Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.57922

Biofunctional properties of the bioactive peptide from protein isolates of jiotilla (*Escontria chiotilla*) and pitaya (*Stenocereus pruinosus*) seeds

Luisaldo SANDATE-FLORES[1,2], Gerardo MÉNDEZ-ZAMORA[1], Marcos Fredy MORALES-CELAYA[3], Jesús Alberto LARA-REYES[1], Victor Eustorgio AGUIRRE-ARZOLA[1], Adriana GUTIÉRREZ-DIEZ[1], Jorge Ariel TORRES-CASTILLO[4], Sugey Ramona SINAGAWA-GARCÍA[1]*

Abstract

In recent years, there has been considerable interest in bioactive peptides derived from food proteins, which might benefit human health. Pitaya and jiotilla are fruits with demonstrated biological functions in its stem, peel, and pulp. However, no studies have been conducted to evaluate the peptides. The aim of this study was to determine biofunctional properties (antioxidant, antihypertensive, and antidiabetic activities) of peptides from cacti seed protein. Antioxidant activity by ABTS, ACE inhibition activity, and α-amylase inhibition activity was carried out in the peptides. Based on the obtained results, hydrolyzed total protein isolate of pitaya showed high antioxidant activity (1328.40 ± 63.50 μM Trolox equivalents/mg protein). Moreover, hydrolyzed glutelin fraction of pitaya has a high α-amylase inhibition activity (76.74 ± 2.15%). On the other hand, hydrolyzed total protein isolate of jiotilla had a good ACE inhibition activity (47.64 ± 3.16). These results demonstrated that pitaya and jiotilla seeds protein hydrolysates had excellent in vitro nutraceutical potential.

Keywords: seeds of cacti; bioactive peptides; antioxidant; anti-hypertensive; anti-diabetic activity.

Practical Application: Peptides from pitaya and jiotilla had in vitro nutraceutical potential.

1 Introduction

The knowledge about bioactive peptides is increasing, and the studies are being related to general health conditions or a reduced risk of certain chronic diseases (Bhandari et al., 2020). The biofunctional properties of these compounds are related to an antihypertensive function associated with ACE inhibition (Yousr & Howell, 2015), antidiabetic property related to α-amylase inhibition (Nair et al., 2013), and antioxidant activity (Zhuang et al., 2013). In the Americas, around 62 million people have diabetes. People with this condition live in low and middle-income countries (Pan American Health Organization, 2021). In 2020, 151,000 people died from diabetes mellitus in Mexico. Peptides from plants can help diabetics through a variety of pathways (Akbarian et al., 2022). It is crucial to find antidiabetic compounds that are accessible to all communities. Peptides can be produced naturally by enzymatic proteolysis during gastrointestinal digestion. In vitro, these compounds can be obtained by hydrolysis of proteins with the use of proteolytic enzymes (Abdel-Hamid et al., 2017). Several plants, microorganisms, and animals proteins sources have been used for generating bioactive peptides (Bechaux et al., 2019). Few studies detail the bioactive peptides from seed protein of cacti, and their biofunctional properties (Loo et al., 2017).

Cactaceae plants grow in arid areas. In Mexico, 47.7% of cacti are endemic (Ramírez-Rodríguez et al., 2020). Cacti fruits are valued for their distinctive organoleptic properties such as taste and color (Chuck-Hernández et al., 2016). Some cacti fruits are underutilized in Mexico (Ramírez-Rodríguez et al., 2020), which are sustainable sources to obtain bioactive peptides and other compounds. The fruits with the greatest potential to obtain these compounds are the "Jiotilla" from *Escontria chiotilla* and "Pitaya" from *Stenocereus pruinosus*. The fruits of *E. chiotilla* are important edible resources with anticancer properties (Sandate-Flores et al., 2020). Furthermore, pitaya has also drawn attention for its potential nutraceutical properties since it has natural pigments (betalains), vitamin C, high contents of iron and phenolic compounds, and antiproliferative activity on human cancer cell lines (García-Cruz et al., 2013, 2017; Martínez et al., 2021). The pitaya and jiotilla are highly valued in the region because the fruits are an important source of income for communities (Chuck-Hernández et al., 2016; García-Suárez et al., 2007). Nevertheless, the short shelf life of cacti fruits after the harvest is a problem (Hernández-Valencia et al., 2019).

Based on the literature, the ways to deal with this obstacle are to produce products such as jams, juices, marmalades (Ali et al., 2020), ice cream, and jellies (Arellano & Casas, 2003) with fruits. Regarding the seeds, cacti can be used as additives in foods (El-Safy et al., 2012). However, the knowledge about peptides of the seeds from the Mexican plants *Escontria Chiotilla* and

Received 03 June, 2022
Accepted 18 July, 2022
[1] *Laboratorio de Biotecnología, Facultad de Agronomía, Universidad Autónoma de Nuevo León, General Escobedo, N.L., México*
[2] *Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León, San Nicolas de los Garza, N.L., México*
[3] *División de Carrera de Agricultura Sustentable y Protegida, Universidad Tecnológica de los Valles Centrales de Oaxaca, San Pablo Huixtepec, Oaxaca, México*
[4] *Instituto de Ecología Aplicada, Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, México*
*Corresponding author: sugey.sinagawagr@uanl.edu.mx

Stenocereus pruinosus is limited. Studies on the potentialities of cacti fruits have increased, but there is still a lack of information regarding bioactive peptides. Based on the above, the proposal of this study was to characterize in vitro biofunctional properties (antioxidant, antihypertensive, and antidiabetic activities) of bioactive peptides from cacti seed flour.

