 kcal/mol. These hydrophobicity values are higher than those reported by other authors for peptides from chickpeas [26] and black sesame [37].

The hydrophobic nature of the identified peptides is particularly relevant, as the presence of hydrophobic amino acids [phenylalanine (F), valine (V), proline (P), and tryptophan (W)] has been associated with strong antioxidant activity [38]. The molecular weight of the peptides ranged from 332.20 to 1096.63 Da, corresponding to peptides with 3 (VKS) and 10 (AAIATREPRL) amino acid residues, respectively. Out of the 28 identified peptides, only 1 had a molecular weight above 1 kDa. This is noteworthy, as 1 kDa molecular weight cut-off centrifugal tubes were used for this fraction, meaning all peptides were expected to have a molecular weight below 1 kDa.

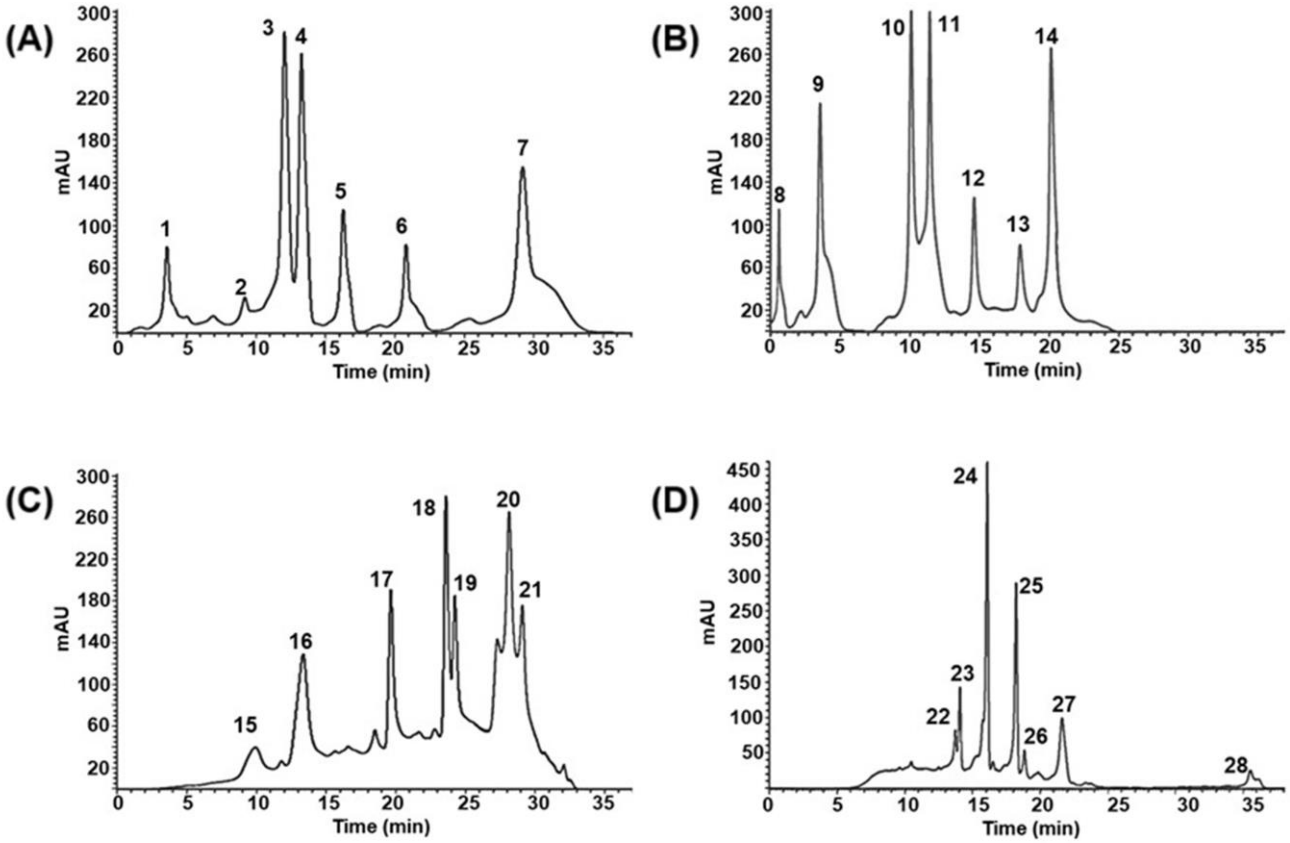


Figure 2. Chromatographic separation of the ALBF < 1 kDa (**A**), GLOF < 1 kDa (**B**), GLUF < 1 kDa (**C**), and PROF < 1 kDa (**D**) fractions of sesame byproduct. 1. VAEEGEEE, 2. MARF, 3. AAAL, 4. EES, 5. AEANQGQ, 6. VKS, 7. HCRCEAL, 8. VAF, 9. AAIATREPRL, 10. EGGTTEL, 11. TIRPNGL, 12. HPS, 13. HQK, 14. VDRNKL, 15. MKTF, 16. AIAATS, 17. PQQPPF, 18. PQQQIP, 19. QQL, 20. PQIPEQS, 21. EAIRAI, 22. PATS, 23. VATL, 24. TITNRCS, 24. PGQTW, 26. GRTGCS, 27. GQKPL and 28. VIDGY. Amino acids are shown in