Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-022-01618-5

ORIGINAL PAPER

Stability of antioxidant and hypoglycemic activities of peptide fractions of Maize (*Zea mays* L.) under different processes

Jennifer Vianey Félix-Medina[1,2] · Adilene Guadalupe Sepúlveda-Haro[1] · María Fernanda Quintero-Soto[2]

Received: 5 February 2022 / Accepted: 6 September 2022

Abstract
Maize proteins are considered of low nutritional value, these proteins when hydrolyzed become a good source of peptides with important biological activity and can be used as supplements or food additives. The objective of this research was to prepare bioactive fractions of maize proteins and evaluate the effect of digestive enzymes (pepsin and pancreatin), temperature and pH on their antioxidant, hypoglycemic activities and their techno-functional properties. In a first stage, the biological activities of total protein hydrolysates of maize, zeins and non-zeins were evaluated. It was observed that the hydrolysate with the highest values of antioxidant (ABTS = 37.09%; DPPH = 33.76%) and hypoglycemic (α-amylase = 46.14%: α-glucosidase = 46.80%) activity was the total protein hydrolysate. In a second stage, the total protein hydrolyzate was fractionated by ultrafiltration to obtain four fractions of different molecular weight (> 10 kDa, 5–10 kDa, 3–5 kDa, and < 3 kDa). The fraction < 3 kDa stood out for presenting better values of ABTS (64.68%), DPPH (40.09%), α-amylase (55.22%) and α-glucosidase (37.21%). This fraction conserved its biological activity when subjected to gastrointestinal digestion and sudden changes in temperature and pH. In addition, this fraction has good techno-functional properties and could be used as a supplement or additive in food formulation.

Keywords Enzymatic hydrolysis · In vitro gastrointestinal digestion · Techno-functional properties · α-Amylase inhibition activity · α-Glucosidase inhibition activity

Introduction

Maize (*Zea mays* L.) is one of the main cereals consumed worldwide. This cereal has a considerable amount of carbohydrates, proteins and bioactive compounds; which give it a nutritional and nutraceutical potential [1, 2]. Currently, proteins of vegetable origin are not only consumed in their entirety but also as peptides, because being hydrolyzed increases their nutraceutical potential [3–5]. Purifying the peptides present in hydrolysates is very time consuming and expensive. So it is necessary to look for alternative analyses to reduce the number of peptides. The fractionation of protein hydrolysates by ultrafiltration is an economical technique and widely used by a large number of researchers around the world [6–8]. Oseguera-Toledo et al. [6] and Vilcacundo et al. [9] evaluated the hypoglycemic activity (HA) of peptide fractions of different molecular weight in common beans (< 1, 1–3, 3–5, 5–10 and > 10 kDa) and quinoa (< 5 and > 5 kDa) observing better activity as the molecular weight of the fractions decreased. On the other hand, Hu et al. [10] elaborated peptide fractions of maize gluten proteins (< 1, 1–3, 3–5, 5–10 and > 10 kDa) hydrolyzed with papain (pH 6.5), ficain (pH 6.0) and bromelain (pH 5.0). Tang and Zhuang [11] evaluated peptide fractions of maize zeins (< 1, 1–3, 3–5, 5–10 and > 10 kDa) hydrolyzed with alkaline protease (pH 9.0), trypsin (pH 8.0), papain (pH 7.0) and flavorzyme (pH 7.0). Both studies used different proteases and hydrolysis conditions, but both agree that the diverse maize proteins are a good alternative for the production of antioxidant peptides. They also observed that most alkaline enzymes were those that generated a greater number

✉ María Fernanda Quintero-Soto
maryferquintero@gmail.com; mquintero@upmys.edu.mx

[1] Ingeniería Bioquímica, Tecnológico Nacional de México-Instituto Tecnológico de Culiacán, Calle Juan de Dios Bátiz 310 pte, Colonia Guadalupe, CP 80220 Culiacán de Rosales, Sinaloa, Mexico

[2] Ingeniería Agroindustrial, Universidad Politécnica del Mar y la Sierra, Carretera a Potrerillos del Norote Km 3, CP 82700 La Cruz, Elota, Sinaloa, Mexico

Published online: 24 September 2022

of peptides with antioxidant activity (AA), showing the antioxidant potential of maize peptide fractions.

The protein hydrolysis process promotes the generation of peptides with good techno-functional properties; the most important properties of hydrolisates and their peptides is the solubility, because this define their applications in the food and pharmaceutical industries [12–14]. These peptide fractions could be used as supplements or food additives having a double effect, protecting food against degradation by oxidation and generating a beneficial effect on the health of the consumer. For that reason, it is important evaluating the stability of the biological activities of peptide fractions after different processes that could generate a negative effect on the activity. The objective of this study was to evaluate the effect of digestive enzymes, temperature, pH on antioxidant, and hypoglycemic activities of peptide fractions of maize and their functional properties.