2 Materials and methods

2.1 Chemicals

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Item A1888-1G), (±)-6-Hidroxy-2,5+,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Item 238813), 2-Mercaptoethanol (Item M3148), N-Hippuryl-His-Leu hydrate (Item H1635-100MG), bovine trypsin (Item T4799-5G) and bovine chymotrypsin (Item C4129-250MG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N, N, N′, N′-Tetramethylethylenediamine (TEMED) (Item 1610801) was acquired from Bio Rad (Hercules, CA). Ammonium Persulfate (Item 009342 A) was purchased from Bio Rad (Japan). Brilliant Blue R-250 (Item 1610400) was acquired from Bio Rad (Canada). Prestained protein ladder (3.5-245 kDa) unstained standards (Item ENZ-ACC131-0500) was purchased Enzo Life Science (Farmingdale, NY). Methyl alcohol RA (Item CTR 01220), glacial acetic acid (Item CTR 138937), and phosphoric acid (H_3PO_4, Item CTR 00696) were acquired from CTR (Mexico). Laemmli (SDS-Sample) (Item 10570018-1) was acquired from bioworld (Dublin, OH). Pepsin (Item 10108057001) from pig gastric mucosa was purchased from Roche (Germany).

2.2 Collection and preprocessing of jiotilla and pitaya

Two batches of jiotilla fruits (10 kg) were collected from a crop field in San Pedro Totolapam, Oaxaca, Mexico 16° 40′latitude (North) and 96°18′ longitude (West). The batch of pitaya fruits (30 kg) was bought in the Puebla market (Central de abastos de Puebla). The collected fruit samples were stored at 5 ◦C for 24 h and processed within 48 h of harvest from the plant. The prickles were removed manually with care. Then, the collected fruits of jiotilla and pitaya were preprocessed by washing with tap water and soap (Axion, Item 7509546017143, Guanajuato, Mexico).

2.3 Preparation of seed flour

The electric extractor (Model 67800, Hamilton Beach, China) was used to separate the seeds from the pulps. Subsequently, 100 g of seeds were washed with tap water, then the seeds were placed in a 600 mL beaker, 400 mL of deionized water was added, and the seeds were agitated for 30 min. The seeds were dried in a hybridization oven (Model 400, Robbins Scientific, Sunnyvale, CA) at 45 °C for 24 h. The seeds were grounded in a blender (Model 4090-014, Osterizer Blender, México). Flour was defatted with a 1:5 (flour: methanol) ratio by stirring (3 rpm, 45 min) and then filtrating to eliminate fatty residues. The mix of flour and methanol from each fruit was placed in 50 mL polypropylene centrifuge tubes. The tubes were subjected to centrifugation (6000 rpm, 4 °C, 10 min, Model Z 400k, Labnet, Wertheim, Germany). The supernatant was removed, and the pellet was used. The flour was dried at 25 ± 2 °C for 24 h. Then flour was sieved through 425 μm meshes and labeled as fine flour.

2.4 Storage protein extraction and fractionation procedure

The storage protein extractions were carried out on base the method of Osborne & Campbell (1898), considering the criteria the solubility (Kumar et al., 2019).

Albumin extraction

For each fine flour, 2 g were weighed and placed in 20 mL beakers; 10 mL of deionized water were added, and then the solutions were agitated for one hour at 4 °C; the solution from each fruit was placed in 15 mL polypropylene centrifuge tubes. The tubes were subjected to centrifugation (5,000 rpm, 4 °C, 15 min, Z 400 k, Labnet, Germany). The supernatants were removed and labeled as albumin fraction. The pellets were used in globulin extraction.

Globulin extraction

The pellets of albumin extraction were placed in 20 mL beakers; 10 mL of the Tris-HCl buffer with 0.3 M NaCl pH 7.8 were added, and then the solutions were agitated for one hour at 4 °C; the solutions were placed in 15 mL polypropylene centrifuge tubes. The tubes were subjected to centrifugation (5,000 rpm, 4 °C, 15 min, Z 400 k, Labnet, Germany). The supernatants were removed and designated as globulin fractions. The pellets were used in the glutelin extraction.

Glutelin extraction

The pellets of globulin extraction were placed in 20 mL beakers; 10 mL of the sodium borate buffer was added, and then the solutions were agitated for one hour at 4 °C; the solutions were placed in 15 mL polypropylene centrifuge tubes. The supernatants were removed and labeled as glutelin fraction.

2.5 Protein quantification

Another technique used for the determination of the protein content was the Bradford (1976) method. A standard curve was prepared with a 2 mg/mL stock solution of BSA. A volume of 30 μL of the sample was added to 1.5 mL spinwin™ microcentrifuge tubes (Tarsons, India) and mixed with 1 mL Bradford reagent. The mixture was then agitated, allowed to stand for 5 min. Finally, absorbance was measured at 595 nm. All measurements were made in triplicate.

2.6 Total protein isolate

For both fine flours, 5 g were weighed and placed in 50 mL beakers. The method followed was Poms et al. (2004) with modification, 25 mL of sodium borate buffer 0.3 M were added, and then the solutions were agitated for 45 min at 4 °C pH 10. Then the solution was subjected to centrifugation at 7,000 rpm for 20 min at 4 °C. The supernatant was recovered and pH was adjusted to pH 4.5 with HCl (Pedroche et al., 2002). The next supernatant was centrifugated at 7,000 rpm for 20 min at 4 °C.

The supernatant was discarded and the precipitate was preserved. The precipitate was labeled as total proteins isolate.

