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Characterization of peptides with antioxidant activity and antidiabetic potential obtained from chickpea (*Cicer arietinum* L.) protein hydrolyzates

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Abstract: Alcalase hydrolyzates were prepared from the albumin (AH) and globulin (GH) fractions of eight chickpea (*Cicer arietinum* L.) genotypes from Mexico and 10 from other countries. Protein content, antioxidant activity (AA) (ABTS, DPPH), and degree of hydrolysis were evaluated and the best genotype was selected by principal component analysis. The hydrolyzates of the chosen genotype were analyzed for its antidiabetic potential measured as inhibition of α-amylase, α-glucosidase, and dipeptidyl peptidase-4 (DPP4). Peptide profiles were obtained by liquid chromatography-mass spectrometry (UPLC-DAD-MS), and the most active peptides were analyzed by molecular docking. The average antioxidant activity of albumin hydrolyzates was higher than that of globulin hydrolyzates. ICC3761 was the selected genotype and peptides purified from the albumin hydrolyzate showed the best antioxidant activity and antidiabetic potential (FEI, FEL, FIE, FKN, FGKG, and MEE). FEI, FEL, and FIE were in the same chromatographic peak and this mixture showed the best ABTS scavenging (78.25%) and DPP4 inhibition (IC_{50} = 4.20 μg/ml). MEE showed the best DPPH scavenging (47%). FGKG showed the best inhibition of α-amylase (54%) and α-glucosidase (56%) and may be a competitive inhibitor based on in silico-predicted interactions with catalytic amino acids in the active site of both enzymes. These peptides could be used as nutraceutical supplements against diseases related to oxidative stress and diabetes.

Practical Application: This study showed that chickpea protein hydrolyzates are good sources of peptides with antidiabetic potential, showing high antioxidant activity and inhibition of enzymes related to carbohydrate metabolism and type 2 diabetes. These hydrolyzates could be formulated in functional foods for diabetes.

1 | INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume worldwide and a good source of nutrients, mainly proteins (Ladizinsky, 1995). The most abundant storage proteins in chickpea are globulins (57%), followed by glutelins (18%), albumins (12%), and prolamins (3%) (Singh & Jambunathan, 1982). Nowadays, proteins are

investigated not only for their nutritional or functional properties, but also as a source of bioactive peptides that can be released by hydrolysis, generating beneficial health effects such as antioxidant and antidiabetic, among others (Ghribi et al., 2015; Milan-Noris et al., 2018). In addition, peptides can be used as natural biological agents in food preservation and the development of functional foods (de Castro & Sato, 2015).

The antioxidant activity (AA) and antidiabetic potential of peptides are a function of their chemical and structural properties. Histidine-containing peptides chelate metal ions and inactivate/remove free radicals, acting as antioxidant peptides (Chen et al., 1996; Torres-Fuentes et al., 2015). Kou et al. (2013) purified peptides from chickpea albumin that showed good AA (ABTS and DPPH), highlighting the peptide RQSHFANAQP that also inhibited the proliferation of breast cancer cells and reduced the total cholesterol and triglyceride levels of hyperlipidemic mice (Xue et al., 2018; Xue et al., 2015). Torres-Fuentes et al. (2015) purified peptides from chickpea globulins and demonstrated their ability to eliminate free radicals and to protect Caco-2 cells exposed to oxidizing agents.

The antidiabetic potential of the peptides is influenced by the hydrophobic and hydrophilic characteristics of the residues (R chains), which allow the peptides to interact with enzymes such as α-amylase, α-glucosidase, and dipeptidyl peptidase-4 (DPP4). α-Amylase and α-glucosidase hydrolyze polysaccharides during digestion and release monosaccharides that are absorbed into the bloodstream (Brayer et al., 1995; Roig-Zamboni et al., 2017). DPP4 is a serine protease that cleaves polypeptides with a penultimate proline or alanine residue at the N terminus. The primary substrates of this enzyme are glucagon-like peptide 1 (GLP-1) and glucose-dependent tropic insulin polypeptide (GIP), which stimulate insulin secretion (Patel & Ghate, 2014). Oseguera-Toledo et al. (2015) observed that bean protein hydrolyzates (alcalase-bromelain) increased the glucose-stimulated insulin secretion from INS-1E cells up to 57% compared to glucose control; they also observed that peptide fractions >1 kDa inhibited enzymes involved in diabetes and carbohydrate metabolism, such as the α-amylase, α-glucosidase, and DPP4.

Mojica and Gonzalez de Mejía (2016) evaluated the antidiabetic potential of black bean hydrolysates produced with different proteases and observed higher inhibition values of α-amylase, α-glucosidase, and DPP4 in alcalase hydrolyzates. The peptide profile obtained with alcalase was similar to that produced by gastrointestinal proteases (pepsin and pancreatin), highlighting the peptides AKSPLF, ATNPLF, FEELN, and LSVSVL that had the best interaction with antidiabetic enzymes evaluated in silico. These peptides reduced glucose absorption in Caco-2 cells by blocking the glucose transporters GLUT2 and SGLT1; furthermore, glucose levels were significantly reduced in mice supplied with different concentrations of alcalase hydrolyzates containing these peptides (Mojica et al., 2017).

The information about the antidiabetic potential of chickpea protein hydrolyzates is scarce. Recently, Chandrasekaran et al. (2020) evaluated the inhibition of α-amylase and DPP4 by chickpea hydrolyzates produced with pepsin-pancreatin and bromelain. Based on in silico analysis, the authors attributed the inhibition of the enzymes to the peptides PHPATSGGGL, YVDGSGTPLT, SPQSPPFATPLW, KMTAGSGVT, GKAAPGSGGGTKA, and GLTQGASLAGSGAPSPLF. However, the individual activity of the peptides was not evaluated to confirm these results. Therefore, it is important to identify bioactive peptides that could be formulated in functional foods. The aim of the present study was to characterize peptides with AA and antidiabetic potential in chickpea protein hydrolyzates produced with alcalase.

2 | MATERIALS AND METHODS

2.1 | Materials

Eighteen chickpea genotypes (*Cicer arietinum* L) were analyzed, including eight desi-type and one kabuli-type from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) and nine kabuli genotypes from the National Institute of Forestry, Agriculture and Livestock Research (INIFAP). These materials were grown at INIFAP Experimental Field in Culiacan, Sinaloa, Mexico, as described by Quintero-Soto et al. (2018).

2.2 | Extraction and quantification of albumins and globulins

Albumins and globulins were extracted from mature seeds flour using the procedure of Dziuba et al. (2014) with some modifications. The flour (100 g) was defatted with 2 L of hexane; the mixture was stirred for 4 hr at 20°C (SP131015, Thermo Fisher Scientific, Waltham, MA, USA), centrifuged at 15,000 *g* for 10 min (5810R, Eppendorf, Germany), and the pellet was recovered to obtain the proteins. The pellet was mixed with 2 L of extraction buffer I (sterile deionized H_2O pH 8.0, 10 mmol/L $CaCl_2$, 10 mmol/L $MgCl_2$, 1 mmol/L PMSF) and stirred for 4 hr at 20°C; the mixture was centrifuged (15,000 *g*, 20 min) and the supernatant (supernatant 1) and the pellet (residue 1) were recovered. The supernatant 1 was mixed with two volumes of ammonium acetate-methanol (0.1 mol/L) and left overnight (16 hr) to precipitate the proteins. The pellet

was washed with 0.1 mol/L ammonium acetate-methanol, methanol, and acetone to eliminate other compounds such as phenolics. The washes were repeated until the supernatant was colorless and the phenolic compounds were not detected with the Folin-Ciocalteau reagent. The dried residue was resuspended in distilled water (5 ml/g dry solid) and dialyzed (cellulose membrane, 6000–8000 Da, 25.5 mm × 30 m, Fisherbrand™, Thermo Fisher Scientific) against distilled water (2 L) for 3 days with three water changes per day. The dialyzate was centrifuged (15,000 *g*, 20 min), the supernatant was recovered and lyophilized (25EL; VirTis Co., Gardiner, NY, USA) to obtain the albumins fraction.

For globulin extraction, the residue 1 and 2 L of extraction buffer II [0.1 mol/L Tris-HCl (pH 8.0), 100 g/L NaCl, 1 mmol/L PMSF, 10 mmol/L EDTA] were mixed, stirred for 16 hr at 20°C, and centrifuged (15,000 *g*, 20 min). The recovered supernatant was treated as described for supernatant 1 for the precipitation, washing, and dialysis of the proteins. Finally, the dialyzate was centrifuged (15,000 *g*, 20 min) and the recovered pellet corresponded to the globulins fraction.

The albumins and globulins fractions were resuspended in water and quantified using the bicinchoninic acid (BCA) protein assay (Pierce, Thermo Fisher Scientific) and bovine serum albumin (BSA) as standard.

2.3 | Preparation of protein hydrolyzates and degree of hydrolysis

Protein hydrolyzates were prepared according to Ghribi et al. (2015) and Kou et al. (2013) with some modifications. One gram of albumins or globulins was resuspended in 10 ml of water (pH 8) and incubated at 80°C for 5 min. The samples were cooled to 50°C, added with alcalase (0.3 U/g protein), and incubated at 50°C for 90 min at pH 8.0. After hydrolysis, samples were heated at 80°C for 20 min to inactivate the enzyme, cooled to 27°C, centrifuged (5000 *g*, 20 min, 4°C), and the supernatants were filtered (0.45 µm; PVDF membrane HPLC certified, Pall Corp., Port Washington, NY, USA). The filtrates were passed through 10 kDa cut-off filters (Vivaspin 20, Sartorius Stedim Biotech, Germany) and lyophilized to obtain the albumin hydrolyzate (AH) and globulin hydrolyzate (GH). The hydrolyzates and the peptide fractions were quantified using the BCA method (section 2.2).

The degree of hydrolysis (DH) was determined according to Adler-Nissen (1986) using the following equations:

$$\mathrm{DH}\,(\%) = ((B \times \mathrm{Nb})/\mathrm{MP}) \times (1/\alpha) \times (1/h_{\mathrm{tot}}) \times 100$$

$$\alpha = (10^{\,\mathrm{pH}\,-pK})/(1 + 10^{\,\mathrm{pH}\,-pK})$$

where B is the volume (ml) of NaOH consumed to keep the pH constant during the proteolysis. Nb is the normality of NaOH, MP is the protein content (g), h_{tot} is the total number of peptide bonds in the substrate, which was assumed to be 7.22 mmol/g protein (Kou et al., 2013), and α is the degree of dissociation of the α-NH_2 groups estimated from the pH and pK values.