Materials and methods

Materials

Maize (*Zea mays* L.) grains were grown in Guamúchil, Sinaloa, Mexico (Fall–winter 2019–2020) and provided by GRACO S.A grain trader. The grains were stored at 4 °C until analysis.

Chemicals and reagents

Folin & Ciocalteu's reagent (F9252), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] (10,102,946,001), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (D9132), Alcalase 2.4 L (P4860), Pepsin (P7000), Pancreatin (P7545), DPP4 inhibitor screening kit (MAK203), α-amylase (A3176) and α-glucosidase (G5003) were purchased from Sigma Aldrich® (St. Louis, MO, USA). Molecular weight cut-off (MWCO) centrifugal tubes of 3 kDa (VS15T92), 5 kDa (VS15T12) and 10 kDa (VS15T02) from Sartorius® (Göttingen, Germany). Micro BCA™ Protein Assay Kit (23,227) and cellulose membrane of 10 kDa (88,245) was purchased from Thermo Fisher Scientific.

Extraction and quantification of maize proteins

The total protein and the fractions of zeins and non-zeins were extracted as described by Wallace et al. [15] modifications. The total protein was obtained by mixing 200 g of previously defatted flour and 1 L of borate buffer [$Na_2B_4O_7{\cdot}10H_2O$ 12.5 mM, pH 10.0, SDS 1% (w/v), β-mercaptoethanol 2% (v/v)] and incubating for 2 h at 37 °C, followed by sonication for 30 min. After the sonication time the samples were centrifuged and the supernatant obtained was placed on a 10 kDa cellulose membrane and dialyzed against water for 48 h with water changes every 8 h. After dialysis the sample was lyophilized (total protein) (25EL, VirTis Co., Gardiner, NY, USA) and stored until use. The total protein (8 g) was mixed with 100 mL of 70% ethanol and incubated for 2 h at 37 °C, followed by another centrifugation to separate the proteins into zeins (supernatant) and non-zeine (precipitate). The zein extract and the non-zeins tablet were freeze-dried and stored at − 20 °C until further use.

The content of total protein, zeins and non-zeins, was determined by bicinchoninic acid (BCA) assay [3]. Samples (0.02 mL) were combined with BCA working reagent [0.2 mL; BCA stock solution: 4% $CuS0_4{\cdot}5H_20$(50:1)] and incubated at 37 °C for 30 min before reading at 550 nm. The concentration of protein was calculated using bovine serum albumin as a standard. The test was performed fivefold.

Preparation of protein hydrolysates and peptide fractions and determination of the degree of hydrolysis

First, the total protein, zeins and non-zeins lyophilized were washed at a controlled temperature with cold methanol and acetone (− 20 °C) to remove other phenolic compounds as indicated by Quintero-Soto et al. [3]. The washes were repeated until the presence of phenolic compounds in the supernatant was not detected when analyzed with the Folin–Ciocalteau reagent. Subsequently, the dry and clean samples (equivalent to 5 g of protein) were resuspended in water (50 mL; pH 8) and placed at 85 °C for 5 min. After the incubation time the samples were taken to a temperature of 50 °C and alcalase (1.5 U) was added keeping a constant temperature and pH for 90 min (50 °C; pH 8.0). Temperature rose to 80 °C for 20 min and samples were centrifuged at 5000 g per 20 min. The supernatant was passed through 0.45 µm PVDF membrane filters (Pall Corp., Port Washington, NY, USA). The filtrate (complete hydrolysate) obtained was concentrated at 1 mg/mL; the AA and HA were determined as described below.

To prepare the bioactive fractions, the complete hydrolysate was used, which showed better values of HA and AA (total protein hydrolyzed), and centrifugal tubes of molecular cutting of 3 kDa, 5 kDa and 10 kDa. The complete hydrolysate was placed in tubes of 10 kDa and centrifuged (40 min; 4000 g). The remnant (> 10 kDa) was stored and the filtrate was placed in 5 kDa tubes and centrifuged again; this procedure was repeated using the 3 kDa tubes. In total, 4 fractions were obtained (> 10 kDa, 5–10 kDa, 3–5 kDa and < 3 kDa). The fractions were concentrated at 1 mg/mL and their AA and HA were determined as described

below. The degree of hydrolysis (DH) of hydrolysates was determined as reported by Xu et al. [12].

