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In vitro inhibition of dipeptidyl peptidase IV by peptides derived from the hydrolysis of amaranth (*Amaranthus hypochondriacus* L.) proteins

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ABSTRACT

Bioactive compounds present in foods could potentially have beneficial effects on human health. In this study we report the *in vitro* inhibitory capacity of peptides released from amaranth seed proteins after enzymatic digestion, against dipeptidyl peptidase IV (DPPIV); an enzyme known to deactivate incretins, hormones involved in insulin secretion. Other seeds, such as soybean, black bean, and wheat were also tested. The highest inhibition of DPPIV was observed with amaranth peptides released after simulated gastrointestinal digestion, showing an IC_{50} of 1.1 mg/mL in a dose-dependent manner. *In silico* tryptic digestion of amaranth globulins was carried out releasing peptides larger than 13 residues. Some of these peptides were used for the *in silico* prediction of their binding modes with DPPIV. Docking models showed that the possible mechanism of globulin peptides to inhibit DPPIV was through blocking the active dimer formation. Peptides were also found inside the major cavity where the natural substrates reach the catalytic site of the enzyme. This is the first report of the identification of inhibitory DPPIV peptides from amaranth hydrolysates and the prediction of their binding modes at the molecular level, leading to their possible use as functional food ingredients in the prevention of diabetes.

1. Introduction

Type 2 diabetes mellitus is a metabolic disorder characterized by a hyperglycemic chronic state due to several fundamental defects such as insulin resistance in muscle and liver and an impaired insulin secretion by pancreatic β-cells (Campbell, 2009; DeFronzo, 2009; Surampudi, John-Kalarickal, & Fonseca, 2009). The World Health Organization (WHO, 2011) has estimated that there are 3.2 million deaths per year because of this disease. Risk factors include sedentary life style, hypercaloric food consumption, obesity, and genetic predisposition (Petrie, Pearson, & Sutherland, 2011).

Incretin-based therapy is actually used to lower the hyperglycemic state in the diabetic patient (Drucker, 2011). Incretins are peptidic hormones released by intestinal enteroendocrine cells to the bloodstream in response to nutrient intake where they stimulate insulin secretion (Nauck, 2011). They are responsible for over 50% of postprandial insulin secretion, especially the hormone glucagon-like peptide 1 (GLP-1) (Ahrén, 2011; Holst, Knop, VilsbØll, Krarup, & Madsbad, 2011; Kim & Egan, 2008). Incretin effect may be reduced in diabetic patients at a more chronic stage of the disease, probably due to the destruction of pancreatic islet (Nauck, 2011). Administration of these hormones has an insulinotropic and a β-cell proliferating response in the diabetic patient with no risk of hypoglycemia, a common side effect of classic antidiabetic drugs (Nauck, 2011; Nauck & Vardarli, 2010). However, the half-life of incretins is very short (<2 min) due to the cleavage and inactivation of these proteins by dipeptidyl peptidase IV (DPPIV). Hence, the use of DPPIV inhibitors increases the time of action of incretins (Conarello et al., 2003; Gerich, 2010; Nauck & Vardarli, 2010; Stephan et al., 2011). There are synthetic DPPIV inhibitory drugs that have shown promising results as antidiabetic agents (Huisamen, Genis, Marais, & Lochner, 2001; Keating, 2010; Kos et al., 2009; Seino, Fukushima, & Yabe, 2010; Wiedeman & Trevillayn, 2003). However, when the enzyme is inhibited there is a risk of presenting side effects, because DPPIV can also inactivate chemokines, neuropeptides and other peptidic hormones; leading to a wide range of physiological effects, aside from its insulintropic response, such as angioedema, pancreatitis and infective disorders (Matteucci & Giampietro, 2011; Mentlein, 1999; Yu et al., 2010).

On the other hand, there are naturally occurring active nutritional components in foods that promote a healthy life (Siró, Kápolna, Kápolna, & Lugasi, 2008). Proteins and peptides have a wide variety of biological activities that may benefit human health by acting like antioxidant, antihypertensive and antithrombotic agents, among

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others activities (Erdmann, Cheung, & Schröder, 2008; Samaranayaka & Li-Chan, 2011). Bioactive peptides are small sequences of amino acids encrypted in food proteins in an inactive form that are released and activated by proteolytic enzymes during food processing or gastrointestinal digestion (Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008). These peptides are present in food sources such as milk, meat, eggs, soybean, wheat, maize, rice, and amaranth (Erdmann et al., 2008; Gibbs, Zougman, Masse, & Mulligan, 2004; Möller et al., 2008; Silva-Sánchez, Barba de la Rosa, & León-Galván, 2008).