2.4 | Purification and identification of peptides showing AA and antidiabetic potential

The AH and GH of the ICC3761 genotype were sequentially separated by UPLC (Accela, Thermo Fisher Scientific) using a Luna C18 column (15 × 4.6 mm, 5 µm particle size, Phenomenex, Inc, Torrence, CA, USA). The mobile phase comprised water–formic acid (1%) (A) and acetonitrile (B), employing a flow of 0.2 ml/min and a semilinear gradient: 0–20 min, 0.5%–4% B; 20–68 min, 4%–20% B; 68–115, 20%–48% B; and 115–120 min, 48%–0% B. The chromatograms were registered at 214, 257, and 280 nm. Peaks eluted during 30 min intervals were pooled to obtain three fractions of each hydrolyzate (AH1, AH2, and AH3; GH1, GH2, and GH3). The six fractions were lyophilized to determine their AA and antidiabetic potential.

The fraction with the best AA and antidiabetic potential (AH1 of ICC3761) was separated by UPLC into six subfractions (AH1-1–AH1-6) using the same column and mobile phase described above and the following gradient: 0–20 min, 0.5%-4% B; 20–35 min, 5%–9% B; and 35–40 min, 9%-0% B. The new fractions were collected based on their absorption maximum at 214, 257, and 280 nm; they were lyophilized to determine their AA and antidiabetic potential.

The peptides were purified from the subfraction with the best AA and antidiabetic potential. A 15-µl aliquot was separated by UPLC using the following gradient: 0–1 min, 0.5%–2% B; 1–15 min, 2%–6% B; 15–18 min, 6%–80% B; 18–20 min, 80%–0% B. The peptides were detected at 214, 257, and 280 nm; they were identified with an LTQ-XL mass spectrometer (Thermo Scientific, USA), using an electrospray ionization source (ESI), operating in positive mode with capillar voltage and temperature of 35 V and 300°C, respectively. Mass spectra were acquired with the Xcalibur 2.1 program (Thermo Scientific) using a mass-charge range (m/z) of 70−2000 and the most intense ions were fragmented by collision induced dissociation (CID). Helium and nitrogen gases were used for collision and drying, respectively. MS/MS data was analyzed with the Proteome Discoverer 1.2 program using the database search tool with the SEQUEST algorithm, which validates and aids in searching the database employing the auto mode.

The chickpea (*Cicer arietinum*) proteome from UniProt was used for the database search. Precursor and fragment mass tolerances were set to 10 ppm and 0.5 Da, respectively. A false discovery rate of 0.01 was used. The carbamidomethylation of cystein and methionine oxidation were selected as variable modifications during the search. Identifications were carried out using three independent samples.

2.5 | Determination of AA

The ABTS and DPPH assays were carried out following the methodologies proposed by Re et al. (1999) and Brand-Williams et al. (1995), with the modifications of Mejri et al. (2017). The ABTS radical was generated by mixing a solution of ABTS (7.4 mmol/L) with potassium persulfate (2.6 mmol/L), followed by incubation for 16 hr in the dark at room temperature. The radical solution was diluted with PBS (10 mmol/L, pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. One microliter of hydrolyzate (1 mg/ml) or peptide fraction (0.2 mg/ml) was mixed with 100 µl of the ABTS radical solution. The mixture was left to stand for 6 min at room temperature in the dark and then the change in absorbance at 734 nm was read using a Multiskan Sky spectrophotometer (Thermo Fisher). The DPPH radical was dissolved in methanol (100 µmol/L) and 50 µl of this solution was mixed with 50 µl of each hydrolyzate (1 mg/ml) or peptide fraction (0.2 mg/ml). After 30 min incubation at room temperature, the absorbance was read at 510 nm using a Multiskan Sky spectrophotometer. The results were expressed as percent inhibition of the radical and µmol of Trolox equivalents (TE)/ 100 g.

2.6 | Assessment of the antidiabetic potential

The α-amylase and α-glucosidase inhibition were determined according to Mojica et al. (2015). For α-amylase, 5 µl of sample (0.1 mg/ml) and 5 µl of α-amylase solution (13 U/ml) were mixed and incubated for 10 min at 25°C, followed by addition of 5 µl of a starch solution (10 g/L) and incubation (10 min, 25°C). The mixture was added with 25 µl of the dinitrosalicylic acid colored reagent and placed in a water bath (W1106, Labnet International, Edison, NJ, USA) at 100°C for 5 min. Finally, the resulting mixture was diluted with 250 µl of distilled water and the absorbance was measured at 520 nm using a Multiskan Sky spectrophotometer.

For α-glucosidase, 25 µl of sample (0.1 mg/ml) was mixed with 50 µl of the α-glucosidase solution (1 U/ml) and the mixture was incubated for 10 min at 25°C. Subsequently, 25 µl of *p*-nitrophenyl-α-*D*-glucopyranoside (5 mmol/L) was added and the mixture was incubated for 5 min. The absorbance was measured at 405 nm using a Multiskan Sky spectrophotometer. The results for α-amylase and α-glucosidase were expressed as percent inhibition in relation to a positive control (acarbose, 1 mmol/L).

The inhibition of dipeptidyl peptidase-4 (DPP4) was determined using the MAK203 kit (Sigma-Aldrich, St. Louis, USA). Forty-nine microliter of sample or reaction buffer (control) were mixed with 1 µl of DPP4 enzyme and the mixture was incubated for 10 min at 37°C in the dark. Subsequently, 23 µl of reaction buffer and 2 µl of substrate (reagents provided by the supplier) were added, followed by incubation for 15 min at 37°C. The fluorescence of the samples was measured (flu1) with a Synergy MX microplate reader (BioTek Instruments, Winooski, VT, USA) using excitation and emission wavelengths of 360 and 460 nm, respectively. The samples were incubated again 15 min and the fluorescence was measured (flu2). The results were expressed as IC_{50} (µg/ml).

The percentage of inhibition of the DPP4 enzyme was determined with the following formulas:

$$\text{Slope} = ((\text{flu2} - \text{flu1})/(T2 - T1)) \times 100$$

$$\%\ \text{inhibition of DPP4} = ((\text{slopeEC} - \text{slopeSM})/\text{slopeEC}) \times 100$$

where $T2$ is the time elapsed until the final reading (30 min) and $T1$ is the first incubation time (15 min); slopeEC and slopeSM are the slopes for control and the sample, respectively.

2.7 | Molecular docking

Molecular docking was carried out as described by Oseguera-Toledo et al. (2015). The binding sites of the purified peptides for α-amylase, α-glucosidase, and DPP4 were located using the GRAMM-X-Protein-Protein (Tovchigrechko & Vakser, 2005; Tovchigrechko & Vakser, 2006) and Rosetta FlexPepDock web servers (London et al., 2011; Raveh et al., 2010) and the Discovery Studio 4.0 program (DS 4.0, Accelrys Software Inc., San Diego, CA, USA). The three-dimensional structures of α-amylase (1HNY), α-glucosidase (5NN8), and DPP4 (1 × 70) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Ligands and water molecules, as well as the monomer B of DPP4 were removed using the Discovery Studio program. The peptide structures were designed in the same program and submitted to the GRAMM-XProtein-Protein molecular alignment

TABLE 1 Protein content of globulin fractions, albumin fractions and hydrolyzates, and degree of hydrolysis of alcalase hydrolyzates from the chickpea genotypes

	Globulins			Albumins		
Genotype	Fraction[x]	Hydrolyzate[y]	Degree of hydrolysis[z]	Fraction[x]	Hydrolyzate[y]	Degree of hydrolysis[z]
Kabuli						
Surutato 77	47.6 ± 4.7^{ab}	95.24 ± 0.52^{ab}	41.74 ± 0.17^{bc}	17.5 ± 1.6^{bc}	92.17 ± 2.74^{a}	33.83 ± 1.02^{b}
Sto. Dom. 82	44.9 ± 1.6^{abc}	94.85 ± 3.63^{ab}	42.14 ± 0.13^{b}	15.0 ± 1.2^{bcd}	88.65 ± 8.97^{a}	35.38 ± 3.40^{b}
Bco. Sin. 92	52.2 ± 1.7^{a}	93.49 ± 2.67^{ab}	41.75 ± 0.15^{bc}	14.4 ± 0.8^{cde}	92.28 ± 4.17^{a}	33.81 ± 1.49^{b}
Progreso 95	34.9 ± 3.7^{c}	95.43 ± 0.75^{ab}	44.65 ± 0.25^{a}	11.2 ± 0.4^{e}	90.21 ± 1.35^{a}	34.54 ± 0.36^{b}
Suprema 03	47.0 ± 2.5^{ab}	93.12 ± 1.36^{ab}	44.30 ± 0.06^{a}	11.8 ± 0.9^{de}	85.53 ± 6.41^{ab}	36.50 ± 1.93^{ab}
Jumbo 2010	46.7 ± 6.1^{ab}	95.74 ± 1.28^{a}	44.37 ± 0.32^{a}	17.6 ± 1.2^{bc}	94.43 ± 1.71^{a}	33.00 ± 0.60^{b}
Blanoro	41.3 ± 2.2^{bc}	93.81 ± 0.42^{ab}	41.80 ± 0.11^{bc}	12.3 ± 0.3^{de}	75.58 ± 3.20^{b}	41.36 ± 1.72^{a}
Hoga 021	50.4 ± 2.3^{ab}	89.68 ± 1.04^{b}	44.15 ± 0.08^{a}	16.3 ± 0.2^{bc}	92.58 ± 4.82^{a}	33.72 ± 1.76^{b}
Hoga 340	51.3 ± 5.0^{ab}	95.64 ± 3.08^{a}	40.16 ± 1.19^{d}	18.1 ± 1.3^{b}	96.03 ± 4.68^{a}	32.50 ± 1.62^{b}
ICC3421	35.4 ± 1.0^{c}	94.50 ± 2.42^{ab}	40.90 ± 0.04 ^{cd}	29.4 ± 1.5^{a}	89.03 ± 3.83^{a}	35.04 ± 1.52^{b}
Average	45.2^{A}	94.15^{A}	42.60^{A}	16.4^{A}	89.70^{A}	34.97^{A}
Desi						
ICC6306	44.7 ± 0.7^{bc}	93.24 ± 0.96^{a}	40.57 ± 0.13^{e}	16.2 ± 0.6^{b}	93.71 ± 4.17^{a}	33.29 ± 1.45^{a}
ICC3761	51.4 ± 1.3^{a}	92.74 ± 0.01^{a}	43.10 ± 0.02^{c}	17.1 ± 1.3^{ab}	96.77 ± 2.52^{a}	32.21 ± 0.84^{a}
ICC4418	47.4 ± 2.4^{ab}	94.09 ± 2.19^{a}	40.51 ± 0.07^{e}	16.7 ± 0.3^{ab}	90.48 ± 6.01^{a}	34.53 ± 2.23^{a}
ICC3512	33.1 ± 0.7^{e}	97.91 ± 1.46^{a}	44.93 ± 0.04^{a}	12.4 ± 0.7^{c}	88.45 ± 7.77^{a}	35.40 ± 2.96^{a}
ICC5383	37.7 ± 0.9 ^{cd}	92.85 ± 3.57^{a}	40.89 ± 0.02^{d}	13.5 ± 0.8^{c}	88.38 ± 3.87^{a}	34.42 ± 1.43^{a}
ICC13124	35.1 ± 0.5^{de}	92.32 ± 1.75^{a}	45.06 ± 0.09^{a}	13.5 ± 1.0^{c}	93.69 ± 1.32^{a}	33.26 ± 0.46^{a}
ICC14872	47.2 ± 3.7^{ab}	94.62 ± 3.37^{a}	44.66 ± 0.12^{b}	18.7 ± 0.9^{a}	97.22 ± 1.80^{a}	32.05 ± 0.58^{a}
ICC5613	40.0 ± 1.3^{de}	94.33 ± 3.89^{a}	40.57 ± 0.10^{e}	12.8 ± 0.2^{c}	89.61 ± 6.31^{a}	34.88 ± 2.45^{a}
Average	42.1^{A}	94.01^{A}	42.54^{A}	15.1^{A}	92.28^{A}	33.76^{A}