Peptide sequence of the total protein fraction < 3 kDa

The peptides were identified from the fraction with the best biological activities. A 50 µL aliquot was separated by Agilent 1100 HPLC–DAD coupled to LC/MSD (Agilent Technologies, USA) using the method described by Quintero-Soto et al. [3]. 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 maize (*Zea mays* L.) 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. Identification was carried out using three independent samples.

Evaluation of antioxidant and hypoglycemic activity

AA was determined by the colorimetric methods ABTS and DPPH as described by Quintero-Soto et al. [3]. The ABTS radical solution (7.4 mmol/L) was prepared by mixing ABTS and potassium persulfate (2.6 mmol/L), followed by incubation overnight (16 h) in the dark at room temperature. The ABTS radical solution was diluted with phosphate buffer (10 mmol/L, pH 7.4) to obtain an absorbance of 0.70 at 734 nm. The solution (0.2 mL) was mixed with 0.02 mL of sample and incubated at room temperature for 6 min before reading at 734 nm. For DPPH, sample (0.05 mL) was mixed with 0.05 mL of DPPH radical solution (0.1 mmol/L in ethanol) and incubated at room temperature for 30 min before reading at 510 mn. Both assays were performed in the dark and the resulting values were expressed as percent inhibition.

The inhibition of the enzymes α-amylase and α-glucosidase was evaluated as reported by Mojica et al. [16] Equal parts of sample and α-amylase solution (10 U/mL) were mixed and incubated (10 min, 25 °C), followed by addition of starch solution (0.05 mL, 10/L) and incubation (10 min, 25 °C). Subsequently, the sample was placed in a water bath (100 °C, 5 min) in the presence of dinitrosalicylic acid (0.025 mL). Finally, the sample was diluted with distilled water (0.25 mL), and the absorbance was read at 520 nm. For α-glucosidase, the α-glucosidase solution (0.05 mL, 1 U/mL) and 0.025 mL of sample were mixted and incubated (10 min, 25 °C). Subsequently, p-nitrophenyl-α-D-glucopyranoside (0.025 mL, 5 mmol/L) was added and the mixture was incubated (5 min, 25 °C) before reading at 405 nm. The results for α-amylase and α-glucosidase were calculated in relation to acarbose (1 mmol/L) and were expressed as percent inhibition.

Stability study of the better peptide fraction

Stability against gastrointestinal digestion in vitro

The bioactive fraction with the best characteristics was again subjected to hydrolysis simulating gastrointestinal digestion to know the stability of the peptides present in the hydrolysate against digestive enzymes. Enzymatic hydrolysis was performed according to Ramírez et al. [17] with modifications. The dry and clean samples (equivalent to 1 g of protein) were resuspended in 20 mL of the pepsin solution (0.75 mg of pepsin/mL of HCl 0.1 M; pH 2.0) and incubated at 37 °C for 3 h. Subsequently the pH was adjusted to 8.0 with NaOH 0.2 N to stop hydrolysis. For the second hydrolysis the samples were mixed with 7 mL of pancreatin solution (5 mg pancreatin/mL phosphate buffer pH 8.0) and incubated again (37 °C; 3 h). Finally, the reaction was stopped (80 °C; 20 min) and the samples were centrifuged (5000 g; 10 min), recovering the supernatant (< 3 kDa P + P). The hydrolysate obtained was concentrated at 1 mg/mL and the biological activities were determined again.

Stability against temperature and pH

The stability of the bioactive fraction against temperature and pH was determined as described by Jang et al. [7]. The sample was incubated at 30, 50, 70, and 90 °C for 10 min. Later, the samples were allowed to cool to room temperature and the pH was adjusted to 7.0. Separately, the sample was also incubated at 2, 4, 6, 8, and, 10 pH value (25 °C, 1 h). Subsequently, the pH was adjusted to 7.0 (1 M HCl or NaOH). AA and HA were then measured as described above.

Techno-functional properties of the better peptide fraction

The protein solubility of the better peptide fraction was determined at pH 2, 4, 6, 8 and 10 following what was reported by Nalinanon et al. [18]. Samples (5 mg) were dispersed in deionised water (4 mL) and pH of the mixture was adjusted to 2, 4, 6, 8, and 10 (1 M HCl or 1 M NaOH). The samples were stirred at 25 °C for 30 min. The volume of samples were made up to 5 mL by distiled water and centrifugated at 5000 g for 15 min. Protein content in the supernatant was measured as described above. Protein solubility was calculated using the following formula:

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times (100)$$

Foam formation (FF) and stability (FS) were determined at a concentration of 1% according to that reported by Nalinanon et al. [18]. Samples (10 mL) were placed in 50 mL-tubes and homogenized at 10,000 rpm for 1 min at room temperature. The samples were allowed to stand for 60 min. FE and FS were then calculated using the following formula:

$$\text{FE (\%)} = \frac{\text{VT}}{\text{Vt}} \times 100$$

$$\text{FS (\%)} = \frac{\text{Vt}}{\text{V0}} \times 100$$

where VT is total volume after whipping; Vt is the original volume before whipping; and V0 is total volume after leaving at room temperature for 60 min.