Amaranth is an American native plant historically cultivated by Mayans, Aztecs, and Incas. In the last two decades, amaranth has re-emerged as an alternative crop not only for its high nutritional value (high lysine and methionine content), but also due to its nutraceutical properties. Amaranth antihypertensive peptides (Barba de la Rosa et al., 2010) and the cancer-preventive lunasin-like peptide (Maldonado-Cervantes et al., 2010) have been characterized. Furthermore, the presence of DPPIV inhibitory peptides encrypted in amaranth seeds has been identified by LC/MS-MS and bioinformatic tools (Silva-Sánchez et al., 2008); however the inhibitory potential of these amaranth peptides against DPPIV has not been characterized. The aim of the present study was to release the bioactive peptides encrypted in amaranth seed storage proteins using *in vitro* tryptic and gastrointestinal digestions, and to evaluate their *in vitro* DPPIV inhibitory activity. Comparison of this activity was also made with peptides derived from other common seed proteins. Furthermore, docking modeling was carried out in order to predict the molecular mechanisms of interaction of amaranth peptides with DPPIV.

2. Materials and methods

2.1. Biological materials

Amaranthus hypochondriacus L. cv nutrisol obtained from INIFAP-Campus Bajío was used in this study. Soybean (*Glycine* max), black bean (*Phaseolus vulgaris*) and wheat (*Tritticum* spp.) seeds, as well as popped amaranth grain were obtained from local market at San Luis Potosi, Mexico.

2.2. Amaranth seed storage proteins extraction

Amaranth protein fractions were extracted according to Barba de la Rosa, Gueguen, Paredes-López, and Viroben (1992) as follows: amaranth hexane-defatted flour was resuspended in distilled water, stirred at 4 °C for 1 h, sonicated in ice for 30 min and then centrifuged at 13,000*g* for 30 min to obtain the albumin fraction in the supernatant. The pellet was resuspended in 0.1 M NaCl, 0.01 M KH_2PO_4, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5 buffer, stirred, sonicated and centrifuged as mentioned above to obtain the 7S globulin fraction. The new pellet was resuspended in 0.8 M NaCl, 0.01 M KH_2PO_4, 1 mM EDTA, pH 7.5 buffer, stirred, sonicated and centrifuged as mentioned above to obtain the 11S globulin fraction. The prolamin fraction was extracted using 70% ethanol as extraction solution and for the glutelin fraction 100 mM Tris pH 8.0 was used, both following the same procedure. The amount of protein was determined using the Bradford Protein-Assay kit (Bio-Rad, Hercules, CA, USA) and bovine serum albumin (BSA) as a standard. All fractions were stored at −20 °C until their analysis.

2.3. Tryptic digestion of amaranth seed storage proteins

Amaranth protein fractions were used for tryptic digestion analysis. Trypsin from porcine pancreas (Sigma–Aldrich, St Louis, MO, USA) was used at several trypsin to protein w/w ratios (1:50, 1:10, 1:5, 1:2.5, 1:1). Digestions were carried out in a 100 mM Tris buffer pH 8.0 incubated in a Thermomixer (Eppendorf, Hamburg, Germany) for 14 h at 37 °C, the reaction was stopped by freezing. SDS–PAGE was carried out according to Laemmli (1970) using 12% acrylamide gels stained with colloidal Coomassie Blue R-250. The enzyme was then removed by ultracentrifugation using a 10 kDa MWCO filters (Millipore, Billerica, MA, USA), the filtrates were collected and the protein concentration was determined by the Lowry-based DC Protein Assay (Bio-Rad) using BSA as a standard.

2.4. Gastrointestinal digestion simulation in vitro

A gastrointestinal digestion *in vitro* model was established as suggested by Wang, Bringe, Berhow, and Gonzalez de Mejia (2008) with slight modifications. Briefly, 1 g of sample (amaranth glutelin, defatted flour from raw amaranth, popped amaranth, black bean, soybean or wheat) was resuspended in 20 mL of 0.03 M NaCl pH 2.0. In order to inactivate bacteria and proteases, the suspensions were heated in a water bath at 80 °C for 5 min and allowed to cool down at room temperature. Porcine pepsin (Sigma–Aldrich) previously dissolved in 0.03 M NaCl pH 2.0 was added in a 1:20 ratio (w/w enzyme to substrate). The samples were digested at constant pH for 3 h at 37 °C. The pH was then adjusted to 7.5. A mixture of trypsin (Sigma–Aldrich) and pancreatin (Sigma–Aldrich) was prepared (1:1 w/w trypsin:pancreatin ratio in 0.1 N $NaHCO_3$), added to the digestive solution and incubated at constant pH for another 3 h (1:20 w/w enzyme to substrate ratio for both the enzymes, trypsin and pancreatin). Digestion was stopped by heating the suspensions at 75 °C for 20 min. The hydrolysates were centrifuged at 13,000*g* for 30 min; the protein concentration in the supernatant was determined by the Lowry-based DC Protein Assay (Bio-Rad) using BSA as a standard, and then stored at −20 °C until analysis next day.