The results are the mean ± SD of three biological replicates and they are expressed on a dry weight basis. [x]The protein contents of globulins and albumins are expressed in g/100 g of total protein. [y]The protein contents of the hydrolyzates are expressed in g/100 g of hydrolyzate. [z]The degree of hydrolysis is reported as percent. Means with different superscript letters in a column within the same type of chickpea are significantly different (Tukey, $p \leq 0.05$). Different letters between the average values of desi and kabuli chickpeas indicate significant differences (t-test; $p \leq 0.05$).

web server (version 12.0). The optimal peptide-enzyme complex was selected based on the minimum global energy value and used as a template to perform the refining molecular dynamics on the Rosetta FlexPepDock web server.

2.8 | Statistic analysis

Data corresponds to the mean of three biological replicates with three technical replicates. It was analyzed using one-way ANOVA and the Tukey test ($p < 0.05$) was used for mean comparisons between genotypes of the same type. The average values of desi and kabuli chickpeas were compared using a t-test. The analyses were carried out with the software STATGRAPHIC plus 5.1 (Statistical Graphics Corp., The Plains, VA, USA). A principal component analysis (PCA) was performed using STATA version 11.1 (Stata Corp, College Station, TX, USA).

3 | RESULTS AND DISCUSSION

3.1 | Content of globulin and albumin fractions in chickpea grains

The average protein content of the globulin fraction was about 2.8 times higher than that of the albumin fraction in both kabuli and desi genotypes (Table 1). The globulins content varied significantly ($p < 0.05$) among the chickpea genotypes and the values ranged from 34.93 (Progreso 95) to 52.16 g/100 g (Bco. Sin. 92) in kabuli chickpeas and from 33.12 (ICC3512) to 51.35 g/100 g (ICC3761) in desi types. These values are lower than those reported by Dhawan et al. (1991) in six kabuli chickpeas (53.44–59.06 g/100 g), but they fall within the range reported by Singh et al. (2008) for five chickpea genotypes from India (39.80–64.21 g/100 g). The albumins content also varied significantly ($p < 0.05$) from 11.23 (Progress 95) to

TABLE 2 In vitro antioxidant activity of alcalase hydrolyzates from the chickpea genotypes

Genotype	Globulins		Albumins	
	ABTS[z]	DPPH[z]	ABTS[z]	DPPH[z]
Kabuli				
Surutato 77	35.3 ± 1.0^{abc} (418.4 ± 19.9^{abc})	23.1 ± 2.2^{b} (8.8 ± 2.2^{b})	47.1 ± 1.3^{c} (687.4 ± 24.2^{c})	45.1 ± 1.5^{cde} (30.4 ± 1.4^{cde})
Sto. Dom. 82	37.4 ± 1.9^{ab} (461.4 ± 40.6^{ab})	28.8 ± 0.7^{a} (14.4 ± 0.6^{a})	50.4 ± 2.6^{bc} (750.3 ± 49.7^{bc})	54.4 ± 3.2^{b} (39.5 ± 3.1^{b})
Bco. Sin. 92	36.7 ± 0.8^{abc} (447.2 ± 16.9^{abc})	25.7 ± 1.5^{ab} (11.3 ± 1.5^{ab})	36.9 ± 1.6^{de} (491.2 ± 30.9^{de})	50.5 ± 3.3^{bc} (35.7 ± 3.2^{bc})
Progreso 95	38.8 ± 1.5^{a} (489.0 ± 30.3^{a})	28.9 ± 1.0^{a} (14.4 ± 1.0^{a})	51.2 ± 0.7^{b} (766.5 ± 13.5^{b})	56.5 ± 0.7^{b} (41.5 ± 0.7^{b})
Suprema 03	34.0 ± 0.8^{bc} (390.4 ± 17.8^{bc})	24.8 ± 2.0^{ab} (10.4 ± 1.2^{ab})	57.0 ± 1.1^{a} (877.5 ± 14.2^{a})	64.5 ± 1.0^{a} (49.3 ± 1.0^{a})
Jumbo 2010	36.2 ± 1.7^{abc} (435.7 ± 33.9^{abc})	23.5 ± 0.8^{b} (9.2 ± 0.8^{b})	40.0 ± 1.5^{d} (551.8 ± 29.3^{d})	41.8 ± 0.5^{de} (27.1 ± 1.1^{de})
Blanoro	32.9 ± 0.9^{c} (369.5 ± 18.4^{c})	23.2 ± 1.3^{b} (8.9 ± 1.3^{b})	21.6 ± 0.4^{g} (197.6 ± 8.3^{g})	40.0 ± 0.3^{e} (25.3 ± 0.4^{e})
Hoga 021	35.3 ± 1.6^{abc} (417.9 ± 33.1^{abc})	25.8 ± 0.5^{ab} (11.5 ± 0.5^{ab})	34.1 ± 0.6^{e} (437.5 ± 11.2^{e})	43.4 ± 2.8^{de} (28.6 ± 2.7^{de})
Hoga 340	37.4 ± 1.1^{ab} (460.1 ± 22.2^{ab})	25.0 ± 1.7^{ab} (10.7 ± 1.7^{ab})	47.4 ± 1.5^{c} (693.2 ± 29.6^{c})	47.0 ± 3.5^{cd} (32.2 ± 3.4^{cd})
ICC3421	37.4 ± 1.2^{ab} (460.5 ± 24.0^{ab})	26.3 ± 1.4^{ab} (11.9 ± 1.4^{ab})	29.5 ± 0.2^{f} (349.2 ± 4.5^{f})	23.0 ± 1.1^{f} (8.68 ± 1.0^{f})
Average	36.1^{A} (435.0^{A})	25.5^{B} (11.1^{B})	41.5^{A} (580.2^{A})	47.6^{A} (31.8^{A})
Desi				
ICC6306	36.6 ± 0.9^{b} (444.1 ± 18.0^{b})	26.5 ± 0.5^{a} (12.1 ± 0.4^{a})	43.4 ± 2.7^{cd} (616.2 ± 51.7^{cd})	45.4 ± 1.1^{c} (30.6 ± 1.5^{c})
ICC3761	40.3 ± 0.5^{a} (518.7 ± 10.6^{a})	27.0 ± 1.7^{a} (12.6 ± 1.6^{a})	41.5 ± 0.2^{de} (579.1 ± 2.2^{dd})	40.2 ± 0.6^{d} (25.5 ± 1.0^{d})
ICC4418	35.0 ± 0.8^{b} (412.2 ± 15.9^{b})	24.8 ± 1.0^{a} (10.4 ± 1.0^{a})	35.2 ± 1.8^{f} (459.6 ± 35.3^{f})	40.3 ± 1.5^{d} (25.6 ± 1.4^{d})
ICC3512	36.8 ± 0.8^{b} (447.7 ± 16.4^{b})	27.4 ± 1.2^{a} (13.0 ± 0.6^{a})	53.0 ± 0.8^{a} (799.4 ± 14.9^{a})	54.4 ± 1.1^{a} (39.5 ± 1.1^{a})
ICC5383	35.7 ± 1.8^{b} (425.5 ± 37.7^{b})	27.2 ± 2.1^{a} (12.8 ± 2.0^{a})	49.5 ± 0.1^{ab} (733.2 ± 1.4^{ab})	49.4 ± 2.3^{b} (34.6 ± 2.3^{b})
ICC13124	34.2 ± 1.3^{b} (395.3 ± 26.9^{b})	27.6 ± 0.4^{a} (13.2 ± 1.0^{a})	38.5 ± 1.4^{ef} (521.6 ± 28.1^{ef})	52.4 ± 1.5^{ab} (37.5 ± 1.4^{ab})
ICC14872	36.1 ± 1.7^{b} (434.8 ± 36.6^{b})	27.0 ± 1.0^{a} (12.7 ± 1.6^{a})	42.4 ± 1.0^{d} ($597.2 \pm 19.5^{d)}$)	45.3 ± 1.5^{c} (30.5 ± 1.0^{c})
ICC5613	36.0 ± 1.1^{b} (431.3 ± 22.6^{b})	26.4 ± 1.6^{a} (12.0 ± 1.2^{a})	47.2 ± 0.9^{bc} (689.5 ± 17.7^{bc})	53.3 ± 1.0^{ab} (38.4 ± 1.2^{ab})
Average	36.3^{A} (438.7^{A})	26.7^{A} (12.4^{A})	43.8^{A} (624.5^{A})	47.6^{A} (32.8^{A})

The results are the mean ± SD of three biological replicates. [z]The antioxidant activity was evaluated at 1 mg of protein/ml and expressed as percent inhibition and Trolox equivalents (TE)/100 g of protein (values in parentheses) on a dry weight basis. Means with different superscript letters in a column within the same type of chickpea are significantly different (Tukey, $p \leq 0.05$). Different letters between the average values of desi and kabuli chickpeas indicate significant differences (t-test; $p \leq 0.05$).