Emulsifying activity index (EAI) and emulsion stability index (ESI) were evaluated at 1% as described by Xu et al. [12]. Samples (5 mL) were mixed with maize oil (1 mL) and the mixture was homogenized at 10,000 rpm for 2 min. Fifty microliters emulsion were collected from near the bottom of the tube at 0 and 10 min after homogenization and the aliquot was dispensed into 5 mL sodium dodecyl sulphate solution (0.1%). The absorbance of diluted samples was measured at 500 nm. EC and ES were calculated using the following formula:

$$\text{EAI}\left(\frac{\text{m}^2}{\text{g}}\right) = \frac{(2)\,(2.303)\,(\text{A}_0)\,(\text{dilution factor})}{(\phi)\,(\text{protein concentration})\,(1000)}$$

$$\text{ESI (min)} = \frac{\text{A}_0}{\Delta\text{A}} \times (\text{t})$$

where A_0 is the absorbance of the diluted emulsion at 0 min after the homogenization; dilution factor = 100; ϕ is the fraction of oil to form the emulsion (0.17); ΔA is the change in the absorbance between 0 and 10 min ($A_0 - A_{10}$), and t is the time interval between initial and final measurements (10 min).

Statistical analysis

Statistical analysis consisted of a unifactorial design using the STATGRAPHIC plus version 5.1 program (Statistical Graphics Corporation TM, Rockville, Maryland, USA). The fisher test was used with a significance level of 5% to compare the means between hydrolysates of the same type/bioactive fractions.

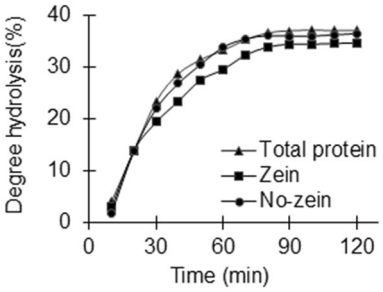

Fig. 1 Degree hydrolysis as a function of time the enzymatic hydrolysis

Results and discussion

Total protein content, maize zeins and non-zeins

Cereals such as maize are known to have relatively low protein content. Félix-Medina et al. [19] report values of 6.25 g/100 g of flour for the same variety of maize analyzed in this study (DEKABL-2038) and cultivated in Culiacán, Sinaloa. On the other hand, Gonzalez-Gongora et al. [20] observed values of 9.90 g/100 g of flour in the FR-28 maize variety cultivated in Buenaventura, Cuba. The values of total protein content (7.07 g/100 g) observed in this study were in line with those reported by the researchers mentioned above. The differences observed by Gonzalez-Gongora et al. [20] could be attributed to genetic differences between the maize studied and also the environmental conditions in which they were grown.

It is possible to group maize proteins in zeins and non-zeins. In this study, the content of these fractions was determined (zeins = 66.83 g/100 g of protein; non-zeins = 37.95 g/100 g of protein) obtaining observed values that are within the range reported by Moro et al. [21] for 93 maize accessions (52.30–68.14 g/100 g for zeins and 14.13–33.83 g/100 g for non-zeins). The fraction of zeins is the majority in the variety of maize studied ($p = 0.05$). It has been reported that this fraction is poor in the amino acids lysine and tryptophan but rich in other essential amino acids such as histidine and leucine [22]. These amino acids present important activities when released from the protein [3].

Characterization of protein hydrolysates

Degree of hydrolysis

The production of hydrolysates with endogenous enzymes such as alcalase is a technique that has been widely used by other researchers to produce hydrolysates [3, 8, 23]. Herein, the total proteins (TPH), zeins (ZH) and non-zeins (NZH) of maize were hydrolyzed. During the first 50 min a high rate

of hydrolysis is observed (Fig. 1), then the hydrolysis rate kept stable reaching a DH of 35% at min 90.