2.7 Protein analysis by SDS-PAGE

The protein profiles of storage protein were visualized on 10% SDS-PAGE (Laemmli, 1970). A total of 20 µL of protein sample in buffer Laemmli were loaded. Electrophoresis was run at 80 V for 30 min for the first step to concentrate the sample in the gel well and then at 120 V for 90 min to resolve proteins according to their molecular weight.

2.8 In vitro enzymatic hydrolysis

The hydrolysis treatments were performed with pepsin, trypsin, and chymotrypsin to simulate gastric fluid. Pepsin was used to simulate the gastric phase, then protein digestion was carried out with trypsin and chymotrypsin (Verhoeckx et al., 2015). Sequential digestion using pepsin and trypsin-chymotrypsin for 5 h of total hydrolysis was performed. The methodology followed was Garza et al. (2017) with modifications.

In the first, 2 mL of glutelin fraction were dissolved in 23 mL deionized water, and the second 200 mg of total proteins isolate were dissolved in 25 mL deionized water. After the aforementioned, the following steps were the same for both methods. Then 10 mg of digestive enzymes were dissolved and added to the previous solution and digestion was performed at 37 °C. The solution was adjusted to pH 2.0 for the pepsin digestion process and 7.8 for trypsin-chymotrypsin digestions. The temperature and pH were monitored during the process. After 2.5 h of hydrolysis with pepsin and 2.5 h with trypsin-chymotrypsin, the reaction was stopped by heating at 80 °C for 10 min and then centrifuged at 7,000 g for 10 min; the supernatant was recovered and labeled as hydrolysate of glutelin and hydrolysate of total glutelin isolate.

2.9 Bio functionality property evaluations of peptides

Antioxidant activity by ABTS

For the ABTS method, the reported method of Re et al. (1999) was used. The ABTS, a single electron transfer (ET) reaction-based assay, was carried out following a solution of ABTS at 7 mM and 2.45 mM potassium persulfate were prepared and mixed. The solutions were left mixing in the dark for 16 h. A dilution of the reagent (absolute ethanol) was needed until it has an absorbance of 0.7 at 734 nm. Thereafter, the absorbance was read using Trolox as a standard in concentrations ranging from 100 to 1200 ppm. Reactions were prepared with 10 µL of each sample or standard and 1 mL of reagent; absorbance at 734 nm was determined after 6 min.

ACE inhibition activity

The antihypertensive property was performed based on the procedure described Hayakari et al. (1978). The Angiotensin-Converting Enzyme (ACE) was extracted from rabbit lungs and dissolved in 0.1 M buffer of KH_2PO_4, pH 8.3 and His-His-Leu (HHL) tripeptide was used as substrate. Absorbance at 382 nm was determined in the supernatant obtained after centrifugation at 10,000 g for 10 min (Minispin®, Eppendorf AG, Germany). The percentages of ECA activity and inhibition of ECA were quantified using Equation 1 and Equation 2, respectively.

$$ECA\,activity\ \% = \frac{ESM - BEM}{ES - BES} x100 \quad (1)$$

$$ECA\,inhibition\ \% = 100 - ECA\,activity\ \% \quad (2)$$

Where *ESM* is the absorbance of the sample substrate enzyme, *BEM* is the absorbance of the blank sample enzyme, *ES* is the absorbance of the enzyme sample. *BES* is the absorbance of the blank substrate enzyme.

α-amylase inhibition activity

In order to determine the antidiabetic activity, the Nair et al. (2013) procedure was used. A milliliter of human saliva was centrifuged (10,000 rpm, 25 °C, 10 min, Minispin®, Eppendorf AG, Germany). The supernatant was removed, and 2 mL buffer NaH_2PO_4 at pH 6.8 were added to the pellet. In a beaker, it was added 8 mL of NaOH 2M and 12 g of potassium sodium tartrate. The solution was heated at 70 °C and mixed until homogeneous. In another beaker, it was added 20 mL of distilled water and 0.4374 g of 3,5-dinitrosalicylic acid (DNS). The DNS solution was mixed until homogeneous. The aforementioned solutions were mixed and heated at 70 °C until homogeneous. Then 100 µL of 1% starch and 100 µL of the human enzyme were added to each sample and incubated at 37 °C for 30 min. Subsequently, 100 µL of DNS solution were added and heated at 90 °C for 5 min. Then samples were cooled and diluted with 900 µL of deionized water after recording the absorbance at 540 nm. The percentages of α-amylase activity and inhibition of α-amylase were quantified using Equation 3 and Equation 4, respectively. Where *SSE* is the absorbance of the sample substrate enzyme, *BSE* is the absorbance of the blank sample enzyme, *SE* is the absorbance of the enzyme sample. *BSE* is the absorbance of blank substrate enzyme.

$$\alpha - \text{Amylase}\ activity\ \% = \frac{SSE - BSE}{SE - BSE} x100 \quad (3)$$

$$\alpha - \text{Amylase}\ inhibition\ \% = 100 - \alpha\ \text{amylase}\ activity\ \% \quad (4)$$

2.10 Statistical analysis

All experiments were carried out in triplicates. The variables were analyzed using the following statistical model (Equation 5):

$$y_{ij} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijk} \quad (5)$$

Where y_{ij} = variables measured; µ = treatments global mean; τ_i = effect of the factor fine flour of pitaya; β_j = effect of the fine flour of jiotilla j; $\tau\beta_{ij}$ = effect of the interaction between the fine flours; ε_{ijk} = residual error. The significance level used for all statistical analyses was 5%; the Tukey statistical test was performed to compare the means between treatments.