one-letter nomenclature. V: Valine, L: Leucine, T: Threonine, K: Lysine, W: Tryptophan, H: Histidine, F: Phenylalanine, I: Isoleucine, R: Arginine, M: Methionine, A: Alanine, P: Proline, G: Glycine, S: Serine, C: Cysteine, N: Asparagine, Q: Glutamine, Y: Tyrosine, D: Aspartic acid, E: Glutamic acid.

Among the potential biological activities of the identified peptides are antihypertensive, antidiabetic, antioxidant, antibacterial, anti-inflammatory, antihyperglycemic, and anti-obesity activities (Table 3). These potential activities are attributed to specific amino acid sequences within the peptides. Félix-Medina et al. [27] identified 12 peptides (PTATPY, VGGNW, NPAAY, VQTIRAQQR, QGMRY, GPCACAS, MQTHS, ETAAF, AAANRAS, QQQCCHQIRQ, HHIMAGAD, and VEDL) in corn proteins with some of the same activities as those found in this study. Furthermore, some peptide fragments identified by these authors (AA, EE, TIR, QQ, and QQL) match fragments of the peptides identified here. Similarly, Du et al. [37] identified eight peptides (ITAPHW, SLPNYHPSPR, QYLPR,

IRPNGL, YHNAPIL, LSYPR, GFAGDDAPRA, and LDPNPRSF) in fermented black sesame with amino acid fragments similar to those in this study (PR, HP, and PNGL).

Table 3. Bioactive peptides identified in the peptide fractions <1 kDa, and their predicted biological activity and physicochemical properties.