Antioxidant activity of protein hydrolysates

The AA of hydrolysates were shown in Table 1. NZH (38.24%) was the one that presented the highest percentage of inhibition for the ABTS radical. This value was comparable to those observed by Quintero-Soto et al. [3] in hydrolysates of chickpea globulins hydrolyzed with alcalase (32.9% to 40.3%). On the other hand, the current values were higher than those observed by Jin et al. [24] who evaluated AA by ABTS in hydrolyzed maize gluten hydrolysates also with alcalase (42.32%; 5 mg/mL). This difference was mostly related to the fact that the DH reported by Jin et al. [24] was lower than the current one mostly corroborated with AA [6, 25]. As for the AA by the DPPH assay, values of 33.76%, 26.75%, and 30.74% were obtained for TPH, ZH, and NZH, respectively. These values was lower than those obtained by Li et al. [26] in total protein and maize zeins (40%) hydrolyzed with alcalase. In addition, these values was similar to those reported by Memarpoor-Yazdi et al. [27] for Zizyphus Jujuba protein hydrolyzed with trypsin (33.1%), papain (22.4%), and the trypsin-papain combination (26.7%). The decrease in AA when combining trypsin and papain demonstrates that this is not always a good option [27], because it could generate the production of free amino acids, which have little or no activity.

Hypoglycemic activity of protein hydrolysates

Diabetes is a disease of global importance that can be controlled with various treatments [16]. An alternative is the use of peptide hydrolysates that inhibit the action of enzymes involved in carbohydrate metabolism [1, 3]. No significant difference ($p = 0.5$) was observed between the percentages of α-amylase inhibition of the 3 hydrolysates analyzed (Table 1). These results were lower than those obtained by Vilcacundo et al. [9] in quinoa protein hydrolysate by gastrointestinal simulation. The differences observed between these results could be due to the different hydrolysis conditions as well as the nature of the protein and enzymes used. On the other hand, Connolly et al. [28] produced protein hydrolysates with residual barley grains obtained after the brewing process. These authors used the same enzyme as in this study (alcalase) at different temperature conditions (60 °C) and pH (7 and 9), obtaining percentages of inhibition of the enzyme α-glucosidase lower than those obtained in this study (pH 7 = 20% y pH 9 = 5%; 2.5 mg/mL). ZH had the lowest percentage of α-glucosidase inhibition. Uraipong and Zhao [29] also reported a lower inhibition of α-glucosidase by zeins fraction (prolamins) of rice.

Characterization of bioactive fractions of total protein hydrolysates

In vitro *antioxidant and hypoglycemic activities of bioactive fractions*

The fractionation of protein hydrolysates by ultrafiltration is a widely used technique to reduce the number of peptides in samples [6]. The fraction < 3 kDa was the one that presented the greatest activity (64.68% and 40.46% for ABTS and DPPH, respectively). Similar results have been reported by other researchers [6, 12]. Hu et al. [10] evaluated the AA of maize gluten peptide fractions with different molecular weights, observing ABTS values of 52%, 55%, and 57% for the 1–3 kDa (1 mg/mL) fraction hydrolyzed with papain, ficain, and bromelain, respectively. Hu et al. [10] also determined the AA of peptide fractions (5 mg/mL) by DPPH, observing values of 80–90% for hydrolyzed fractions with papain; 35–55% for fractions hydrolyzed with ficain; and 40–78% for fractions hydrolyzed with bromelain. These values are lower than those reported in this study (Table 2), demonstrating the efficiency of the alcalase enzyme in the production of peptides with AA. This is because the enzyme used in this study works well in the alkaline conditions, and it has also been observed that most alkaline enzymes were those that generated a greater number of peptides with AA [11].

The capacity of bioactive fractions to inhibit the enzyme α-amylase and α-glucosidase is shown in Table 2. The inhibition percentages for α-amylase were around 50% for the 4 fractions evaluated. These values are similar to those observed by Vilcacundo et al. [9] for a peptide fraction of quinoa < 5 kDa (IC50 = 1.09 mg/mL) and lower than those

Table 1 In vitro antioxidant and hypoglycemic activity of protein hydrolysates

Hydrolysate[a]	ABTS[b]	DPPH[b]	α-Amilasa[b]	α-Glucosidasa[b]
Total protein	37.09 ± 1.07^{a}	33.76 ± 1.75^{a}	46.14 ± 1.96^{a}	46.80 ± 2.56^{a}
Zein	33.58 ± 3.03^{b}	26.75 ± 1.08^{c}	47.20 ± 1.03^{a}	36.13 ± 2.37^{b}
Non-zein	38.24 ± 2.59^{a}	30.74 ± 1.22^{b}	46.19 ± 1.08^{a}	40.37 ± 0.54^{c}