2.5. DPPIV activity assay

DPPIV activity assay was carried out based on the manufacturer's protocol by Sigma–Aldrich (protocol SSGPNA01 Revised 08/26/99). The chromogenic substrate Gly-Pro-pNA, and dipeptidyl peptidase IV isolated from porcine kidney (Sigma–Aldrich) were used. Reactions were performed in 96 well plates using 100 ng/mL of enzyme, 500 μM substrate and increasing amounts of hydrolysates in a 100 mM Tris pH 8.0 reaction buffer for 1 h at 37 °C and the readings were taken at 415 nm in a microplate reader (Bio-Rad). Diprotin A, a protease inhibitor (Sigma–Aldrich) was used as a positive control of inhibition.

2.6. Characterization of encrypted peptides in amaranth glutelins fraction

Glutelins fraction was digested with trypsin and analyzed by LC–ESI/MS/MS in search of peptides with interesting activities as reported by Silva-Sánchez et al. (2008). This analysis was carried out on a SYNAPT-HDMS (Waters Corp., Milford, MA, USA) coupled with a nano-UPLC System (Supplementary information). The identification of bioactive peptides was conducted using the peptide database (http://www.uwm.edu.pl/biochemia).

2.7. Docking analysis of DPPIV and amaranth globulins tryptic peptides

Due to the fact that the crystal structure of amaranth glutelins is not known, we have used the information in databases about 11S amaranth globulins for prediction of the amaranth binding modes with DPPIV. Models of amaranth globulins peptides I and II (Table 1) were built using the I-TASSER server which employs a fragment-based method. Peptide fragments were obtained from

multiple template structures and reassembled, based on threading alignments (Roy, Kucukural, & Zhang, 2010). The best ranking model for peptide I and peptide II, as judged from the C-score value, were selected for docking simulations as described below. Peptide III and peptide IV (Table 1) were generated based on their homology to the 3D-11S globulin structure from amaranth and 3D-11S *Pisum sativum* globulin (Protein Data Bank, PDB codes: 3QAC and 3KSC, respectively) using the program COOT (Emsley, Lohkamp, Scott, & Cowtan, 2010). A rigid body docking analysis of the peptides and DPPIV enzyme (PDB code 1R9 M) was performed using ClusPro 2.0 (http://cluspro.bu.edu) (Comeau, Gatchell, Vajda, & Camacho, 2004). The most strongly interacting models were chosen based on the cluster size and used as an input on a high resolution docking protocol as implemented on the FlexPepDock server (London, Raveh, Cohen, Fathi, & Schueler-Furman, 2011). The CASTp database was used to identify and measure the surfaces accessible pockets of DPPIV (Liang & Woodward, 1998). Visual representation of the interactions in the final models was generated with the program CHIMERA (Pettersen et al., 2004). Binding energies were calculated using the program FoldX (Schymkowitz et al., 2005) and amino acid interactions information was obtained using the PDBsum program (Laskowski, 2009).

2.8. Statistical analysis

All experiments were performed in triplicate. Statistical analyses were performed by using the GraphPad Prism 5 software (GraphPad Software Inc., La Joya, CA, USA). Paired Student's *t* tests ($P < 0.05$), one-way ANOVA tests ($P < 0.0001$) and Tukey's tests ($P < 0.05$) were performed to detect statistical differences. All data are expressed as mean ± SD.

3. Results and discussion

3.1. Amaranth glutelins digestion

It is well known that glutelins constitute the main protein fraction in amaranth seeds (Barba de la Rosa et al., 1992). It has also been reported that this fraction contains peptides with a potential inhibitory activity against the DPPIV (Silva-Sánchez et al., 2008), but this activity has not been tested before. Glutelin tryptic digestions were conducted at different enzyme to protein ratios, as shown in Fig. 1. As trypsin concentrations were increased, high molecular bands disappeared while the peptides lower than 10 kDa increased until no bands were detected by Coomassie stain. High amounts of trypsin (1:1 enzyme to protein ratio) was needed in order to completely hydrolyze the glutelins, this may indicate a certain resistance to hydrolysis as reported for other amaranth proteins (Condés, Scilingo, & Añón, 2009).