3 Results and discussion

3.1 Protein quantification

After protein isolation and hydrolysis procedures from fine flour, it was important to determine the concentration of protein at each step in the purification procedure. The method used was Bradford. The results show in Table 1. The storage proteins were classified as albumins, globulins and glutelins. The glutelins were the main fraction present and for this reason, the glutelin fraction was chose for later analyses of bifunctional properties. Compared with other flours, in Chia seeds, the glutelin fraction is the principal fraction (Segura-Campos, 2020). In rice, the major storage proteins are glutelins (Singh et al., 2021; Takahashi et al., 2019); but in dicots, albumins and globulins occupy a major proportion (Kumar et al., 2019).

3.2 Protein analysis by SDS-PAGE

The protein profiles of albumins extraction showed a band with molecular weights between 25 and 35 KDa (Figure 1) in jiotilla. The protein profile of globulin extraction exhibited a band with molecular weights between 25 and 35 KDa in both fruits. The protein profile of glutelin extraction showed bands with molecular weights between 25 and 75 kDa in pitaya and jiotilla. Antioxidant and other biological activities of protein hydrolysates are correlated with the type of enzyme, the reaction time and degree of hydrolysis (Wang et al., 2018), and the sequence of peptides (Mojica & Mejía, 2016). The antioxidant activity of peptides is related to their amino acid composition (Wong et al., 2020). Some amino acids, such as isoleucine, leucine, proline, and tyrosine had been reported to exhibit antioxidant properties (Jiang et al., 2018; Sun et al., 2019). Glutenins are rich in hydrophobic amino acids like proline, tyrosine and leucine (Ewart, 1967). The amino acids before mentioned could be present in the glutelins of cacti biopeptides and these compounds could give the biological activities presented.

Table 1. Protein concentration in the different fractions of fine.

	Fraction	Bradford* (mg/mL)
Pitaya	Albumin fraction	$3.19 \pm 0.15^{b;\,C}$
	Globulin fraction	$4.08 \pm 0.06^{b;\,C}$
	Glutelin fraction	$86.45 \pm 1.40^{a;\,A}$
Jiotilla	Albumin fraction	$1.29 \pm 0.00^{b;\,D}$
	Globulin fraction	$1.23 \pm 0.00^{b;\,D}$
	Glutelin fraction	$82.94 \pm 0.25^{a;\,B}$

*Bradford methods to determine the concentration of protein. $^{a\text{-}b}$means (n = 3/method of quantification of proteins/fraction) within the same fruit at each fraction with different lowercase superscripts differ significantly when the p-value of (τ_i) < .05; $^{A\text{-}D}$means (n = 3/method of quantification of proteins/fraction) within the same column, for both methods and for all fractions, with different uppercase superscripts differ significantly when the p-value of $\tau\beta_{ij}$ < .05.

3.3 Bio functionality property evaluations of peptides

The common method for producing hydrolysates is based on in vitro hydrolysis with proteolytic enzymes that simulate the gastrointestinal process (Garza et al., 2017). The hydrolysis with enzymes can generate bioactive peptides that have been studied in terms of their biofunctional or nutraceutical potential (Bhandari et al., 2020).

Antioxidant activity by ABTS

The results of antioxidant properties assays in general, showed that both glutelin fraction and total protein isolates could have antioxidant properties in both pitaya (Table 2) and jiotilla. The hydrolysates had higher antioxidant properties than the non-hydrolyzed proteins i.e. in pitaya an increasing until 241% and 384.8% for hydrolyzed glutelin fraction and hydrolyzed total protein isolate was found, respectively compared with their non-hydrolyzed protein. Similar behavior in the antioxidant property in the glutelin fraction and total protein isolate of jiotilla was observed (Table 2). Based on the literature, this behavior has been reported in bioactive peptide of walnut (Moghadam et al., 2020) and soy (Coscueta et al., 2019). The rise in antioxidant properties is probably due to the increased usability of hydrogen ions obtained by bioactive peptides released after hydrolysis (Guan et al., 2018). Comparing antioxidant properties with the total hydrolysates from protein of moringa seeds with 5,260 μM Trolox equivalent/mg protein, (Garza et al., 2017), protein hydrolysates of pitaya and jiotilla had lower antioxidant properties. But the pitaya seeds total protein isolate hydrolysates

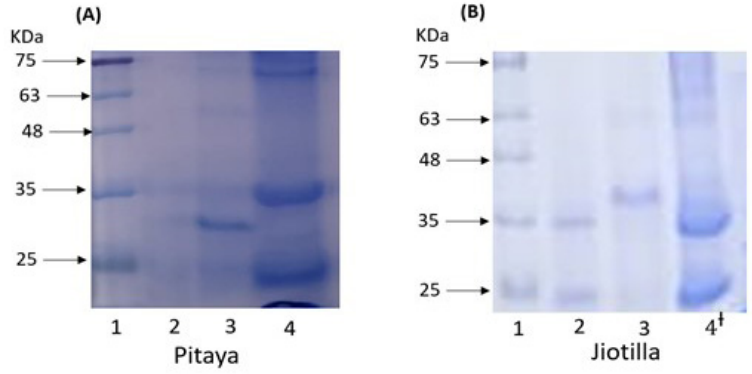

Figure 1. Polyacrylamide gel electrophoresis profile in 10% SDS- PAGE. (A) Pitaya. (B) Jiotilla. Lane 1 molecular weight marker. Lane 2 albumin fraction. Lane 3 globulin fraction. Lane 4 glutelin fraction. The jiotilla glutelin fraction was diluted by a factor of 4.

Table 2. Biofunctional properties of bioactive peptides of pitaya and jiotilla seeds.