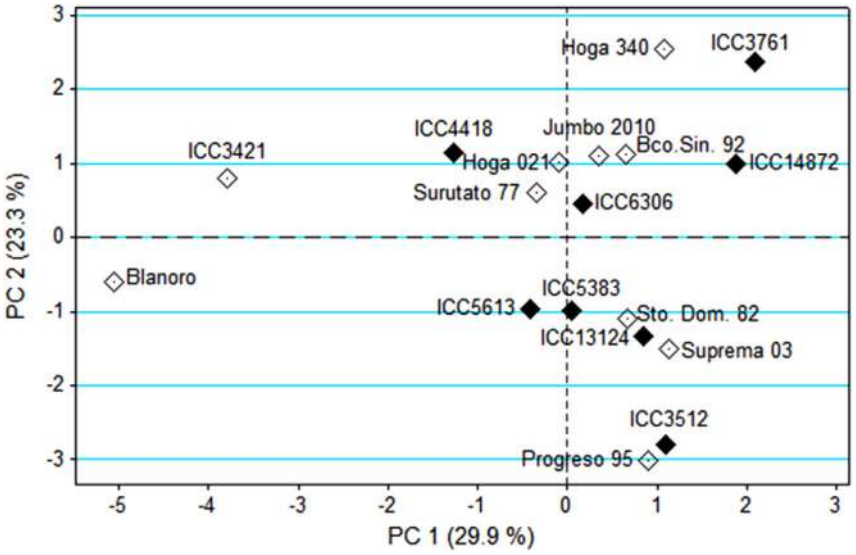

FIGURE 1 Principal component analysis of 18 chickpea genotypes based on the antioxidant activity and degree of hydrolysis (PC 1) of alcalase hydrolyzates from albumin and globulin fractions and protein content (albumins and globulins) (PC 2). Kabuli (white diamond) and desi (black diamond)

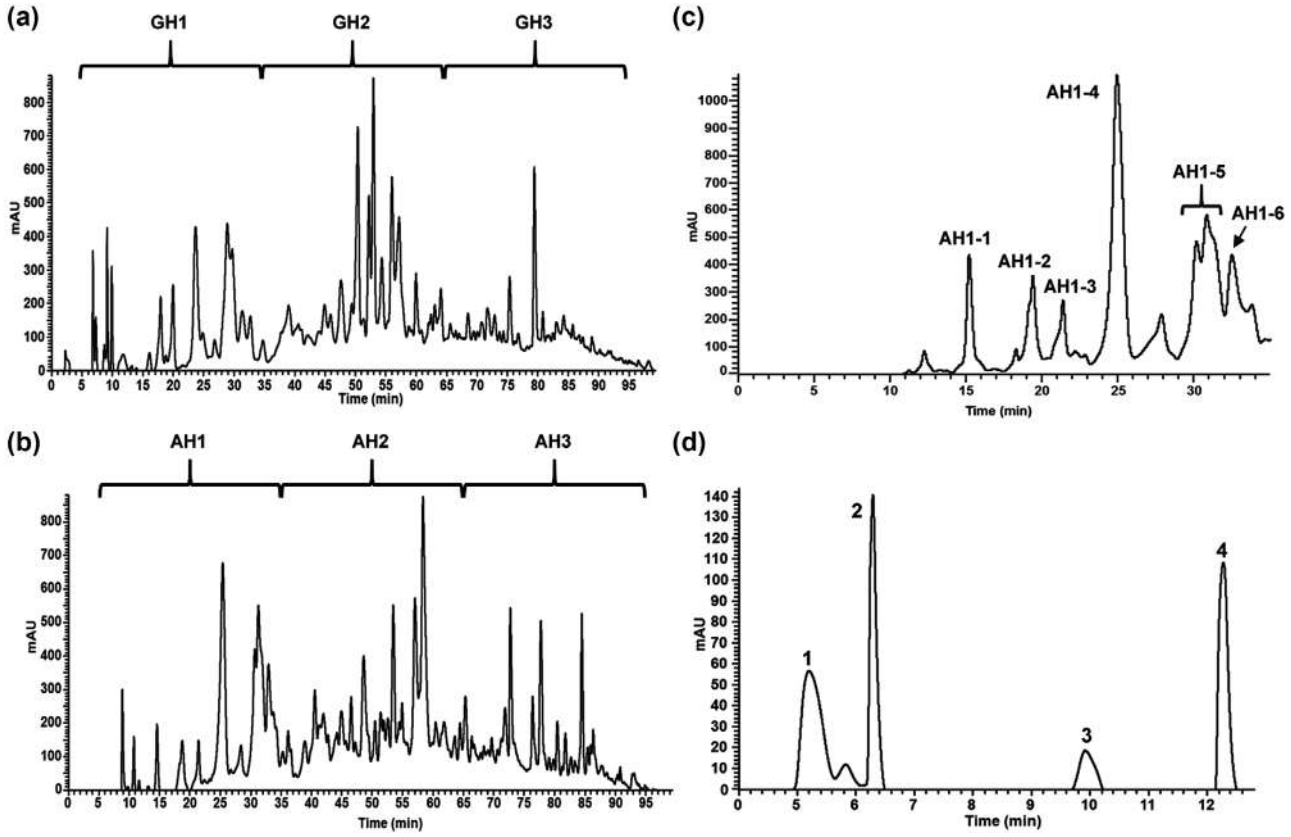

FIGURE 2 Chromatographic separation of alcalase hydrolyzates and peptide fractions from chickpea ICC3761. Globulins hydrolyzate (a), Albumins hydrolyzate (b), Fraction AH1 (c) and Subfraction AH1-5 (d)

29.38 g/100 g (ICC3421) in kabuli genotypes and from 12.41 (ICC3512) to 18.66 g/100 g (ICC13124) in desi chickpeas; these values corresponded with those reported by Singh et al. (2008) (11.63–24.23 g/100 g) in chickpeas from India.

3.2 | Production of albumin and globulin hydrolyzates

Alcalase is an inexpensive endopeptidase with a broad catalytic activity and has been widely used to produce protein

TABLE 3 In vitro antioxidant (AA) and antidiabetic potential of peptide fractions purified from alcalase hydrolyzates (AH) of the ICC3761 chickpea proteins

Fraction	ABTS[y]	DPPH[y]	α-Amylase[z]	α-Glucosidase[z]
GH1	20.0 ± 1.1^{c} (272.0 ± 37.8^{c})	31.0 ± 1.8^{b} (18.8 ± 2.1^{c})	7.9 ± 0.5^{b}	21.3 ± 1.5^{c}
GH2	13.2 ± 1.0^{e} (61.5 ± 8.2^{e})	26.9 ± 1.8^{c} (13.8 ± 2.2^{e})	15.2 ± 1.6^{a}	23.0 ± 2.1^{c}
GH3	16.1 ± 0.9^{d} (140.8 ± 28.8^{d})	27.1 ± 2.0^{c} (15.4 ± 1.0 ^{cd})	14.1 ± 0.7^{a}	34.7 ± 1.2^{b}
AH1	34.8 ± 0.6^{a} (767.1 ± 21.7^{a})	48.9 ± 0.2^{a} (40.2 ± 1.3^{a})	13.7 ± 1.1^{a}	50.5 ± 1.9^{a}
AH2	26.6 ± 1.1^{b} (490.9 ± 34.1^{b})	45.4 ± 0.4^{a} (36.0 ± 1.4^{b})	14.9 ± 1.2^{a}	32.2 ± 1.7^{b}
AH3	28.5 ± 0.6^{b} (556.5 ± 20.6^{b})	48.0 ± 0.4^{a} (39.2 ± 0.5^{ab})	10.5 ± 1.1^{b}	33.9 ± 1.7^{b}
AH1-1	66.6 ± 1.7^{c} (1981.6 ± 63.3^{c})	72.5 ± 1.2^{a} (44.5 ± 1.2^{a})	44.0 ± 1.1^{c}	47.4 ± 4.1^{b}
AH1-2	71.2 ± 1.6^{b} (2154.2 ± 60.4^{b})	70.0 ± 0.8^{ab} (42.0 ± 0.9^{ab})	37.3 ± 1.0^{d}	45.6 ± 1.7^{b}
AH1-3	73.1 ± 1.7^{b} (2223.4 ± 65.3^{b})	71.8 ± 1.2^{ab} (43.9 ± 0.9^{ab})	57.7 ± 3.1^{b}	60.5 ± 0.4^{a}
AH1-4	45.5 ± 2.0^{e} (1200.8 ± 76.5^{e})	69.3 ± 0.9^{b} (41.4 ± 1.0^{b})	34.9 ± 1.1^{d}	45.9 ± 1.7^{b}
AH1-5	78.8 ± 0.9^{a} (2433.2 ± 36.3^{a})	69.4 ± 1.5^{b} (41.4 ± 1.1^{b})	66.6 ± 1.5^{a}	57.0 ± 1.5^{a}
AH1-6	61.6 ± 1.7^{d} (1797.9 ± 66.4^{d})	69.9 ± 0.6^{ab} (42.6 ± 1.5^{ab})	63.3 ± 1.1^{a}	49.4 ± 1.9^{b}

The results are the mean ± SD of three replicates and they are expressed on a dry weight basis. [y]The antioxidant activity was evaluated at 0.2 mg of protein/ml and reported as percent inhibition and Trolox equivalents (TE)/100 g of protein (values in parentheses). [z]The samples were evaluated at 0.1 mg of protein/ml and the values are reported as percent inhibition in relation to acarbose (1 mmol/L). Different letters in the same column for fractions (lowercase) or subfractions (uppercase) indicate significant differences ($p < 0.05$) between the means according to the Tukey test.

hydrolyzates and bioactive peptides in legumes, including chickpea (Ghribi et al., 2015; Kou et al., 2013; Xu et al., 2020). The hydrolysis curves of the globulin and albumin protein extracts treated with alcalase showed a high rate of hydrolysis during the first 30–40 min (Figure S1). After 90 min, the DH of the chickpea genotypes varied significantly ($p < 0.05$) from 40.2% to 45.1% in the globulin extracts and from 32.1% to 41.4% in the albumin extracts (Table 1). These values correspond with those reported previously in chickpea protein extracts treated with alcalase (Kou et al., 2013; Xu et al., 2020).

The protein content of the hydrolyzates ranged from 89.7 to 97.9 g/100 g for the globulin fractions and from 75.6 to 96.8 g/100 g for albumins (Table 1). These values were slightly higher than that reported by Ghribi et al. (2015) (83.8%) and suggested that the hydrolyzates were suitable for the characterization of peptides with antioxidant and antidiabetic potential.