3.2. Characterization of DPPIV inhibitory activity of tryptic amaranth peptides

Diprotin A (Ile-Pro-Ile), which had an IC_{50} (half of the maximal inhibitory concentration) of 17 μM was used as control (Fig. S1).

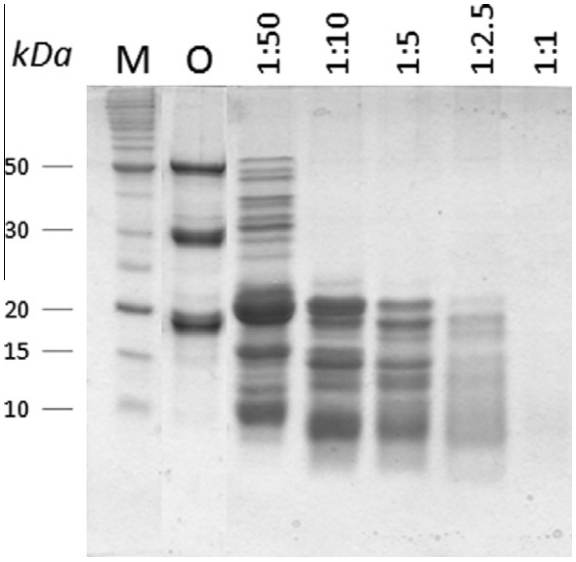

Fig. 1. SDS–PAGE gel of amaranth glutelins digested with trypsin from porcine pancreas. Glutelins (5 mg) were digested with different enzyme:protein ratios (1:50, 1:10, 1:5, 1:2.5, 1:1) in 100 mM Tris–HCl pH 8.0 for 14 h. Proteins were revealed using the colloidal Coomassie blue stain. M = molecular mass marker, O = native glutelins.

This value was similar to values reported in the literature (Wiedeman & Trevillayn, 2003). Fig. 2A shows that the inhibitory activity of amaranth glutelins hydrolysates against DPPIV decreased in a dose-dependent manner with an IC_{50} ranging from 1.2 to 2.0 mg/mL, depending on the enzyme to substrate ratio. Interestingly, when the fragments larger than 10 kDa were removed by ultrafiltration, the IC_{50} of the ultrafiltrated samples ranged from 1.0 to 1.6 mg/mL, indicating that peptides smaller than 10 kDa were able to inhibit the DPPIV activity (Fig. 2B). Similar IC_{50}'s have been reported by Li-Chan, Hunag, Jao, Ho, and Hsu (2012) using salmon skin gelatin hydrolysates. This concentration of peptides required to inhibit DPPIV suggests that, in order to achieve a real long-term beneficial effect, it may be necessary to include these foods in the regular diet. Further research is needed to determine *in vivo* the concentration to obtain a significant inhibition of DPPIV activity.

Amaranth albumins and globulins were digested with trypsin at a 1:5 ratio and released peptides were tested for their capacity to inhibit the DPPIV activity. As shown in Fig. 3A, all the protein fractions were able to inhibit the enzyme at least 20% at 1500 μg/mL following a dose–response pattern. Glutelins fraction showed the highest inhibitory activity and 11S globulins showed the lowest. These results indicate that all amaranth protein fractions contain peptides with inhibitory activity against DPPIV.

3.3. In vitro gastrointestinal digestion of amaranth and other seeds

We have established an *in vitro* digestion model using the main enzymes responsible for protein degradation in the gastrointestinal

Table 1
Molecular docking within the dipeptidyl peptidase IV (DPPIV) potential binding site and 11S amaranth globulin peptides.

No.	Peptide sequence	Residues (color)[a]	Binding energy (kCal/mol)[b]	pI/MW[c]
I	STHASGFFFFHPT	1–13 (yellow)	−7.27	6.6/1482.6
II	STNYFLISCLLFVLFNGCMGEG	18–39 (magenta)	−9.48	4.0/2428.7
III	GLTEVWDSNEQEF	69–81 (blue)	−5.98	3.5/1553.6
IV	TIEPHGLLLPSFTSAPELIYIEQ GNGITGMMIPGCPETYESGSQ QFQGGEDE	92–143 (orange)	−7.34	3.7/5571.1

[a] The color is the one used on Fig. 5.
[b] Energy calculated using the FoldX program.
[c] Theoretical data; MW in Da.