	Treatment	ABTS* µM	%IAA**	%IECA***
Pitaya	Glutelin fraction	367.30 ± 26.50^{bC}	56.43 ± 0.81^{bBC}	21.23 ± 2.35^{bCD}
	Hydrolyzed glutelin fraction	1254.00 ± 119.30^{aA}	$76.74 \pm 2.15^{a\,A}$	28.16 ± 4.47^{abBCD}
	Total protein isolate	274.00 ± 59.00^{bCD}	$29.41 \pm 3.29^{c\,F}$	20.06 ± 7.75^{bD}
	Hydrolyzed total protein isolate	$1328.40 \pm 63.50^{a\,A}$	52.34 ± 2.24^{bCD}	$40.39 \pm 8.43^{a\,AB}$
Jiotilla	Glutelin fraction	420.70 ± 64.30^{cC}	47.17 ± 2.01^{bDE}	0.93 ± 0.05^{cE}
	Hydrolyzed glutelin fraction	1236.90 ± 112.30^{aA}	$58.65 \pm 1.42^{a\,B}$	35.17 ± 3.55^{bABC}
	Total protein isolate	68.40 ± 8.39^{dD}	$17.19 \pm 2.31^{d\,G}$	2.88 ± 0.05^{cE}
	Hydrolyzed total protein isolate	944.00 ± 58.60^{bB}	$42.00 \pm 1.83^{c\,E}$	47.64 ± 3.16^{aA}

*ABTS is antioxidant activity. Trolox were used in calibration curves. The units used in ABTS were trolox equivalent/mg protein; **%IAA is percentage of α-amylase inhibition of α-amylase; ***%IECA is percentage of inhibition of angiotensin-converting enzyme. $^{a\text{-}d}$means (n = 3/biofunctionality evaluation method/treatment) within the same column and within each bio functionality evaluation method and at each treatment with different lowercase superscripts differ significantly when the p-value of (τ_i) < .05; $^{A\text{-}E}$means (n = 3/bio functionality evaluation method/treatment) within the same column, for all bio functionality evaluation method and for all treatments, with different uppercase superscripts differ significantly when the p-value of $\tau\beta_{ij}$ < .05. The assays were carried out at the concentration of 1 mg/mL of proteins.

showed an antioxidant property of 1,328.4 µM Trolox equivalent/mg protein, which is 772 ± 27 µM trolox/g sample superior to the value presented by the digestion of proteins extracted from plum seeds with thermolysin (González-García et al., 2014).

α-amylase inhibition activity

α-Amylase hydrolyzed the starch to simple sugar; therefore, the inhibition of this enzyme is related to decrements in blood glucose levels (Visvanathan et al., 2016). In general, the protein hydrolysates had higher inhibition activity of α-amylase than the non-hydrolyzed proteins. The highest α-amylase inhibition activity 76.74% was found in the glutelin fraction hydrolysates of pitaya seeds (Table 2). The α-amylase inhibition activity increased until 144.32% in total protein isolate hydrolysates of jiotilla compared to the non-hydrolyzed proteins. However, in the pitaya α-amylase inhibition activity increased by 78% in hydrolyzed total protein isolate compared to non-hydrolyzed proteins. The α-amylase-inhibitory activities in pitaya and jiotilla seeds were slightly lower than reported by hydrolysates of moringa oleifera seed flour with pepsin (77.59 ± 0.17%) (Olusola et al., 2018). Nevertheless, protein hydrolysate with tryptic and alcalase obtained from the seed of *Luffa cylindrica* (27.96 ± 0.06% and 36.36 ± 0.71% respectively) (Arise et al., 2019), demonstrated a lower α-amylase inhibition than all hydrolyzed samples of pitaya and jiotilla. The hydrolyzed glutelin fraction from pitaya was higher than protein hydrolysates with trypsin from Pawpaw (*Carica papaya*) seeds (63.64 ± 1.55%) (James et al., 2020).

ACE inhibition activity

ACE activity increases is correlated to the production of a vasoconstrictor angiotensin II derived from angiotensin I, resulting in high blood pressure. Hydrophobic amino acid residues in peptides are linked to ACE inhibitors (Daskaya-Dikmen et al., 2017; Nuchprapha et al., 2020). The no-hydrolyzed glutelin fraction and total protein isolate from jiotilla showed the lowest ACE inhibition activity. This behavior is similar to published work on longan seed protein isolates, where ACE inhibition activity was only detected after the enzymatic hydrolysis (Nuchprapha et al., 2020). The ACE inhibition activity of hydrolyzed total protein isolate of pitaya and jiotilla were 40.4% and 47.6% respectively, resulting higher than their no hydrolyzed proteins. In pitaya, the ACE inhibition activity increased until 33% in hydrolyzed glutelin fraction compared to the non-hydrolyzed fraction (Table 2). However, surprisingly in the jiotilla, ACE inhibition activity increased 3,682% in hydrolyzed glutelin fraction compared with non-hydrolyzed fraction. When compared with other seeds, ACE inhibitory activity was lower than the values reported for *Lens culinaris* 89.74 ± 0.56% (Park et al., 2019) and fruit seeds of peach (*Prunus* genus) 90.3 ± 0.2 (González-García et al., 2018). A direct comparison of our results with other seeds such as *Avena sativa* (26.01 ± 1.90%) and *Arachis hypogaea* (21.14 ± 1.79) (Park et al., 2019), jiotilla, and pitaya represents higher ACE inhibition activity than seeds mentioned before.