[a]Hydrolysates were evaluated at a concentration of 1 mg/mL

[b]Results are reported as percent radical/enzyme inhibition. Different letters in the same column indicate significant differences ($p < 0.05$) between the means according to Fisher test

Table 2 In vitro antioxidant and hypoglycemic activity of peptide fractions of the total protein hydrolyzate

Fraction[a]	ABTS[b]	DPPH[b]	α-Amylase[b]	α-Glucosidase[b]
> 10 kDA	28.06 ± 1.57[d]	29.96 ± 0.49[c]	46.37 ± 1.64[c]	30.85 ± 0.90[c]
5–10 kDa	32.61 ± 0.75[c]	34.40 ± 1.89[b]	47.79 ± 0.41[c]	31.37 ± 0.40[c]
3–5 kDa	45.20 ± 1.61[b]	35.48 ± 2.54[b]	50.09 ± 0.66[b]	33.38 ± 1.00[b]
< 3 kDa	64.68 ± 1.58[a]	40.09 ± 0.46[a]	55.22 ± 1.28[a]	37.21 ± 1.09[a]

[a]Fractions were evaluated at a concentration of 1 mg/mL

[b]Results are reported as percent radical/enzyme inhibition. Different letters in the same column indicate significant differences ($p < 0.05$) between the means according to Fisher test

reported by Quintero-Soto et al. [3] for 6 peptide fractions (34.9 to 66.6%) of chickpea purified by reverse phase chromatography and evaluated at 0.1 mg/mL. Oseguera-Toledo et al. [6] determined the percentages of α-glucosidase inhibition of 5 peptide fractions of pinto beans, observing values of 58% (> 10 kDa, 56% (5–10 kDa), 65% (3–5 kDa), 70% (1–3 kDa) and 80% (< 1 kDa) (0.1 mg/mL). These values are higher than those observed in this study (Table2). This suggests that peptides generated from legume proteins (chickpeas and beans) may have a better affinity for the enzymes α-amylase and α-glucosidase compared to peptides generated from cereals or pseudocereals (maize and quinoa).

Peptide sequence of the total protein fraction < 3 kDa

Table 3 show the 12 peptides sequence identified in the fraction < 3 kDa. All peptides identified showed potential as inhibitors of ACE and DPP4. Furthermore, the peptides released from the 19 kDa alpha-zein protein showed potential as antioxidants. Various researchers have reported that the most common bioactivities of alcalase-generated peptides are ACE and DPP4 inhibition [3, 6, 16]. The peptides PTATPY and HHIMAGAD showed the potential to inhibit the enzyme α-glucosidase. These peptides were released from the 27 kDa gamma-zein and GBSS proteins. The HHIMAGAD peptide is of interest, because it has the most bioactivities (ACE inhibitor, DPP4 inhibitor, α-glucosidase inhibitor, antioxidant, and anti-inflammatory). All peptides identified or part of their sequence had already been identified by other researchers in different foods [3, 4, 6, 10, 12, 14, 16, 30].

The peptides identified were mostly of low molecular weight (< 10 Da), hydrophilic (negative grand average of hydropathicity values), and low aliphatic [31, 32]. These characteristics are important in the future uses of these peptides in the industry.

Table 3 Peptide profile of the fraction < 3 kDa of the total protein hydrolyzate

Peptide	PM (Da)	pI	AI	GRAVI	Possible activity	Parental protein
PTATPY	648.3105	5.17	16.67	− 0.683	ACE, DPP4, Anti-inflammatory	27 kDa gamma-zein
VGGNW	531.2435	5.69	58.00	− 0.200	ACE, DPP4	TPI
NPAAY	534.2431	5.27	40.00	− 0.560	ACE, DPP4, AOX	19 kDa alpha-zein
VQTIRAQQL	1055.6070	11.11	130.00	− 0.156	ACA, DPP4, AOX	19 kDa alpha-zein
QGMRY	653.2947	9.60	0.00	− 1.560	ACE, DPP4, AOX	GBSS
GTPCACAS	708.2563	5.01	25.00	0.637	ACE, DPP4, AOX	GBSS
MQTHS	602.2475	7.66	0.00	− 1.260	ACE, DPP4	Brittle-1
ETAAF	537.2427	3.14	40.00	0.440	ACE, DPPA, AOX	16.9 kDa HSP
AAANRAS	659.3342	11.18	57.14	− 0.229	ACE, DPPA, AOX	19 kDa alpha-zein
QQQCCHQIRQ	1270.5613	8.22	39.00	− 1.570	ACE, DPP4, AOX	16 kDa gamma-zein
HHIMAGAD	850.9512	5.97	73.75	− 0.039	ACE, DPP4, α-Glu, AOX, Anti-inflammatory	GBSS
VEEDL	603.2741	2.90	136.00	− 0.500	ACE, DPP4, α-Glu	G3PC1