3.3 | In vitro AA of albumin and globulin hydrolyzates

In general, the AH showed higher AA (ABTS and DPPH) than the GH in both kabuli and desi genotypes (Table 2). This may be associated with the higher number of peptides and amino acids with potential AA identified in the albumin hydrolyzates compared to the globulin hydrolyzates: albumins (303 peptides; Phe = 78, His = 24, Trp = 12, Pro = 97) and globulins (170 peptides; Phe = 42, His = 20, Trp = 10, Pro = 58) (Table S1). The ABTS values for AH ranged from 197.62 (Blanoro) to 877.50 μmol ET/L (Suprema 03) in kabuli genotypes and from 459.57 (ICC4418) to 799.4 μmol ET/L (ICC3512) in desi types. In the case of the GH, the chickpea genotypes with the highest AA by ABTS were Progreso 95 (488.95 μmol ET/L) and ICC3761 (518.69 μmol ET/L), while the genotypes with the lowest values were Blanoro

TABLE 4 Peptide profile, antioxidant activity (AA), and antidiabetic potential of AH1-5 subfractions

Peak	pM (Da)[1]	Peptide	pI	Hydrophobicity	Net charge	Possible Activity	ABTS[x]	DPPH[x]	α-Amylase[y]	α-Glucosidase[y]	DPP4[z]
1	407.2038 (2.93)	FEI	3.2	8.30	− 1	Antihypertensive Antidiabetic	78.25 ± 3.7[a]	64.63 ± 5.7[b]	39.96 ± 0.3[d] (27.70 ± 0.5[c])	53.56 ± 0.7[b] (16.41 ± 0.4[a])	4.20 ± 0.47[a]
	407.2027 (5.63)	FEL	3.2	8.57	− 1	Antioxidant					
	407.2030 (4.89)	FIE	3.0	8.70	− 1	Antihypertensive					
2	407.2151 (2.93)	FKN	9.9	9.84	1	–	40.09 ± 2.4[c]	63.78 ± 0.4[b]	46.84 ± 1.0[b] (31.81 ± 1.2[b])	43.29 ± 0.4[c] (13.27 ± 0.4[b])	12.08 ± 0.23[c]
3	407.2155 (1.95)	FGKG	9.9	11.29	1	Antihypertensive Antidiabetic	37.39 ± 2.5[c]	73.65 ± 1.0[a]	54.12 ± 0.5[a] (37.01 ± 0.9[a])	56.01 ± 0.5[a] (17.16 ± 0.4[a])	7.29 ± 0.55[b]
4	407.13.57 (2.20)	MEE	2.9	14.49	− 2	Antihypertensive Antidiabetic	48.02 ± 1.6[b]	74.14 ± 2.9[a]	43.44 ± 0.7[c] (29.60 ± 1.0[bc])	41.97 ± 1.5[c] (12.87 ± 0.9[b])	7.65 ± 0.17[b]

[1]The values in parentheses indicate the mass error (ppm). The isoelectric point (pI), hydrophobicity (kcal/mol), and net charge of the peptides were obtained from PepDraw (http://www.tulane.edu/~biochem/WW/PepDraw/). The possible activities were obtained from BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/en/biopep) and attributed to the complete peptide or part of it. Amino acids are shown in one letter nomenclature. The activity values are the mean ± SD of three replicates. [x]The antioxidant activity was evaluated at 0.2 mg of protein/ml and reported as percent inhibition. [y]The samples were evaluated at 0.1 mg of protein/ml (0.24 mmol/L) and the values are reported as percent inhibition in relation to acarbose (1 mmol/L) and percent inhibition (values in parenthesis). [z]The values are reported as IC_{50} (μg/ml).

(369.54 μmol ET/L) and ICC13124 (395.29 μmol ET/L). The values obtained were similar to those reported by Esfandi et al. (2019) in oat alcalase hydrolyzates (320 to 450 μmol ET/L) and by Garcia-Mora et al. (2016) in alcalase hydrolyzates from pinto bean albumins (240–410 μmol ET/L).

The AA by DPPH was also higher in AH (22.99%–64.46%) than GH (23.07%–28.85%) (Table 2). For AH, the genotypes with the highest AA were Suprema 03 (64.46%) and ICC3512 (54.21%), whereas for GH the most outstanding kabuli genotype was progreso 95 (28.85%) and there were no significant differences ($p < 0.05$) among desi chickpeas (average = 26.73%). The DPPH values in this study corresponded to those reported in chickpea alcalase hydrolyzates (25%–45%) (Ghribi et al., 2015; Xu et al., 2020).

Principal component analysis (PCA) considering protein content, degree of hydrolysis, and AA showed that the best chickpea genotypes were located in the upper right quadrant: ICC3761, ICC14872, ICC6306, Bco. Sin. 92, Jumbo 2010, and Hoga 340 (Figure 1). ICC3761 was selected for further analysis.

3.4 | In vitro antioxidant and antidiabetic potential of protein fractions purified from the ICC3761 genotype

The chromatographic separation of GH (Figure 2a) and AH (Figure 2b) from ICC3761 showed several peaks that contained more than one peptide; the profiles were reproducible between the replicate samples. The AA and antidiabetic potential of the fractions (GH1, GH2, and GH3; AH1, AH2, and AH3) (Table 3) showed the best values for AH1 (ABTS, 34.8%; DPPH, 48.9%; α-amylase, 13.7%; and α-glucosidase, 50.5%) (Table 3). The AA values are similar to those reported by Kou et al. (2013) and Torres-Fuentes et al. (2015) in fractions of chickpea hydrolyzates. The α-amylase inhibition value corresponded to those reported by Chandrasekaran et al. (2020) in pepsin-pancreatin and bromelain chickpea hydrolyzates (11.0%–38.4%); however, those values were obtained using a much higher concentration of hydrolyzates (10 mg/ml).

AH1 was separated into six subfractions (AH1-1–AH1-6) (Figure 2c). AH1-5 had the best AA (ABTS, 78.8%; DPPH, 69.4%) and antidiabetic potential (α-amylase, 66.6%; α-glucosidase, 57.0%) (Table 3) and was selected for the purification and characterization of bioactive peptides.

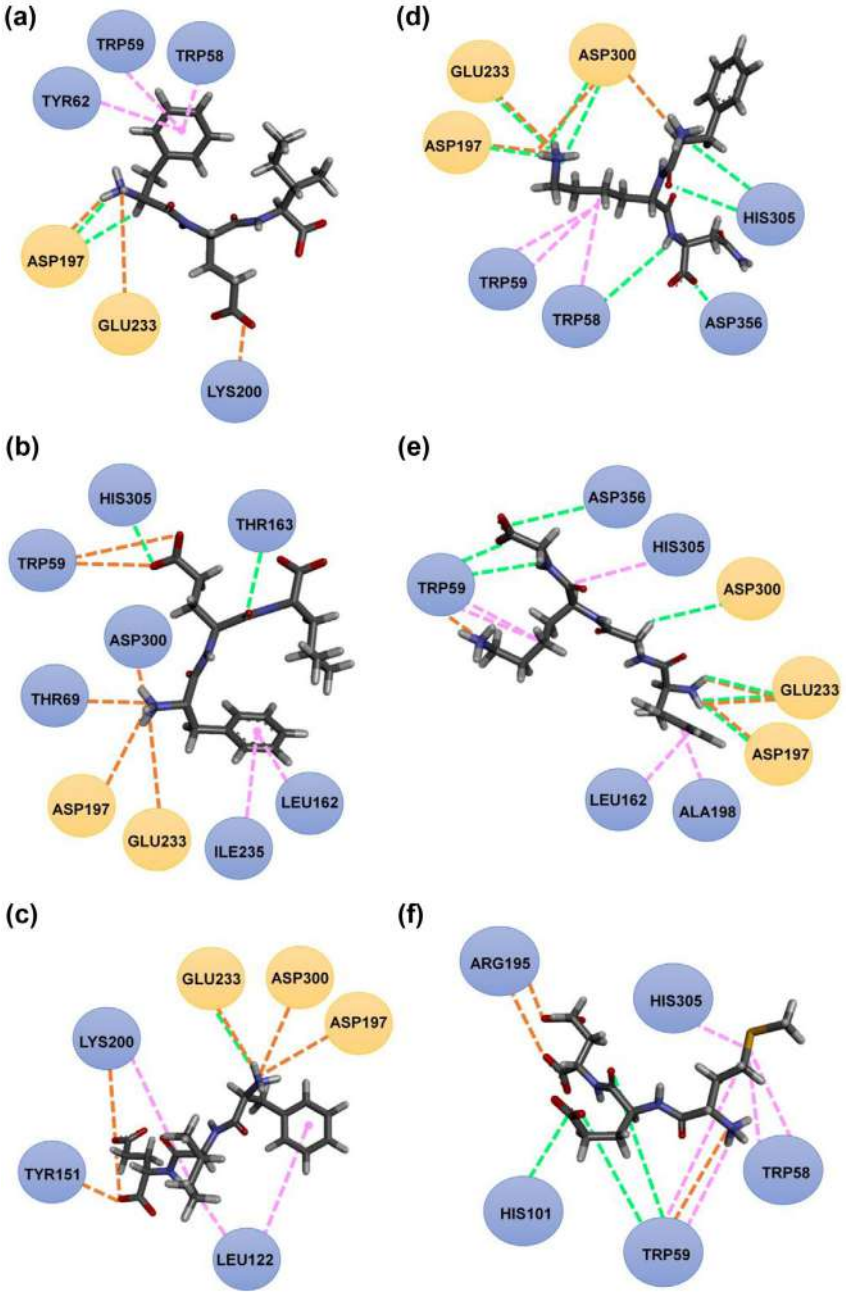

FIGURE 3 Molecular docking of the purified peptides with α-amylase. FEI (a), FEL (b), FIE (c), FKN (d), FGKG (e), and MEE (f). Type of interactions: Electrostatic (orange dashed lines), hydrogen bridge (green dashed lines), and hydrophobic (pink dashed lines)

3.5 | Peptide profiles, AA, and antidiabetic potential of purified subfractions from AH1-5

RP-UPLC-DAD analysis of AH1-5 showed four peaks (Figure 2d) whose peptides were identified by mass spectrometry (Table 4; Figures S2–S7); the elution order of the peptides corresponded with their hydrophobicity values. Only peak 1 was a mixture of three peptides with similar compositions (FEI, FEL, FIE). In silico analysis (BIOPEP platform) of the AH1-5 peptides suggested their activities: ROS scavenging and inhibition of enzymes of carbohydrate metabolism and blood pressure. Therefore, the AA and antidiabetic potential were evaluated in the purified peptides.

The AA by ABTS of the peptides purified from AH1-5 was evaluated at 0.2 mg/ml and ranged from 37.39% to 78.25% (Table 4); these values were higher than those reported by Zhang et al. (2019) for four synthetic peptides designed from sequences found in soy hydrolyzates (54.1%–70.8%; evaluated at 1 mg/ml). In the AA by ABTS, peptides with negative charge showed the highest values (Table 4). Another important parameter was the presence of aromatic amino acids (F) since the benzene ring acts as a free radical stabilizer by electron donation (Sánchez & Vázquez, 2017). The presence of leucine at the C-terminus

TABLE 5 Total energy, interaction energy, and total number of interactions obtained by molecular docking of the purified peptides with the enzymes α-amylase, α-glucosidase, and DPP4

Peptide	Global energy (kcal/mol)	Binding energy (kcal/mol)	Total Interactions	Hydrogen bonds
α-Amylase				
FEI	− 908.83	− 7.32	7	2
FEL	− 904.63	− 5.30	10	2
FIE	− 909.87	− 6.62	8	1
FKN	− 904.43	− 9.37	12	8
FGKG	− 907.14	− 10.04	13	7
MEE	− 900.88	− 7.84	11	4
α-Glucosidase				
FEI	− 1547.59	− 7.18	3	1
FEL	− 1531.95	− 6.48	4	1
FIE	− 1550.09	− 7.00	4	1
FKN	− 1529.74	− 6.33	8	5
FGKG	− 1545.99	− 7.06	8	5
MEE	− 1543.58	− 6.32	9	3
DPP4				
FEI	− 1400.48	− 8.31	8	2
FEL	− 1398.89	− 12.70	9	5
FIE	− 1395.33	− 13.05	10	1
FKN	− 1336.11	− 8.19	6	4
FGKG	− 1408.46	− 11.69	14	6
MEE	− 1394.64	− 11.30	11	9

has also been reported to increase the AA (Chen et al., 1996). Thus, this combination of factors explains the highest ABTS values for peptides in peak 1 (FEI, FEL, and FIE).