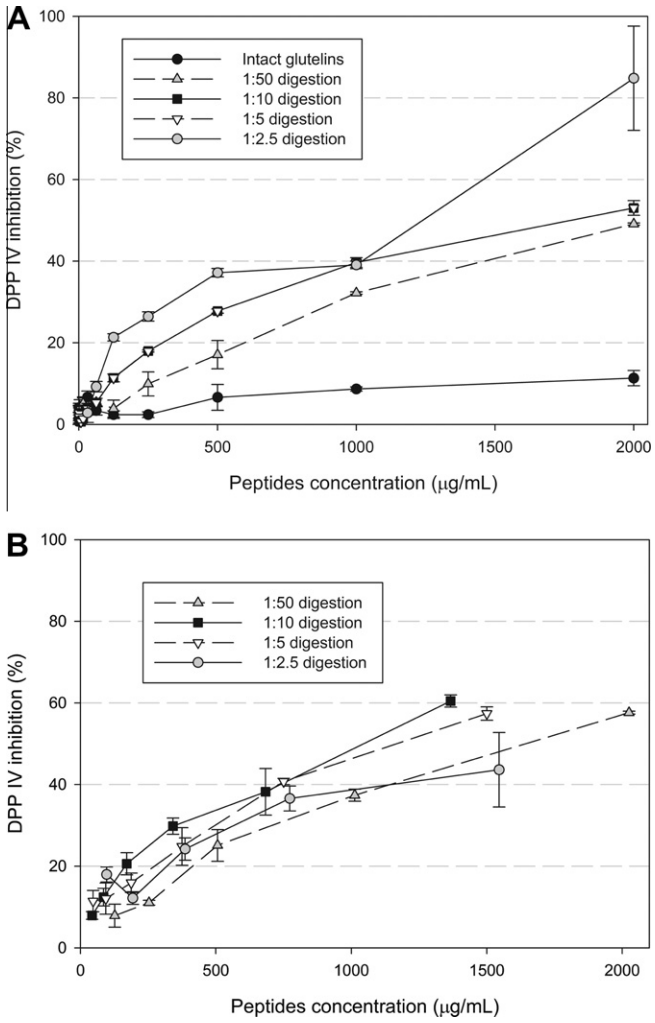

Fig. 2. DPPIV inhibitory activity of amaranth tryptic glutelins hydrolysates. (A) Amaranth glutelins were digested at different enzyme:protein ratios; (B) fragments bigger than 10 kDa were removed by ultracentrifugation, and the DPPIV activity was measured. DPPIV concentration: 100 ng/mL, substrate concentration: 500 μM. Error bars ± SD ($N = 5$).

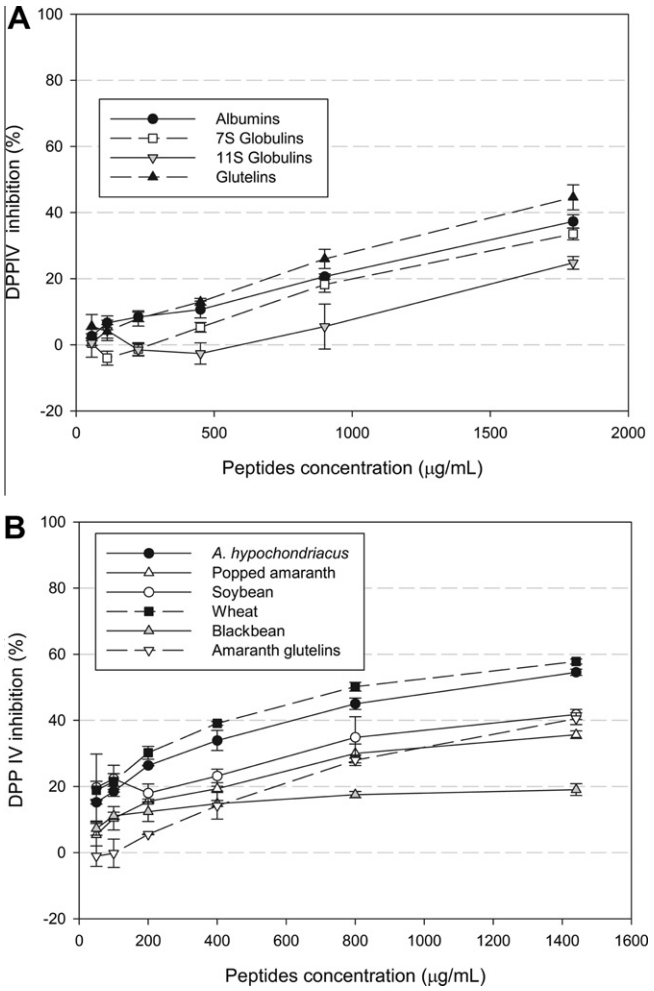

Fig. 3. (A) DPPIV inhibitory activity of different amaranth seed storage protein fractions. Amaranth proteins were digested with trypsin in 1:5 ratio (enzyme:substrate w/w) for 14 h, peptides with MW lower than 10 kDa were used. (B) Gastrointestinal digestion *in vitro* releases DPPIV inhibitory peptides from amaranth proteins and other seeds. Different seed flours were digested with pepsin, pancreatin and trypsin for 6 h. DPPIV concentration: 100 ng/mL, substrate concentration: 500 μM. Error bars ± SD ($N = 3$).