PM (Da)	Peptide *	PI	Hydrophobicity (kcal/mol)	Net Charge	Possible Bioactivity
			ALBF < 1 kDa		
890.36	VAEEGEEE	2.83	27.24	−5	ACE, DPP4
523.29	MARF	10.90	7.82	1	ACE, DPP4
344.23	AAAL	5.60	8.15	0	ACE, DPP4, AB
363.16	EES	2.92	15.62	−2	DPP4
716.31	AEANQGQ	3.21	16.07	−1	ACE, DPP4, α-GLU
332.24	VKS	9.88	10.70	1	ACE, DPP4
830.37	HCRCEAL	7.01	14.88	0	ACE, DPP4, α-GLU, AOX, AI
			GLOF < 1 kDa		
335.23	VAF	5.56	6.23	0	ACE, DPP4
1096.71	AAIATREPRL	11.02	14.67	1	ACE, DPP4, AB
705.32	EGGTTEL	2.92	16.1	−2	ACE, DPP4, AOX
769.45	TIRPNGL	11.11	9.73	1	ACE, DPP4, AOX
339.17	HPS	7.63	10.83	0	ACE, DPP4
411.26	HQK	9.80	13.80	1	ACE
743.46	VDRNKL	10.14	15.29	1	ACE, DPP4
			GLUF < 1 kDa		
525.27	MKTF	9.93	8.57	1	ACE, DPP4, AO
532.28	AIAATS	5.51	8.99	0	ACE, DPP4, AB
712.37	PQQPPF	5.19	8.15	0	ACE, DPP4, α-GLU
709.39	PQQQIP	5.25	9.37	0	ACE, DPP4,
387.23	QQL	5.44	8.19	0	DPP4
791.38	PQIPEQS	3.01	12.69	−1	ACE, DPP4, α-GLU
671.41	EAIRAI	6.85	12.10	0	ACE, DPP4, α-GLU, AOX
			PROF < 1 kDa		
374.18	PATS	5.18	9.25	0	ACE, DPP4
402.27	VATL	5.63	6.94	0	DPP4
793.38	TITNRCS	8.76	10.38	1	ACE
587.27	PGQTW	5.21	8.12	0	ACE, DPP4, AOX, AT
579.27	GRTGCS	8.76	12.70	1	ACE, DPP4, AOX
541.34	GQKPL	10.15	11.51	1	ACE, DPP4, AOX
565.28	VIDGY	3.15	10.40	−1	ACE, DPP4

MW: Molecular weight. * Peptides with intensity greater than 70%. Isoelectric point (IP), hydrophobicity, and net charge were calculated using the PepDraw platform [https://www.pepdraw.com/ (accessed on 10 March 2025)]. Potential peptide activity was obtained from the BioPep platform [https://biochemia.uwm.edu.pl/en/biopep-uwm-2/ (accessed on 20 January 2025)] and corresponds to the activity of the entire peptide or part of it. Amino acids are shown in single-letter nomenclature. V: Valine, L: Leucine, T: Threonine, K: Lysine, W: Tryptophan, H: Histidine, F: Phenylalanine, I: Isoleucine, R: Arginine, M: Methionine, A: Alanine, P: Proline, G: Glycine, S: Serine, C: Cysteine, N: Asparagine, Q: Glutamine, Y: Tyrosine, D: Aspartic acid, E: Glutamic acid. ACE: Anguitensin-converting enzyme inhibitor, DPP4: Dipeptidyl peptidase IV enzyme inhibitor, AB: antibacterial, AOX: antioxidant, AI: Anti-inflammatory peptide, α-Glu: α-Glucosidase enzyme inhibitor, AO: pancreatic lipase enzyme inhibitor.

3.2.2. Biological Activities of Peptides

Figure 3 illustrates the antioxidant power of the peptides identified in ALBF < 1 kDa (Figure 3A), GLOBF < 1 kDa (Figure 3B), GLUF < 1 kDa (Figure 3C), and PROF < 1 kDa (Figure 3D). For DPPH radical inhibition, IC_{50} values ranged from 90.18 to 220.39 µg/mL for VATL and PQIPEQS, respectively. The peptides with the best IC_{50} values were VATL (90.18 µg/mL), VAF (90.54 µg/mL), VIDGY (91.37 µg/mL), AAAL (92.45 µg/mL), and PGQTW (94.13 µg/mL). These peptides contain the aromatic amino acids phenylalanine (F), tyrosine (Y), and tryptophan (W) in their sequences. Their R-groups possess a benzene ring, which can donate electrons due to the conjugated double bonds in its structure. Similarly, the presence of the amino acid leucine (L) at the C-terminal end has been reported to

enhance the antioxidant activity of peptides [26]. Cheng et al. [39] investigated rice peptides with antioxidant and anti-inflammatory activity, observing that substituting any of the amino acid residues with alanine considerably improved both activities. The antioxidant activity values of the identified peptides are superior to those reported by Lu et al. [34] for seven sesame peptides obtained by sequential hydrolysis with Alcalase + Trypsin (105 to 6760 µg/mL). They are also comparable to values reported by Quintero-Soto et al. [26] for the MEE peptide (200 µg/mL).