The highest AA by DPPH was observed for the peptides with the highest hydrophobicity (MEE, 74.14%; FGKG, 73.65%) (Table 4). This pattern corresponds with the results of Wang et al. (2015) who evaluated the AA of nine peptides from duck meat hydrolyzates and observed that peptides with more than 50% hydrophobic amino acids showed high DPPH inhibition values (54%–93%), while peptides with 29% hydrophobic amino acids showed low values (16%). The DPPH values of the present study were higher than those reported by Zhang et al. (2019) for four synthetic peptides derived from soybean hydrolyzates (40%–47% at 1 kg/L); these synthetic peptides showed lower hydrophobicity values than those in the AH1-5 subfractions (Table 4).

The purified peptides were evaluated for their antidiabetic potential: inhibition of α-amylase, α-glucosidase, and DPP4 (Table 4). The inhibition of these enzymes is considered an effective strategy in the control of type 2 diabetes mellitus. The α-amylase inhibition varied from 39.96% (FEI, FEL, FIE) to 54.12% (FGKG). These values were similar to those reported by Oseguera-Toledo et al. (2015) in protein hydrolyzates (>1 kDa) obtained with alcalase-bromelain from pinto bean Durango and black bean 8025 (30-50%). The inhibition values of α-glucosidase varied from 41.97% (MEE) to 56.01% (FGKG) and were lower than those found in peptide fractions (>1 kDa) from pinto (75%) and black beans (70%) (Oseguera-Toledo et al., 2015). The purified peptides were more efficient in the inhibition of these enzymes than other nutraceuticals such as the phenolics quercetin (α-amylase, 16.6% at 0.66 mmol/L) (Jaishree & Narsimha, 2020) and ferulic acid (α-amylase, 14.6%; α-glucosidase, 17% at 0.51 mmol/L) (Zheng et al., 2020).

The peptides inhibited DPP4 (Table 4) with IC_{50} values (4.2–12.08 μg/ml) close to those reported for commercial inhibitors such as Diprotin A (ILE-PRO-ILE, IC_{50} = 1.1 μg/ml) and Diprotin B (VAL-PRO-ILE, IC_{50} = 5.5 μg/ml) (Umezawa et al., 1984), and lower than that reported for the commercial drug Sitagliptin ($C_{16}H_{15}F_6N_5O$, IC_{50} = 54.3 μg/ml) (Chandrasekaran et al., 2020). The peak 1 peptides (FEI, FEL, and FIE) were the best inhibitors of DPP4 (IC_{50} = 4.20 μg/ml).

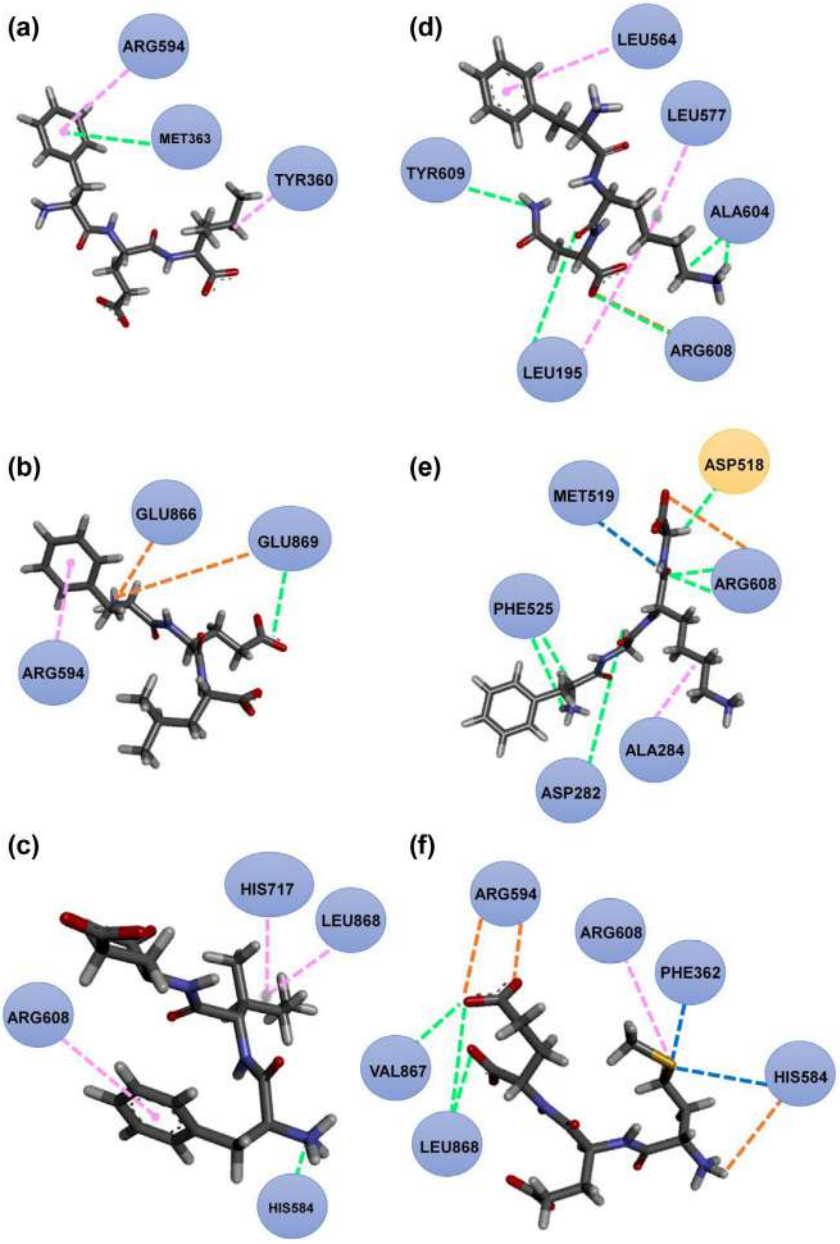

FIGURE 4 Molecular docking of the purified peptides with α-glucosidase. FEI (a), FEL (b), FIE (c), FKN (d), FGKG (e), and MEE (f). Type of interactions: Electrostatic (orange dashed lines), hydrogen bridge (green dashed lines), hydrophobic (pink dashed lines), and sulfur-X (blue dashed lines)

3.6 | Molecular docking between the peptides and α-amylase, α-glucosidase, and DPP4

The α-amylase contains three domains (A, B, C) and a catalytic triad in domain A (ASP^{197}, GLU^{233}, ASP^{300}) (Brayer et al., 1995). The specific activity of α-amylase is reduced up to one million times when replacing/changing/blocking ASP^{197}, whereas the substitution of GLU^{233} and ASP^{300} decreases the enzyme efficiency a thousand times (Brayer et al., 2000). Molecular docking analysis (Figure 3) indicated that all purified peptides interact with ASP^{197} of α-amylase, except for MEE that did not interact with any residue of the catalytic site; however, MEE interacted with other amino acids of the active site such as ARG^{195}, TRP^{59}, HIS^{101}, and HIS^{305}. FGKG and FKN interacted with the three catalytic amino acids, suggesting they act through competitive inhibition. The differences in enzyme inhibition could be due to interactions with other amino acids and the stability of the interactions. FGKG interacted with TRP^{59}, LEU^{162}, ALA^{198}, and HIS^{305}, while FKN interacted with TRP^{58}, TRP^{59}, and HIS^{305}; these amino acids have been shown to interact with acarbose and bean protein inhibitors (Nahoum et al., 2000). In addition, the hydrophobic interactions LEU^{162}-PHE^{1} and ALA^{198}-PHE^{1} for FGKG appear to contribute significantly to stabilize the inhibitor-enzyme complex, since FGKG showed the highest percent inhibition and the lowest binding energy (−10.047 kcal/mol) (London et al., 2011). The peptides FEI, FEL, and FIE showed 7, 10, and 8 interactions,

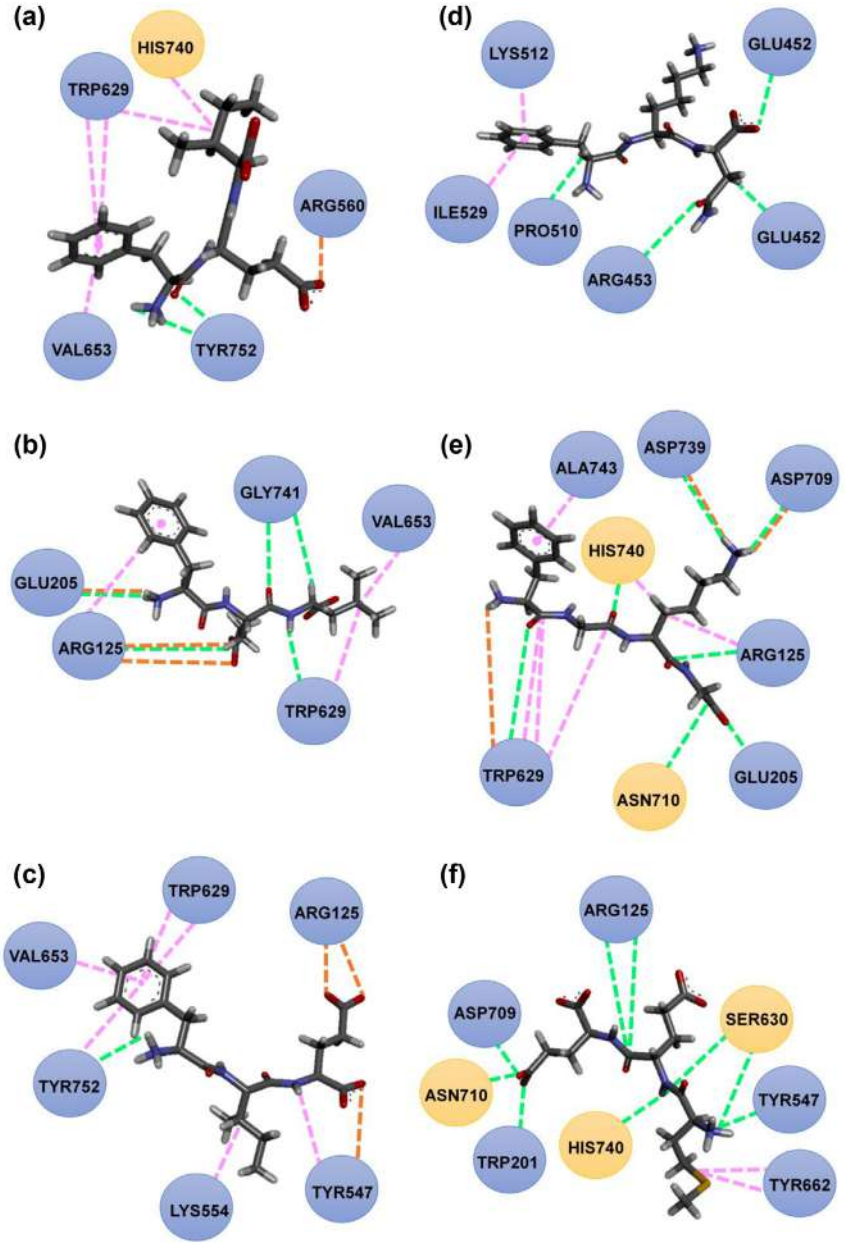

FIGURE 5 Molecular docking of the purified peptides with DPP4. FEI (a), FEL (b), FIE (c), FKN (d), FGKG (e), and MEE (f). Type of interactions: Electrostatic (orange dashed lines), hydrogen bridge (green dashed lines), hydrophobic (pink dashed lines), and sulfur-X (blue dashed lines)

respectively; the main interactions were electrostatic, followed by hydrophobic and hydrogen bridges. These tripeptides showed the highest binding energy values (Table 5) and the lowest α-amylase inhibition percentages (Table 4).