4 Conclusion

Seed storage protein of Pitaya and Jiotilla were quantified, and the major and more abundant were the glutelin's fraction. Both glutelin fraction as total isolated protein in both cacti fruits exhibit the best biofunctional properties when they are hydrolyzed sequentially with pepsin, trypsin-chymotrypsin for 5 h of total hydrolysis, which increases their nutraceutical activities as antioxidants, antihypertensives, and antidiabetics. In recent years, the research in cacti fruits have increased. However, more investigation is needed to identify other potential health benefits of bioactive peptides from seed proteins of pitaya and jiotilla as well as to validate through in vivo studies the bioactive capacities established.

Funding

This work was supported by Programa de Apoyo a la Investigacion Cientifica y Tecnologica (PAICYT) of the Universidad Autonoma de Nuevo Leon (Grant number 65-CAT-2022).

Acknowledgements

The authors gratefully acknowledge support from México's CONACYT Postdoctoral Fellowship No. 898698 awarded to author LS-F.

References

Abdel-Hamid, M., Otte, J., Gobba, C., Osman, A., & Hamad, E. (2017). Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. *International Dairy Journal*, 66, 91-98. http://dx.doi.org/10.1016/j.idairyj.2016.11.006.</jrn>puor, S.,

Akbarian, M., Khani, A., Eghbalpour, S., & Uversky, V. N. (2022). Bioactive peptides: synthesis, sources, applications, and proposed mechanisms of action. *International Journal of Molecular Sciences*, 23(3). https://doi.org/10.3390/ijms23031445.

Ali, R. F. M., El-Anany, A. M., Mousa, H. M., & Hamad, M. E. (2020). Nutritional and sensory characteristics of bread enriched with roasted prickly pear (Opuntia ficus-indica) seed flour. *Food & Function*, 11(3), 2117-2125. http://dx.doi.org/10.1039/C9FO02532D. PMid:32073013.

Arellano, E., & Casas, A. (2003). Morphological variation and domestication of Escontria chiotilla (Cactaceae) under silvicultural management in the Tehuacán Valley, Central Mexico. *Genetic Resources and Crop Evolution*, 50(4), 439-453. http://dx.doi.org/10.1023/A:1023902704131.

Arise, R. O., Idi, J. J., Mic-Braimoh, I. M., Korode, E., Ahmed, R. N., & Osemwegie, O. (2019). In vitro Angiotesin-1-converting enzyme, α-amylase and α-glucosidase inhibitory and antioxidant activities of Luffa cylindrical (L.)M. Roem seed protein hydrolysate. *Heliyon*, 5(5), E01634. http://dx.doi.org/10.1016/j.heliyon.2019.e01634. PMid:31193002.

Bechaux, J., Gatellier, P., Page, J., Drillet, Y., & Sante-Lhoutellier, V. (2019). A comprehensive review of bioactive peptides obtained from animal byproducts and their applications. *Food & Function*, 10(10), 6244-6266. http://dx.doi.org/10.1039/C9FO01546A. PMid:31577308.

Bhandari, D., Rafiq, S., Gat, Y., Gat, P., Waghmare, R., & Kumar, V. (2020). A review on bioactive peptides: physiological functions, bioavailability and safety. *International Journal of Peptide Research and Therapeutics*, 26(1), 139-150. http://dx.doi.org/10.1007/s10989-019-09823-5.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

Chuck-Hernández, C., Parra-Saldívar, R., & Sandate-Flores, L. (2016). Pitaya (*Stenocereus* spp.). In B. Caballero, P. M. Finglas & F. Toldrá (Eds.), *Encyclopedia of food and health* (Vol. 4, pp. 385-391). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-12-384947-2.00775-3.

Coscueta, E. R., Campos, D. A., Osório, H., Nerli, B. B., & Pintado, M. (2019). Enzymatic soy protein hydrolysis: a tool for biofunctional food ingredient production. *Food Chemistry: X*, 1, 100006. PMid:31432006.

Daskaya-Dikmen, C., Yucetepe, A., Karbancioglu-Guler, F., Daskaya, H., & Ozcelik, B. (2017). Angiotensin-I-Converting Enzyme (ACE)-inhibitory peptides from plants. *Nutrients*, 9(4), 316. http://dx.doi.org/10.3390/nu9040316. PMid:28333109.

El-Safy, F. S., Salem, H. R., & El-Ghany, M. E. A. (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein. *World Journal of Dairy & Food Sciences*, 7(1), 59-65.

Ewart, J. A. D. (1967). Amino acid analyses of glutenins and gliadins. *Journal of the Science of Food and Agriculture*, 18(3), 111-116. http://dx.doi.org/10.1002/jsfa.2740180307. PMid:6042168.

García-Cruz, L., Dueñas, M., Santos-Buelgas, C., Valle-Guadarrama, S., & Salinas-Moreno, Y. (2017). Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (Stenocereus spp.) fruit from two species (S. Pruinosus and S. stellatus). *Food Chemistry*, 234, 111-118. http://dx.doi.org/10.1016/j.foodchem.2017.04.174. PMid:28551213.

García-Cruz, L., Valle-Guadarrama, S., Salinas-Moreno, Y., & Joaquín-Cruz, E. (2013). Physical, chemical, and antioxidant activity characterization of pitaya (Stenocereus pruinosus) fruits. *Plant Foods for Human Nutrition*, 68(4), 403-410. http://dx.doi.org/10.1007/s11130-013-0391-8. PMid:24142131.

García-Suárez, F., Carreto-Montoya, L., Cárdenas-Navarro, R., Pérez, J. C. D., & López-Gómez, R. (2007). Pitaya (Stenocereus stellatus) fruit growth is associated to wet season in Mexican dry tropic. *International Journal of Experimental Botany*, 76, 19-26.