tract including pepsin from stomach, pancreatin (which includes proteases and other lytic enzymes) and trypsin released by pancreas into the small intestine. Using this gastrointestinal simulated digestion model, we tested the amaranth glutelins fraction, defatted flour from amaranth, popped amaranth, soybean, black bean, and wheat (Fig. 3B). The sample that caused the lowest inhibition upon DPPIV was the black bean flour, a leguminous rich in protein and other phytochemicals (Reynoso-Camacho, Ramos-Gomez, & Loarca-Piña, 2006), however, the present conditions of hydrolysis may have been ineffective for releasing DPPIV inhibitory peptides from black bean proteins and further work should be done in this directon (Fig. 3B). The popped amaranth flour presented a lower effect than the non-processed flour. This may indicate that the heating process during seed popping is affecting the hydrolysis and the availability of bioactive peptides, may be due to the denaturalization of the proteins, spontaneous hydrolysis, peptide cross-linking, peptide fragmentation or Maillard reactions between carbohydrates and proteins (Van Lanker, Adams, & De Kimpe, 2011).

The samples with the highest inhibitory activity (up to a 50%) were the raw amaranth flour (IC_{50} 1.1 mg/mL) and the wheat flour with an IC_{50} of 0.8 mg/mL, reported here for the first time. Wheat is an important source of protein worldwide, which has also been reported to contain bioactive peptides such as lunasin and ACE inhibitors (Jeong, Lee, Jeong, & Park, 2007; Motoi & Kodama, 2003). In wheat, the gliadins (prolamins fraction) are an important fraction responsible for the allergic and inflammatory responses and the small intestine mucosa damage or celiac disease in patients sensitive to gluten (Dewar et al., 2006; McGough & Cummings, 2005). Amaranth, as a pseudo-cereal, has the advantage to be a prolamin-free seed, and thus is an excellent alternative for preparation of gluten-free food for celiac disease patients (Alvarez-Jubete, Arendt, & Gallagher, 2010). The peptides released from amaranth glutelins using the simulated gastrointestinal digestion conserved the DPPIV inhibitory activity, but the effect was lower than that observed with the whole flour. It is possible that this effect is due to the sum of inhibitory peptides released from all amaranth protein fractions (albumins, globulins and glutelins).

Because there is no information about the DPPIV inhibitory activity of soybean, we tested the effect of a soybean protein hydrolysate. Surprisingly it was observed that soybean flour was able to inhibit the enzyme up to 40%, (1.4 mg/mL) following a pattern similar to the one observed with amaranth glutelins. There are several evidences of the presence of bioactive peptides in soybean proteins with different activities, such as anticancer, hypolipidem-