The active site of α-glucosidase is mainly formed by acid (ASP^{518}, GLU^{521}, and ASP^{616}) and basic residues (ARG^{600} and HIS^{674}); ASP^{518} acts as a nucleophile, while GLU^{521} and ASP^{616} stabilize the transition state by acid/base catalysis in conjunction with ARG^{600} and HIS^{674} (Hermans et al., 1991; Roig-Zamboni et al., 2017; Ur Rehman et al., 2019). Only FGKG showed interactions with amino acids of the α-glucosidase catalytic site (Figure 4), forming unconventional hydrogen bonding between ASP^{518} and GLY^{4} (O—H) and between ARG^{600} and LYS^{3} (O—H). ARG^{600} is essential for α-glucosidase activity (Ur Rehman et al., 2019). The binding energy of the peptides with α-glucosidase (Table 5) was lower than that reported between acarbose and α-glucosidase (−4.42 kcal/mol) (Aispuro-Pérez et al., 2019).

DPP4 has two binding sites S1 and S2. Site 1 (S1) consists of hydrophobic amino acids (TYR^{547}, TYR^{631}, VAL^{656}, TRP^{659}, TYR^{662}, TYR^{666}, and VAL^{711}) that allow the interaction of uncharged amino acids with the catalytic triad (SER^{630}, ASP^{708}/ASN^{710}, HIS^{740}). Site 2 (S2) involves key interactions with GLU^{205}, GLU^{206}, and ARG^{125}, and is made up of a long hydrophobic chain and an aromatic chain that increases the enzyme-substrate affinity. DPP4 also has a large cavity known as subsite S2' that is surrounded by VAL^{207}, SER^{209}, ARG^{358}, and PHE^{357} (De et al., 2019). Molecular docking analysis suggested all

peptides are competitive inhibitors of DPP4, except for FKN that did not interact with any amino acid in the active site (Figure 5). The binding energies of the interactions between DPP4 and the peptides were in the following order: FIE < FEL < FGKG < MEE < FEI < FKN (Table 5), which corresponded to the DPP4 inhibition values, except for the FEI peptide (Table 4). The binding energy values were lower than those reported for chickpea peptides generated with pepsin-pancreatin (−5.0 to −8.2 kcal/mol) and bromelain (−5.1 to −7.3 kcal/mol) (Chandrasekaran et al., 2020), indicating a high affinity between the purified peptides and DPP4. FEI showed hydrophobic interactions pi-alkyl type with the catalytic amino acid HIS^{740}, and with TRP^{629} and VAL^{653}, as well as pi–pi stacking type with TRP^{629}. PHE^{1} in the peptide formed hydrogen bonds with TYR^{752} and GLU^{2} and an electrostatic interaction with ARG^{560}. On the other hand, FEL interacted with amino acids from DPP4 S2 (ARG^{125} and GLU^{205}), forming saline bridges between the carboxyl group of GLU^{2} and the guanidium group of ARG^{125}. This linkage was also observed between GLU^{3} of FIE and ARG^{125} of DPP4. ARG^{125} is responsible for the binding of the inhibitor to the active site (Hiramatsu et al., 2004). FKN formed only six interactions with DPP4, four hydrogen bonds (ARG^{453}, PRO^{510}, and two with GLU^{452}) and two hydrophobic pi-alkyl type (LYS^{512} and ILE^{529}). FGKG showed the highest number of interactions (14), including those with catalytic amino acids such as ASN^{710} (hydrogen bridge) and HIS^{740} (hydrophobic), as well as with S2 amino acids such as ARG^{125} (hydrophobic) and GLU^{205} (hydrogen bridges). GLU^{205} is essential for DPP4 to recognize the proline residue in the N-terminus of the substrate (Abbott et al., 1999). Only MEE formed bonds with the three amino acids of the DPP4 catalytic triad (SER^{630}, ASN^{710}, and HIS^{740}), showing hydrogen bonds with MET^{1}, GLU^{3}, and MET^{1}, respectively. MEE also formed hydrogen bonds with the amino acid ARG^{125} of S2 and hydrophobic interactions with the phenol ring of TYR^{662} of S1. The interactions between the purified peptides and DPP4, except for FKN, have been reported for commercial inhibitors such as Diprotin A and Sitagliptin (Hiramatsu et al., 2004; Patel & Ghate, 2014). Thus, these chickpea peptides are good alternatives for commercial use and this is the first study reporting the inhibition of α-glucosidase and DPP4 by peptides purified from chickpea.

4 | CONCLUSION

The chickpea hydrolyzates showed wide variability in peptide content and AA, reflecting the great genetic diversity of the genotypes analyzed. The average AA of albumin hydrolyzates was higher than that of globulin hydrolyzates. The purified peptides (FEI, FEL, FIE, FKN, FGKG, and MEE) of the alcalase-hydrolyzates obtained from the albumin fraction of the ICC3761 genotype showed high radical scavenging (ABTS and DPPH) and inhibition of enzymes related to carbohydrate metabolism (α-amylase and α-glucosidase) and type 2 diabetes (DPP4). These results suggest the potential of chickpea hydrolyzates as nutraceutical supplements to prevent/treat diseases related to oxidative stress and diabetes.

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AUTHOR CONTRIBUTIONS

María F. Quintero-Soto collected most of the data, interpreted the results and drafted the manuscript. Jeanett Chávez-Ontiveros performed the antioxidant assays. José A. Garzón-Tiznado coordinated the field work, provided resources and reviewed the manuscript. Nancy Y. Salazar-Salas performed the UPLC-DAD-MS analyses. Karen V. Pineda-Hidalgo analyzed the data. Francisco Delgado-Vargas reviewed and edited the manuscript. José A. Lopez-Valenzuela designed and supervised the study, reviewed and edited the manuscript.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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REFERENCES

Abbott, C. A., McCaughan, G. W., & Gorrell, M. D. (1999). Two highly conserved glutamic acid residues in the predicted B propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. *FEBS Letters*, *458*(3), 278–284. https://doi.org/10.1016/S0014-5793(99)01166-7

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*. Elsevier Applied Science Publishers.

Aispuro-Pérez, A., López-Ávalos, J., García-Páez, F., Montes-Avila, J., Picos Corrales, L., Ochoa-Terán, A., Bastidas, P., Montaño, S., Zamora, L., Osuna-Martínez, L., & Sarmiento Sanchez, J. I. (2019). Synthesis and molecular docking studies of imines as α-glucosidase and α-amylase inhibitors. *Bioorganic Chemistry*, *94*, 103491. https://doi.org/10.1016/j.bioorg.2019.103491

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brayer, G. D., Luo, Y., & Withers, S. G. (1995). The structure of human pancreatic alpha-amylase at 1.8 a resolution and comparisons with related enzymes. *Protein Science*, *4*(9), 1730–1742. https://doi.org/10.1002/pro.5560040908

Brayer, G. D., Sidhu, G., Maurus, R., Rydberg, E. H., Braun, C., Wang, Y., Nguyen, N. T., Overall, C. M., & Withers, S. G. (2000). Subsite mapping of the human pancreatic alpha-amylase active site through structural, kinetic, and mutagenesis techniques. *Biochemistry*, *39*(16), 4778–4791. https://doi.org/10.1021/bi9921182

Chandrasekaran, S., Luna-Vital, D., & de Mejia, E. G. (2020). Identification and comparison of peptides from chickpea protein hydrolysates using either bromelain or gastrointestinal enzymes and their relationship with markers of type 2 diabetes and bitterness. *Nutrients*, *12*(12), 3843. https://doi.org/10.3390/nu12123843

Chen, H.-M., Muramoto, K., Yamauchi, F., & Nokihara, K. (1996). Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *Journal of Agricultural and Food Chemistry*, *44*(9), 2619–2623. https://doi.org/10.1021/jf950833m

De Castro, R. J. S., & Sato, H. H. (2015). Biologically active peptides: Processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. *Food Research International*, *74*, 185–198. https://doi.org/10.1016/j.foodres.2015.05.013

De, S., Banerjee, S., Kumar, S. K. A., & Paira, P. (2019). Critical role of dipeptidyl peptidase IV: a therapeutic target for diabetes and cancer. *Mini Reviews in Medicinal Chemistry*, *19*(2), 88–97. https://doi.org/10.2174/1389557518666180423112154

Dhawan, K., Malhotra, S., Dahiya, B. S., & Singh, D. (1991). Seed protein fractions and amino acid composition in gram (*Cicer arietinum*). *Plant Foods for Human Nutrition*, *41*(3), 225–232. https://doi.org/10.1007/bf02196390

Dziuba, J., Szerszunowicz, I., Nałęcz, D., & Dziuba, M. (2014). Proteomic analysis of albumin and globulin fractions of pea (*Pisum sativum* L.) seeds. *Acta Scientiarum Polonorum Technologia Alimentaria*, *13*, 181–190. https://doi.org/10.17306/J.AFS.2014.2.7

Esfandi, R., Willmore, W. G., & Tsopmo, A. (2019). Peptidomic analysis of hydrolyzed oat bran proteins, and their in vitro antioxidant and metal chelating properties. *Food Chemistry*, *279*, 49–57. https://doi.org/10.1016/j.foodchem.2018.11.110

Garcia-Mora, P., Peñas, E., Frias, J., Zieliński, H., Wiczkowski, W., Zielińska, D., & Martínez-Villaluenga, C. (2016). High-pressure-assisted enzymatic release of peptides and phenolics increases angiotensin converting enzyme i inhibitory and antioxidant activities of pinto bean hydrolysates. *Journal of Agricultural and Food Chemistry*, *64*(8), 1730–1740. https://doi.org/10.1021/acs.jafc.5b06080