Garza, N. G. G., Koyoc, J. A. C., Castillo, J. A. T., Zambrano, E. A. G., Ancona, D. B., Guerrero, L. C., & García, S. R. S. (2017). Biofunctional properties of bioactive peptide fractions from protein isolates of moringa seed (Moringa oleifera). *Journal of Food Science and Technology*, 54(13), 4268-4276. http://dx.doi.org/10.1007/s13197-017-2898-8. PMid:29184233.

González-García, E., García, M. C., & Marina, M. L. (2018). Capillary liquid chromatography-ion trap-mass spectrometry methodology for the simultaneous quantification of four angiotensin-converting enzyme-inhibitory peptides in Prunus seed hydrolysates. *Journal of Chromatography. A*, 1540, 47-54. http://dx.doi.org/10.1016/j.chroma.2018.02.003. PMid:29428219.

González-García, E., Marina, M. L., & García, M. C. (2014). Plum (Prunus Domestica L.) by-product as a new and cheap source of bioactive peptides: extraction method and peptides characterization. *Journal of Functional Foods*, 11, 428-437. http://dx.doi.org/10.1016/j.jff.2014.10.020.

Guan, H., Diao, X., Jiang, F., Han, J., & Kong, B. (2018). The enzymatic hydrolysis of soy protein isolate by Corolase PP under high hydrostatic pressure and its effect on bioactivity and characteristics of hydrolysates. *Food Chemistry*, 245, 89-96. http://dx.doi.org/10.1016/j.foodchem.2017.08.081. PMid:29287456.

Hayakari, M., Kondo, Y., & Izumi, H. (1978). A rapid and simple spectrophotometric assay of angiotensin-converting enzyme. *Analytical Biochemistry*, 84(2), 361-369. http://dx.doi.org/10.1016/0003-2697(78)90053-2. PMid:204217.

Hernández-Valencia, C. G., Román-Guerrero, A., Aguilar-Santamaría, Á., Cira, L., & Shirai, K. (2019). Cross-linking chitosan into hydroxypropylmethylcellulose for the preparation of neem oil coating for postharvest storage of pitaya. *Molecules*, 24(2), 219. http://dx.doi.org/10.3390/molecules24020219. PMid:30634411.

James, J. I., Aisami, A., Maijama, M. S., Adams, B. D., & Zira, S. G. (2020). Invitro a-amylase inhibitory activity and antioxidant profile of carica papaya seed protein hydrolysate. *International Journal of Innovative Approaches in Science Research*, 4(1), 1-14. http://dx.doi.org/10.29329/ijiasr.2020.237.1.

Jiang, Y., Zhang, M., Lin, S., & Cheng, S. (2018). Contribution of specific amino acid and secondary structure to the antioxidant property of corn gluten proteins. *Food Research International*, 105, 836-844. http://dx.doi.org/10.1016/j.foodres.2017.12.022. PMid:29433280.

Kumar, A., Agarwal, D. K., Kumar, S., Reddy, Y. M., Chintagunta, A. D., Saritha, K. V., Pal, G., & Kumar, S. P. J. (2019). Nutraceuticals derived from seed storage proteins: implications for health wellness. *Biocatalysis and Agricultural Biotechnology*, 17, 710-719. http://dx.doi.org/10.1016/j.bcab.2019.01.044.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), 680-685. http://dx.doi.org/10.1038/227680a0. PMid:5432063.

Loo, S., Kam, A., Xiao, T., & Tam, J. P. (2017). Bleogens: cactus-derived anti-candida cysteine-rich peptides with three different precursor

arrangements. *Frontiers if Plant Science*, 8, 2162. http://dx.doi.org/10.3389/fpls.2017.02162. PMid:29312404.

Martínez, E. M. M., Sandate-Flores, L., Rodríguez-Rodríguez, J., Rostro-Alanis, M., Parra-Arroyo, L., Antunes-Ricardo, M., Serna-Saldívar, S. O., Iqbal, H. M. N., & Parra-Saldívar, R. (2021). Underutilized mexican plants: screening of antioxidant and antiproliferative properties of mexican cactus fruit juices. *Plants*, 10(2), 368. http://dx.doi.org/10.3390/plants10020368. PMid:33672994.

Moghadam, M., Salami, M., Mohammadian, M., Emam-Djomeh, Z., Jahanbani, R., & Moosavi-Movahedi, A. A. (2020). Physicochemical and bio-functional properties of walnut proteins as affected by trypsin-mediated hydrolysis. *Food Bioscience*, 36, 100611. http://dx.doi.org/10.1016/j.fbio.2020.100611.

Mojica, L., & Mejía, E. G. (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. http://dx.doi.org/10.1039/C5FO01204J. PMid:26824775.

Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology*, 3(1), 128-132.

Nuchprapha, A., Paisansak, S., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Reamtong, O., Choowongkomon, K., & Karnchanatat, A. (2020). Two novel ACE inhibitory peptides isolated from longan seeds: purification, inhibitory kinetics and mechanisms. *RSC Advances*, 10(22), 12711-12720. http://dx.doi.org/10.1039/D0RA00093K. PMid:35492113.

Olusola, A. O., Ekun, O. E., David, T. I., Olorunfemi, O. E., & Oyewale, M. B. (2018). Moringa oleifera seed protein hydrolysates: kinetics of α-amylase inhibition and antioxidant potentials. *Global Advanced Research Journal of Medicine and Medical Sciences*, 7(9), 190-201.