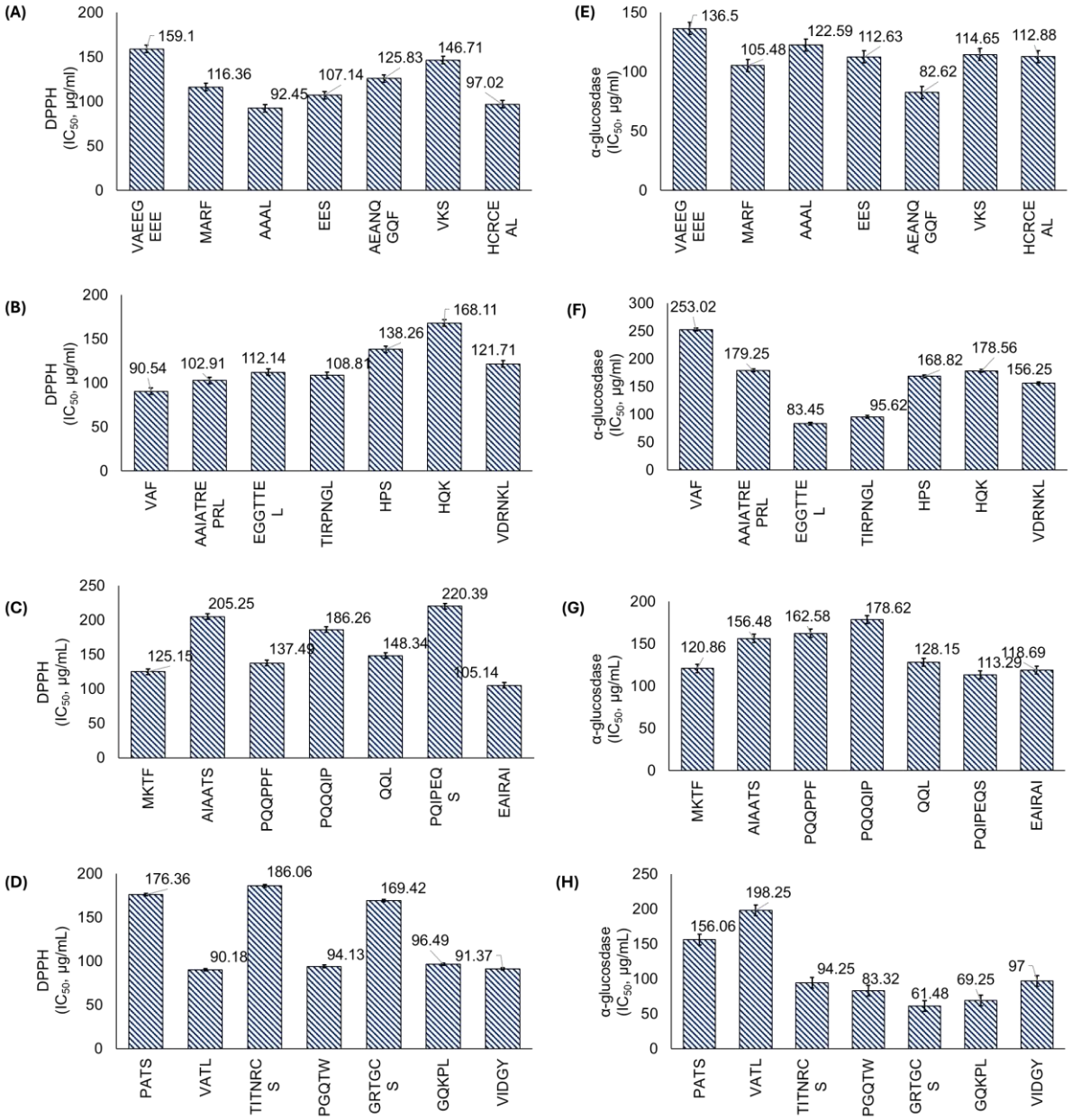


Figure 3. Antioxidant (DPPH) and antihyperglycemic (α-glucosidase) activity of the purified peptides. ALBF < 1 kDa (**A**,**E**), GLOBF < 1 kDa (**B**,**F**), GLUF < 1 kDa (**C**,**G**), PROF < 1 kDa (**D**,**H**). Amino acids are shown in single-letter nomenclature. V: Valine, L: Leucine, T: Threonine, K: Lysine, W: Tryptophan, H: Histidine, F: Phenylalanine, I: Isoleucine, R: Arginine, M: Methionine, A: Alanine, P: Proline, G: Glycine, S: Serine, C: Cysteine, N: Asparagine, Q: Glutamine, Y: Tyrosine, D: Aspartic acid, E: Glutamic acid. Results are the average of three biological replicates and are expressed as dry weight. The horizontal lines (I) in the bars of each graph indicate significant differences (Tukey, $p < 0.05$).

The exact mechanism by which peptides inhibit carbohydrate metabolism enzymes, such as α-glucosidase, is not fully understood. However, some drugs are known to exert their inhibitory activity by binding to the enzyme's active site through hydrophobic interactions [35]. In this study, we observed IC_{50} values for α-glucosidase enzyme inhibition ranging from 61.48 μg/mL (GRTGCS) to 253.02 μg/mL (VAF) (Figure 3E–H). Interestingly, the peptides with the best potential to inhibit α-glucosidase were those containing glycine (G) residues at their N-terminal end (GRTGCS and GQKPL), followed by those that contained this amino acid within their sequence. This could be because glycine (G) has the ability to form hydrogen bonds with the ASP^{518} amino acid in the enzyme's active site [26], leading to its inhibition. Similarly, peptides such as NPVSLPGR and LSAERGFLY, with glycine (G), arginine (R), and serine (S) residues, inhibit the