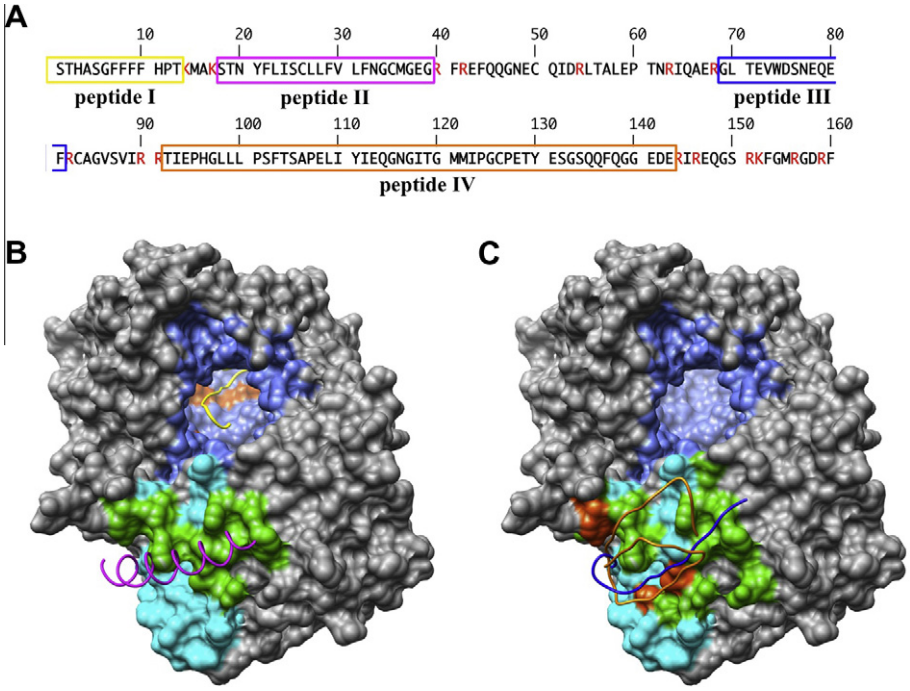

Fig. 4. Molecular modeling of the interaction between DPPIV and amaranth 11S globulin peptides. Only one monomer of the biological unit of DPPIV, which is a dimer, is represented at the surface. The internal cavity of the enzyme is colored blue; residues that mediate dimerization of DPPIV are shown in cyan (PDB accession code 1R9M). (A) Amaranth 11S globulin primary structure. Amino acids in red show the site for trypsin cleavage. The sequences inside the colored boxes correspond to the peptides used for docking with DPPIV, panels (B) and (C). (B) For simplicity, only residues of DPPIV forming h-bonds with peptide-I (yellow) are colored orange. Residues of DPPIV forming hydrophobic and h-bond interactions with peptide-II (magenta) are shown in green. (C) As in panel (B) only residues of DPPIV forming h-bonds with peptide-III (blue) are shown in orange. Residues of DPPIV forming hydrophobic and h-bonds contacts with peptide IV (orange) are shown in green. Detailed interactions of peptides I-IV with DPPIV are shown in Fig. 5.

ic, antiadipogenic, and antidiabetic effects (Gonzalez de Mejia, Martinez-Villaluenga, Roman, & Bringe, 2010; Inoue et al., 2011; Kwon, Daily, Kim, & Park, 2010; Wang et al., 2008). Whether the antidiabetic effect of soybean is due to the presence of peptides with inhibitory activity against DPPIV needs to be determined. Lacroix and Li-Chan (2012) found through an *in silico* analysis that different proteins from soybean and wheat contain DPPIV inhibitory peptides within their seeds, but this information have not been experimentally confirmed. Our results corroborate those findings although we cannot asseverate that the sequences they report are the ones responsible for the observed inhibition.

3.4. Characterization of amaranth peptides with inhibitory activity of DPPIV

By LC-ESI/MS/MS and bioinformatics analysis we identified the amino acid sequences PPPP, GP, PP, MP, VA, MA, KA, LA, FA, AP, FP, PA, LP, VP, LL, VV, HA, IPA, IPI that have displayed an inhibition of DPPIV activity (Table S1). Among these peptides, the sequence IPI (Ile-Pro-Ile) known as diprotin A is a well-known inhibitor of DPPIV (Li-Chan et al., 2012; Rahfeld, Schierhorn, Hartrodt, Neubert, & Heins, 1991; Umezawa et al., 1984). At molecular level of action, diprotin A functions as non-covalent inhibitor that transitorily binds to the catalytic site of the enzyme and blocks the degradation of larger polypeptidic chains, such as incretins (Wiedeman & Trevillayn, 2003).

Until now, no information about amaranth glutelins structure is reported, but amaranth globulins have been cloned and sequenced and recently its 3D structure has been reported (Barba de la Rosa, Herrera-Estrella, Utsumi, & Paredes-López, 1996; Tandag-Silvas et al., 2012). For this reason, we used globulin structure to perform an *in silico* trypsin digestion analysis. Fig. 4A shows that tryptic digestion released at least four peptides larger than 4 residues (Table 1). Peptides larger than 13 residues have been reported as novel DPPIV inhibitors (Uchida, Ohshiba, & Mogami, 2011), but mechanism of action have not been reported yet. For this reason we have used the bioinformatics tools to predict the mode of action of these large peptides.

3.5. Molecular docking simulations of amaranth globulins inhibitory action

Tools for drug design or the ligand-enzyme docking simulations were used to analyze the binding modes of amaranth tryptic peptides with the DPPIV enzyme (Fig. 4B and C). DPPIV active structure (PDB ID code: 1R9 M) exists as a dimer with two domains forming an eight-bladed propeller domain with a 30–45 Å cavity between each monomer where the inhibitors bind next to the catalytic site (Wiedeman, 2007). This dimeric structure forms two openings access to the cavity, the large opening, charged negatively, was found between the hydrolase domain and the propeller domain and is believed to be the favored entry route of the substrates (Hiramatsu et al., 2003). As observed in Table 1, the smallest peptide (residues 1–13) shown in yellow in Fig. 4, has a predicted a near neutral isoelectric point (p*I*). This peptide was located inside the DPPIV large cavity blocking the access of the natural substrate. The other three peptides with acidic p*I* (Table 1); were located at the dimerization interface, therefore blocking the formation of DPPIV dimeric active form. The predicted interaction energy (kCal/mol) of the peptides ranged from −5.98 to −9.48 kCal/mol (Table 1), these are values in the range reported for stable interactions (Schymkowitz et al., 2005). The potential interaction of amaranth globulin peptides with the DPPIV domain is shown in Fig. 5. Peptide I interacts with residues Phe_{208} through Trp_{216} of DPPIV, peptide II has interactions with DPPIV from residues Glu_{731} to His_{750}, while peptide II with residues Thr_{706} to Thr_{753}, and peptide IV from Phe_{730} to Gln_{749}