The isoelectric point (IP), aliphatic index (AI), and grand average of hydropathicity (GRAVI) of the peptides were obtained from Expaxy Server-ProtParam (https://web.expasy.org/protparam/). The possible activities of the peptides 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.

pM molecular weight. *ACE* Angiotensin-converting enzyme inhibitors, *DPP4* Dipeptidyl-peptidase-IV inhibitors, *AOX* antioxydant, *α-Glu* α-glucosidase inhibitors. *TPI* triosephosphate isomerase, *GBSS* granule-bound starch synthase I, *HSP* 16.9 kDa heat shock protein, *G3PC1* glyceraldehyde-3-phosphate dehydrogenase 1

Stability of the biological activity of the total protein fraction < 3 kDa

The fraction of the total protein hydrolyzate < 3 kDa was selected for presenting the best AA and HA. The results obtained are shown in Fig. 2. No significant difference (p = 0.05) was observed in AA (ABTS and DPPH) and HA (inhibition of α-amylase and α-glucosidase) before and after gastrointestinal digestion; which indicates that the active structures of the peptides were not compromised by intestinal proteases.

The stability of bioactive components in protein hydrolysate must be ensured during the formulation or preparation of the food [7]. Jang et al. [7] reported a decrease in AA at temperatures above 50 °C, contrary to what was observed in this study where AA by the two methods used (ABTS and DPPH) remained at 97% or higher (101%) after being subjected to different temperatures (Fig. 3). Similarly, the ability to inhibit enzymes α-amylase and α-glucosidase remained stable, with slight increases and decreases observed without having a statistically significant effect (p = 0.05). The stability of the biological activities of the < 3 kDa fraction against temperature could be due to the fact that temperatures of up to 80 °C are used in the preparation of the hydrolysates.

The pH plays an essential role in the biological activity of hydrolysates, since it strongly influences the interactions between the amino acids of the peptides causing the loss of the structure and consequently its chemical properties and biological activities [33]. No significant difference in AA by ABTS was observed after subjecting the peptide fraction to different pH (p = 0.05) (Fig. 4). This could be because the bioactive region of the peptides was not affected or the new structures formed have the ability to capture the ABTS radical similar to the initial structure. On the other hand, AA by

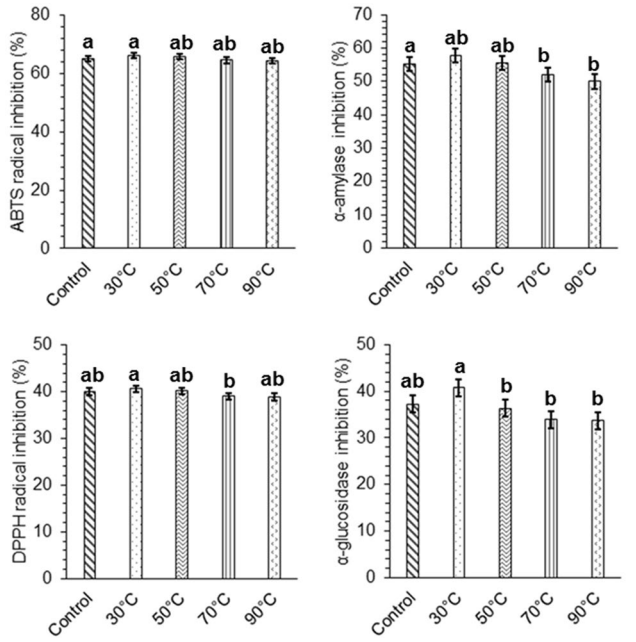

Fig. 3 Stability of biological activity of < 3 kDa fraction (control) under different temperature