Osborne, T. B., & Campbell, G. F. (1898). Proteins of the pea. *Journal of the American Chemical Society*, 20(5), 348-362. http://dx.doi.org/10.1021/ja02067a006.

Park, H.-J., Kim, J., Kim, H. S., Lee, S.-H., Jang, J. S., & Lee, M. H. (2019). Synergistic effect of fruit – seed mixed juice on inhibition of angiotensin I-converting enzyme and activation of NO production in EA. hy926 cells. *Food Science and Biotechnology*, 28(3), 881-893. http://dx.doi.org/10.1007/s10068-018-0512-0. PMid:31093447.

Pedroche, J., Yust, M. M., Girón-Calle, J., Alaiz, M., Millán, F., & Vioque, J. (2002). Utilisation of chickpea protein isolates for production of peptides with angiotensin I-converting enzyme (ACE) -inhibitory activity. *Journal of the Science of Food and Agriculture*, 82(9), 960-965. http://dx.doi.org/10.1002/jsfa.1126.

Poms, R. E., Capelletti, C., & Anklam, E. (2004). Effect of roasting history and buffer composition on peanut protein extraction efficiency. *Molecular Nutrition & Food Research*, 48(6), 459-464. http://dx.doi.org/10.1002/mnfr.200400052. PMid:15508181.

Ramírez-Rodríguez, Y., Martínez-Huélamo, M., Pedraza-Chaverri, J., Ramírez, V., Martínez-Tagüeña, N., & Trujillo, J. (2020). Ethnobotanical, nutritional and medicinal properties of Mexican drylands Cactaceae fruits: recent findings and research opportunities. *Food Chemistry*, 312, 126073. http://dx.doi.org/10.1016/j.foodchem.2019.126073. PMid:31901824.

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. http://dx.doi.org/10.1016/S0891-5849(98)00315-3. PMid:10381194.

Sandate-Flores, L., Romero-Esquivel, E., Rodríguez-Rodríguez, J., Rostro-Alanis, M., Melchor-Martínez, E. M., Castillo-Zacarías, C., Ontiveros, P. R., Celaya, M. F. M., Chen, W. N., Iqbal, H. M. N., & Parra-Saldívar, R. (2020). Functional attributes and anticancer potentialities of chico (Pachycereus weberi) and jiotilla (escontria chiotilla) fruits extract. *Plants*, 9(11), 1623. http://dx.doi.org/10.3390/plants9111623. PMid:33266445.

Segura-Campos, M. R. (2020). Isolation and functional caharacterization of chia (Salvia hispanica) proteins. *Food Science and Technology*, 40(2), 334-339. http://dx.doi.org/10.1590/fst.41618.

Singh, A., Kumar, Y., & Arora, B. (2021). Storage proteins profile in diploid and tetraploid seeds of oryza sativa L. *Cutting-Edge Research in Agricultural Sciences*, 8, 41-52. http://dx.doi.org/10.9734/bpi/cras/v8/8102D.

Sun, C., Tang, X., Ren, Y., Wang, E., Shi, L., Wu, X., & Wu, H. (2019). Novel antioxidant peptides purified from mulberry (*Morus atropurpurea* Roxb.) leaf protein hydrolysates with hemolysis inhibition ability and cellular antioxidant activity. *Journal of Agricultural and Food Chemistry*, 67(27), 7650-7659. http://dx.doi.org/10.1021/acs.jafc.9b01115. PMid:31241944.

Takahashi, K., Kohno, H., Kanabayashi, T., & Okuda, M. (2019). Glutelin subtype-dependent protein localization in rice grain evidenced by immunodetection analyses. *Plant Molecular Biology*, 100(3), 231-246. http://dx.doi.org/10.1007/s11103-019-00855-5. PMid:30911876.

Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., & Wichers, H. (2015). *The impact of food bioactives on health: in vitro and ex vivo models*. Cham: Springer. http://dx.doi.org/10.1007/978-3-319-16104-4.

Visvanathan, R., Jayathilake, C., & Liyanage, R. (2016). A simple microplate-based method for the determination of α-amylase activity using the glucose assay kit (GOD method). *Food Chemistry*, 211, 853-859. http://dx.doi.org/10.1016/j.foodchem.2016.05.090. PMid:27283705.

Wang, X., Qiu, N., & Liu, Y. (2018). Effect of different heat treatments on in vitro digestion of egg white proteins and identification of bioactive peptides in digested products. *Journal of Food Science*, 83(4), 1140-1148. http://dx.doi.org/10.1111/1750-3841.14107. PMid:29577301.

Pan American Health Organization – PAHO, World Health Organization – WHO. (2021). *Diabetes*. Washington, D.C.: PAHO/WHO. Retrieved from https://www.paho.org/en/topics/diabetes.

Wong, F.-C., Xiao, J., Wang, S., Ee, K.-Y., & Chai, T.-T. (2020). Advances on the antioxidant peptides from edible plant sources. *Trends in Food Science & Technology*, 99, 44-57. http://dx.doi.org/10.1016/j.tifs.2020.02.012.

Yousr, M., & Howell, N. (2015). Antioxidant and ACE inhibitory bioactive peptides purified from egg yolk proteins. *International Journal of Molecular Sciences*, 16(12), 29161-29178. http://dx.doi.org/10.3390/ijms161226155. PMid:26690134.

Zhuang, H., Tang, N., & Yuan, Y. (2013). Purification and identification of antioxidant peptides from corn gluten meal. *Journal of Functional Foods*, 5(4), 1810-1821. http://dx.doi.org/10.1016/j.jff.2013.08.013.