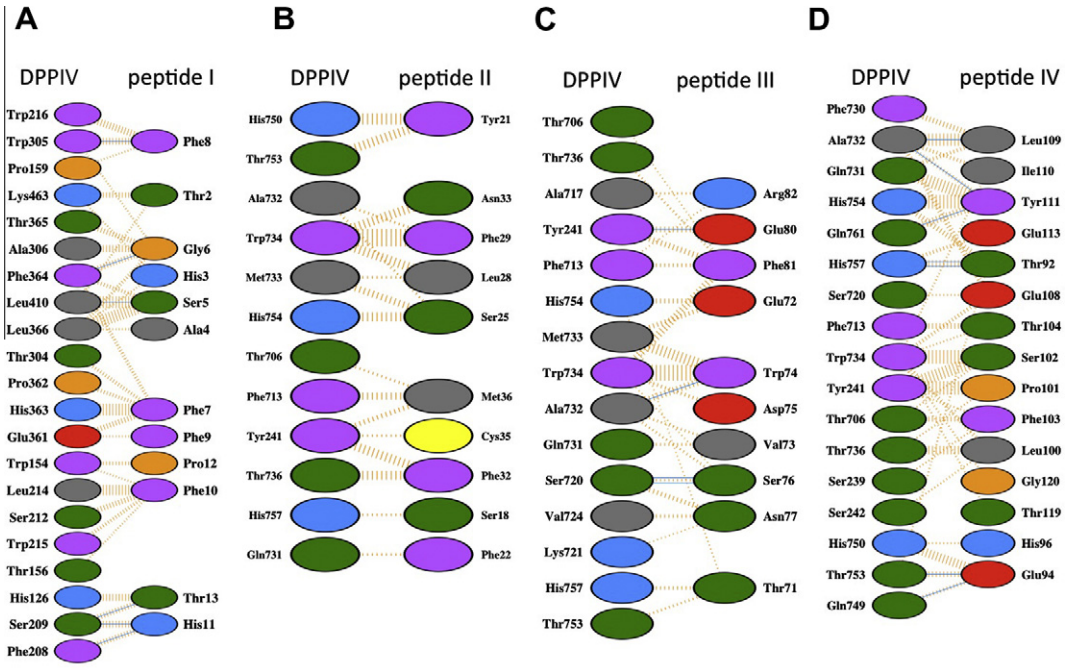

Fig. 5. Detailed interaction between DPPIV enzyme with amaranth peptides, calculated using the PDBsum server (38). Orange striped lines represent hydrophobic interactions, blue lines hydrogen bonds. Colored circles describe amino acid properties; positive residues (H,K,R) are shown in blue; negative residues (D,E) in red; neutral residues (S, T, N, Q) in green; aliphatic residues (A, V, L, I, M) in grey; aromatic residues (F, Y, W) in magenta; P and G are colored brown and C is in yellow. The number of hydrogen-bonds/non-bonded interactions identified is shown in parenthesis for DPPIV residues interacting with (A) amaranth peptide-I (6/91), (B) amaranth peptide-II (0/52), (C) amaranth peptide-III (4/91) and (D) amaranth peptide IV (7/107).

(Fig. 5). The interactions between DPPIV and amaranth peptides are mainly via hydrophobic interactions (orange lines bonds) and hydrogen-bonds (blue lines bonds).

4. Conclusions

The information presented here, confirms the presence of two types of DPPIV inhibitory peptides encrypted in amaranth seed storage proteins, the short peptides such as IPI, and larger peptides. Using docking analysis we have shown that some tryptic peptides released from amaranth globulins are peptides larger than 13 residues, these peptides interact with DPPIV at enzyme dimerization sites. This is the first report that evaluates the DPPIV inhibitory activity from food hydrolysates using a gastrointestinal digestion model. These results represent a new perspective of cereal benefits beyond their nutritional properties. It is also important to be aware that food heat processing could damage the protein structure, thus affecting the ability of enzymes to release the active peptides. Therefore, novel alternatives in food processing should be developed to preserve their bioactivity for their potential use as novel compounds in the prevention and management of type 2 diabetes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.08.032.

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