Ghribi, A. M., Sila, A., Przybylski, R., Nedjar-Arroume, N., Makhlouf, I., Blecker, C., Attia, H., Dhulster, P., Bougatef, A., & Besbes, S. (2015). Purification and identification of novel antioxidant peptides from enzymatic hydrolysate of chickpea (*Cicer arietinum* L.) protein concentrate. *Journal of Functional Foods*, *12*, 516–525. https://doi.org/10.1016/j.jff.2014.12.011

Hermans, M. M., Kroos, M. A., van Beeumen, J., Oostra, B. A., & Reuser, A. J. (1991). Human lysosomal alpha-glucosidase. Characterization of the catalytic site. *The Journal of Biological Chemistry*, *266*(21), 13507–13512. https://www.ncbi.nlm.nih.gov/pubmed/1856189

Hiramatsu, H., Yamamoto, A., Kyono, K., Higashiyama, Y., Fukushima, C., Shima, H., Sugiyama, S., Inaka, K., & Shimizu, R. (2004). The crystal structure of human dipeptidyl peptidase IV (DPPIV) complex with diprotin A. *Biological Chemistry*, *385*(6), 561–564. https://doi.org/10.1515/bc.2004.068

Jaishree, V., & Narsimha, S. (2020). Swertiamarin and quercetin combination ameliorates hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced type 2 diabetes mellitus in wistar rats. *Biomedicine & Pharmacotherapy*, *130*, 110561. https://doi.org/10.1016/j.biopha.2020.110561

Kou, X., Gao, J., Xue, Z., Zhang, Z., Wang, H., & Wang, X. (2013). Purification and identification of antioxidant peptides from chickpea (*Cicer arietinum* L.) albumin hydrolysates. *LWT - Food Science and Technology*, *50*(2), 591–598. https://doi.org/10.1016/j.lwt.2012.08.002

Ladizinsky, G. (1995). Chickpea. In J. Smartt & N. W. Simmonds (Eds.), *Evolution of crop plants* (pp. 258–261). Logman Scientific and Technical.

London, N., Raveh, B., Cohen, E., Fathi, G., & Schueler-Furman, O. (2011). Rosetta flexpepdock web server-high resolution modeling of peptide-protein interactions. *Nucleic Acids Research*, *39*(Web Server issue), W249-W253. https://doi.org/10.1093/nar/gkr431

Mejri, L., Vásquez-Villanueva, R., Hassouna, M., Marina, M. L., & García, M. C. (2017). Identification of peptides with antioxidant and antihypertensive capacities by RP-HPLC-Q-TOF-MS in dry fermented camel sausages inoculated with different starter cultures and ripening times. *Food Research International*, *100*, 708–716. https://doi.org/10.1016/j.foodres.2017.07.072

Milan-Noris, A. K., Gutierrez-Uribe, J. A., Santacruz, A., Serna-Saldivar, S. O., & Martinez-Villaluenga, C. (2018). Peptides and Isoflavones in gastrointestinal digests contribute to the anti-Inflammatory potential of cooked or germinated desi and kabuli chickpea (*Cicer arietinum* L.). *Food Chemistry*, *268*, 66–76. https://doi.org/10.1016/j.foodchem.2018.06.068

Mojica, L., Chen, K., & González de Mejía, E. (2015). Impact of commercial precooking of common bean (*Phaseolus vulgaris*) on the generation of peptides, after pepsin–pancreatin hydrolysis, capable to inhibit dipeptidyl peptidase-IV. *Journal of Food Science*, *80*(1), H188-H198. https://doi.org/10.1111/1750-3841.12726

Mojica, L., & Gonzalez de Mejía, E. (2016). Optimization of enzymatic production of anti-diabetic peptides from black bean (*Phaseolus vulgaris* L.) proteins, their characterization and biological potential. *Food & Function*, *7*(2), 713–727. https://doi.org/10.1039/c5fo01204j

Mojica, L., Gonzalez de Mejia, E., Granados-Silvestre, M. Á., & Menjivar, M. (2017). Evaluation of the hypoglycemic potential of a black bean hydrolyzed protein isolate and its pure peptides using in silico, in vitro and in vivo approaches. *Journal of Functional Foods*, *31*, 274–286. https://doi.org/10.1016/j.jff.2017.02.006

Nahoum, V., Roux, G., Anton, V., Rouge, P., Puigserver, A., Bischoff, H., Henrissat, B., & Payan, F. (2000). Crystal structures of human pancreatic alpha-amylase in complex with carbohydrate and proteinaceous inhibitors. *Biochemical Journal*, *346*(1), 201–208. https://doi.org/10.1042/bj3460201

Oseguera-Toledo, M. E., Gonzalez de Mejia, E., & Amaya-Llano, S. L. (2015). Hard-to-cook bean (*Phaseolus vulgaris* L.) proteins

hydrolyzed by alcalase and bromelain produced bioactive peptide fractions that inhibit targets of type-2 diabetes and oxidative stress. *Food Research International*, *76*(Pt 3), 839–851. https://doi.org/10.1016/j.foodres.2015.07.046

Patel, B. D., & Ghate, M. D. (2014). Recent approaches to medicinal chemistry and therapeutic potential of dipeptidyl peptidase-4 (DPP-4) inhibitors. *European Journal of Medicinal Chemistry*, *74*, 574–605. https://doi.org/10.1016/j.ejmech.2013.12.038

Quintero-Soto, M. F., Saracho-Pena, A. G., Chavez-Ontiveros, J., Garzon-Tiznado, J. A., Pineda-Hidalgo, K. V., Delgado-Vargas, F., & Lopez-Valenzuela, J. A. (2018). Phenolic profiles and their contribution to the antioxidant activity of selected chickpea genotypes from Mexico and ICRISAT collections. *Plant Foods for Human Nutrition*, *73*(2), 122–129. https://doi.org/10.1007/s11130-018-0661-6

Raveh, B., London, N., & Schueler-Furman, O. (2010). Sub-angstrom modeling of complexes between flexible peptides and globular proteins. *Proteins: Structure, Function, and Bioinformatics*, *78*(9), 2029–2040. https://doi.org/10.1002/prot.22716

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Roig-Zamboni, V., Cobucci-Ponzano, B., Iacono, R., Ferrara, M. C., Germany, S., Bourne, Y., Parenti, G., Moracci, M., & Sulzenbacher, G. (2017). Structure of human lysosomal acid alpha-glucosidase - a guide for the treatment of pompe disease. *Nature Communications*, *8*(1), 1111. https://doi.org/10.1038/s41467-017-01263-3

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety*, *1*(1), 29–46. https://doi.org/10.1093/fqsafe/fyx006

Singh, G. D., Wani, A. A., Kaur, D., & Sogi, D. S. (2008). Characterisation and functional properties of proteins of some Indian chickpea (*Cicer arietinum*) cultivars. *Journal of the Science of Food and Agriculture*, *88*(5), 778–786. https://doi.org/10.1002/jsfa.3144

Singh, U., & Jambunathan, R. (1982). Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). *Plant Foods for Human Nutrition*, *31*(4), 347–354. https://doi.org/10.1007/BF01094046

Torres-Fuentes, C., Contreras, M. D. M., Recio, I., Alaiz, M., & Vioque, J. (2015). Identification and characterization of antioxidant peptides from chickpea protein hydrolysates. *Food Chemistry*, *180*, 194–202. https://doi.org/10.1016/j.foodchem.2015.02.046

Tovchigrechko, A., & Vakser, I. A. (2005). Development and testing of an automated approach to protein docking. *Proteins: Structure, Function, and Bioinformatics*, *60*(2), 296–301. https://doi.org/10.1002/prot.20573

Tovchigrechko, A., & Vakser, I. A. (2006). GRAMM-X public web server for protein–protein docking. *Nucleic Acids Research*, *34*(suppl_2), W310-W314. https://doi.org/10.1093/nar/gkl206

Umezawa, H., Aoyagi, T., Ogawa, K., Naganawa, H., Hamada, M., & Takeuchi, T. (1984). Diprotins A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria. *The Journal of Antibiotics*, *37*(4), 422–425. https://doi.org/10.7164/antibiotics.37.422

Ur Rehman, N., Rafiq, K., Khan, A., Ahsan Halim, S., Ali, L., Al-Saady, N., Hilal Al-Balushi, A., Al-Busaidi, H., & Al-Harrasi, A. (2019). α-Glucosidase inhibition and molecular docking studies of natural brominated metabolites from marine macro brown Alga *Dictyopteris hoytii*. *Marine Drugs*, *17*(12), 666. https://doi.org/10.3390/md17120666

Wang, L. S., Huang, J. C., Chen, Y. L., Huang, M., & Zhou, G. H. (2015). Identification and characterization of antioxidant peptides from enzymatic hydrolysates of duck meat. *Journal of Agricultural and Food Chemistry*, *63*(13), 3437–3444. https://doi.org/10.1021/jf506120w

Xu, Y., Galanopoulos, M., Sismour, E., Ren, S., Mersha, Z., Lynch, P., & Almutaimi, A. (2020). Effect of enzymatic hydrolysis using endo- and exo-proteases on secondary structure, functional, and antioxidant properties of chickpea protein hydrolysates. *Journal of Food Measurement and Characterization*, *14*, 343–352. https://doi.org/10.1007/s11694-019-00296-0

Xue, Z., Hou, X., Yu, W., Wen, H., Zhang, Q., Li, D., & Kou, X. (2018). Lipid metabolism potential and mechanism of CPe-III from chickpea (*Cicer arietinum* L.). *Food Research International*, *104*, 126–133. https://doi.org/10.1016/j.foodres.2017.03.016

Xue, Z., Wen, H., Zhai, L., Yu, Y., Li, Y., Yu, W., Cheng, A., Wang, C., & Kou, X. (2015). Antioxidant activity and anti-proliferative effect of a bioactive peptide from chickpea (*Cicer arietinum* L.). *Food Research International*, *77*, 75–81. https://doi.org/10.1016/j.foodres.2015.09.027

Zhang, Q., Tong, X., Li, Y., Wang, H., Wang, Z., Qi, B., Sui, X., & Jiang, L. (2019). Purification and characterization of antioxidant peptides from alcalase-hydrolyzed soybean (*Glycine max* L.) hydrolysate and their cytoprotective effects in human intestinal Caco-2 cells. *Journal of Agricultural and Food Chemistry*, *67*(20), 5772–5781. https://doi.org/10.1021/acs.jafc.9b01235

Zheng, Y., Tian, J., Yang, W., Chen, S., Liu, D., Fang, H., Zhang, H., & Ye, X. (2020). Inhibition mechanism of ferulic acid against alpha-amylase and alpha-glucosidase. *Food Chemistry*, *317*, 126346. https://doi.org/10.1016/j.foodchem.2020.126346

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