α-glucosidase enzyme by blocking substrate entry into the active site and preventing catalysis resulting from binding to the active site [40]. Félix-Medina et al. [27] reported α-glucosidase enzyme inhibition percentages of 37.20% for a mixture of 12 corn protein peptides (1 mg/mL) with molecular weights ranging from 531.24 to 1270.52 Da. These values are higher than those observed in this study.

One of the main causes of type 2 diabetes mellitus is that insulin stimulation is hindered by the inactivation of incretin hormones; dipeptidyl peptidase IV inhibition helps control blood glucose by increasing incretin hormones [glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP)] [24]. Figure 4 present dipeptidyl peptidase IV enzyme inhibition values by the action of purified peptides. The IC_{50} values for the dipeptidyl peptidase IV inhibition ranged from 12.12 μg/mL (VKS) to 92.33 μg/mL (AAIATREPRL). These values are superior to those reported by Quintero-Soto et al. [26] for chickpea peptides (4.20 to 12.80 μg/mL). However, our values are better than those reported by Chaipoot et al. [24] for black sesame peptide fractions (780 μg/mL) and by González-Montoya et al. [41] for germinated soybean (810 to 1040 μg/mL). In an in silico study by Zhang et al. [42], peptides with proline (P) or alanine (A) residues at the second position of the N-terminus were reported to exhibit strong dipeptidyl peptidase IV enzyme inhibitory activity. In our research, two peptides with this characteristic were observed in the GLOBF < 1 kDa fraction: VAF (IC_{50} = 14.52 μg/mL) and HPS (22.05 μg/mL). While these were not the most potent inhibitors, they ranked 2nd and 7th out of the 28 peptides, respectively.

The anti-obesity potential of the identified peptides was evaluated by their ability to inhibit the metabolic enzyme pancreatic lipase; inhibition of this enzyme helps reduce fat absorption in the small intestine, resulting in a decrease in body weight [24]. The obtained data are presented in Figure 4. The peptides that showed the best anti-obesity potential were AAIATERPRL (6.14 mg/mL), AEANQGQF (6.89 mg/mL), PQQQIP (7.01 mg/mL), and PGQTW (7.09 mg/mL). Though there are no direct studies evaluating the presence of alanine on pancreatic lipase inhibitory activity, a recent study on soy indicates that hydrolysates composed of amino acids with small side chains [alanine (A) and glycine (G)], as well as basic amino acid residues [lysine (K), histidine (H), and arginine (R)], have a significant effect on the pancreatic lipase enzyme [43]. Awosika and Aluko [22] evaluated the inhibition of pancreatic lipase by pea peptide fractions of different molecular weights, observing better inhibition percentages with increasing molecular weight (5–10 kDa, 5.9 mg/mL; 3–5 kDa, 6.1 mg/mL; 1–3 kDa, 7.5 mg/mL; <1 kDa, 11.5 mg/mL). This effect is similar to what we observed in our study, where the peptide with the best capacity to inhibit pancreatic lipase was also the peptide with the highest molecular weight (1096.3 Da). This finding aligns with the observed correlation between the size of the identified peptides and the IC_{50} values for this enzyme ($r = -0.737$, $p < 0.0001$).

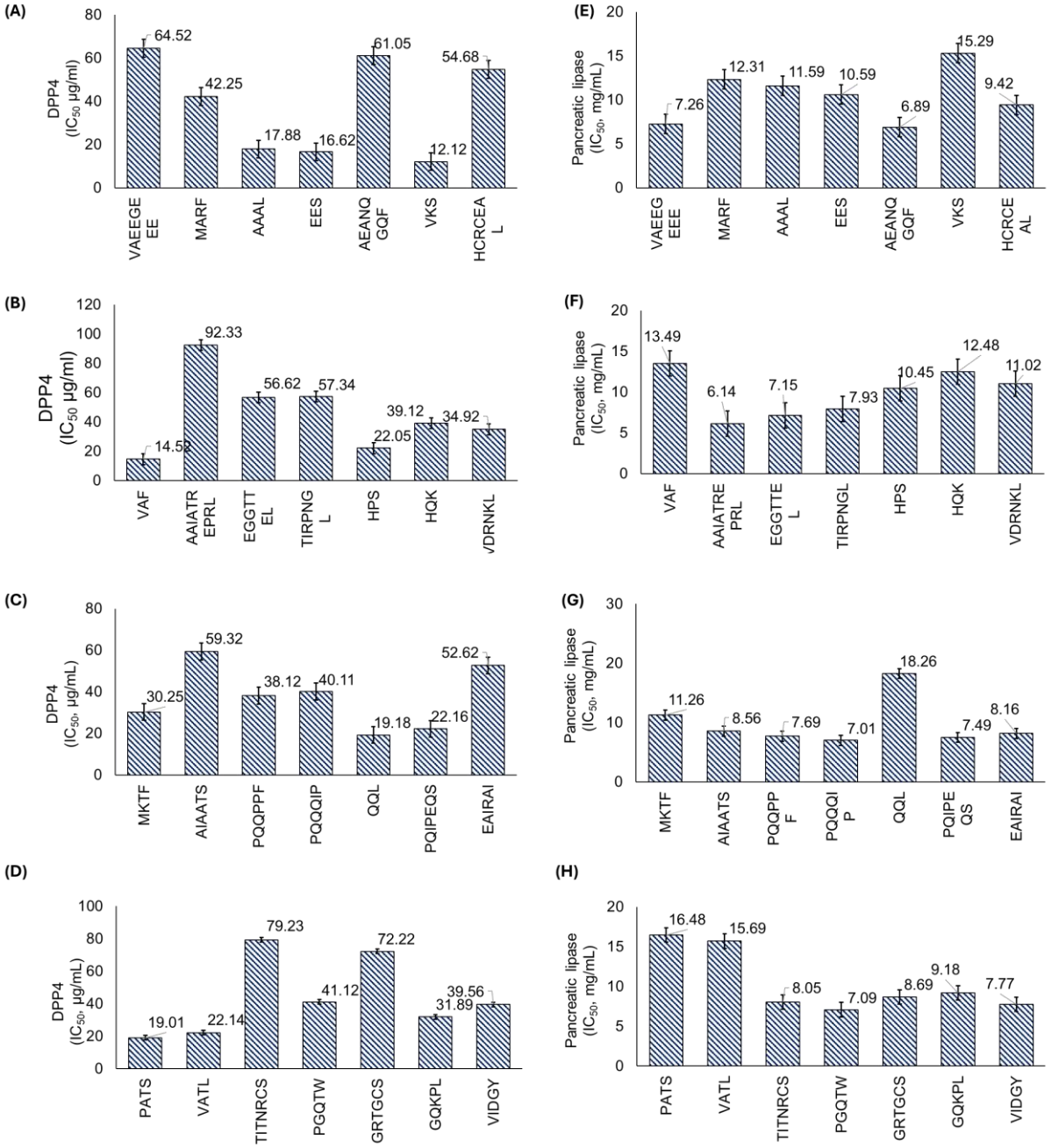


Figure 4. Antidiabetic (DPP4) and antiobesity (pancreatic lipase) activity of the purified peptides. ALBF < 1 kDa (**A**,**E**), GLOBF < 1 kDa (**B**,**F**), GLUF < 1 kDa (**C**,**G**), PROF < 1 kDa (**D**,**H**). Amino acids are shown in single-letter nomenclature. V: Valine, L: Leucine, T: Threonine, K: Lysine, W: Tryptophan, H: Histidine, F: Phenylalanine, I: Isoleucine, R: Arginine, M: Methionine, A: Alanine, P: Proline, G: Glycine, S: Serine, C: Cysteine, N: Asparagine, Q: Glutamine, Y: Tyrosine, D: Aspartic acid, E: Glutamic acid. Results are the average of three biological replicates and are expressed as dry weight. The horizontal lines (I) in the bars of each graph indicate significant differences (Tukey, $p < 0.05$).

4. Conclusions

This research demonstrates that sesame oil extraction byproduct (SOEB) is a good source of proteins with high nutraceutical potential. It can be effectively utilized to generate protein hydrolysates and purify peptides possessing significant nutraceutical properties, including antioxidant and antihyperglycemic properties. The identification of the 28 peptides in the ALBF < 1 kDa, GLOF < 1 kDa, GLUF < 1 kDa, and PROF < 1 kDa fractions

revealed their wide structural variability. Furthermore, the identified peptides showed the ability to inhibit metabolic enzymes such as α-glucosidase, dipeptidyl peptidase IV, and pancreatic lipase, as well as good antioxidant potential. The results obtained demonstrate the added value of the sesame seed oil extraction byproduct as an unconventional raw material in the development of new functional foods and nutraceuticals.

Considering the nutraceutical potential of the identified hydrolysates and peptides, future studies should focus on evaluating the biological properties of SOEB hydrolysates and peptides in in vivo models. Additionally, the potential applications of purified peptides in the formulation of dietary supplements or fortified foods aimed at preventing and managing metabolic diseases such as diabetes and obesity should be explored.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SOEB	Sesame oil extraction byproduct
DPPH	2,2-Diphenyl-1-picrylhydrazyl
PMSF	Phenylmethylsulfonyl fluoride
EDTA	Ethylenediaminetetraacetic acid
ALBH	Albumin hydrolysate
GLOH	Globulin hydrolysate
GLUH	Glutenin hydrolysate
PROH	Prolamin hydrolysate
ALBF	Albumin fraction
GLOF	Globulin fraction
GLUF	Glutelin fraction
PROF	Prolamin fraction

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