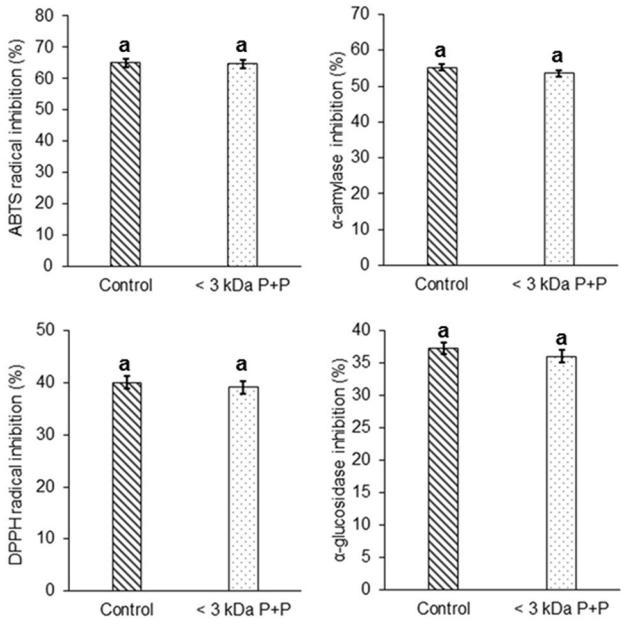

Fig. 2 Stability of biological activity of < 3 kDa fraction (control) during simulated gastrointestinal digestion. < 3 kDa P + P: fraction < 3 kDa after digestion with pepsin and pancreatin

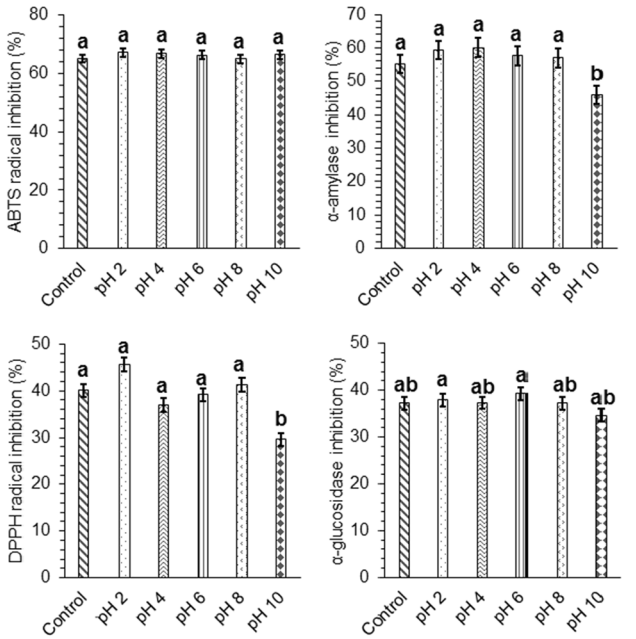

Fig. 4 Stability of biological activity of < 3 kDa fraction (control) under different pH

Table 4 Techno-functional properties of the fraction < 3 kDa of the total protein hydrolyzate

Techno-functional properties	< 3 kDa
Water solubility* (%)	
pH 2	96.36 ± 1.19[a]
pH 4	95.48 ± 1.20[a]
pH 6	96.89 ± 1.26[a]
pH 8	97.73 ± 2.11[a]
pH 10	97.97 ± 1.00[a]
Foam formation (%)	201.66 ± 7.63
Foam stability (%)	52.57 ± 4.53
Emulsion activity index (m^2/g)	197.87 ± 12.03
Emulsion stability index (min)	38.42 ± 0.98

*Different letters in the same column for de same propertie indicate significant differences ($p < 0.05$) between the means according to Fisher test

DPPH and inhibition of α-amylase was significantly affected at pH 10 ($p = 0.05$), these activities showed a decrease of 26% and 7% for DPPH and α-amylase, respectively. This could be attributed to the basic pH. The hydroxide ion can attract charged peptides and break hydrogen bonds, causing the loss of biological activity [33].

Techno-functional properties of < 3 kDa peptide fraction

The techno-functional properties of the peptide fraction < 3 kDa is shown in Table 4. The protein solubility values for the different pH evaluated (pH 2, 4, 6, 8 and 10) were above 95%. The lowest solubility value was found at pH 4 (95.48%) and the highest at pH 10 (97.97%), however, no significant difference was observed between these values ($p = 0.05$). The latter were comparable to those reported by Nalinanon et al. [18] for fish muscle protein hydrolysates with different DH (71.2–99.3%) and Zheng et al. [34] for maize gluten hydrolysates (91.4–94.6%). The results observed indicated that the pH didn't affect the solubility of the peptide fraction analyzed, because the peptides were mostly polar amino acids with the ability to form hydrogen bonds with water (Table 3). This suggests that this fraction < 3 kDa can be used as an additive in the formulation of foods with a wide pH range.

FF is influenced by three factors: transport, diffusion and reorganization of molecules [35]. Zheng et al. [34] observed FF values between 100 and 200% for maize gluten hydrolysates. These values are comparable with those obtained for the total protein fraction < 3 kDa. After repose (60 min) the foam decreased by 50%. This could be attributed to the fact that the peptides in the fraction are of low molecular weight (Table 3), which prevents the formation of a continuous film of proteins at the water/air interface to stabilize the foam.

The EAI of the total maize protein fraction < 3 kDa was relatively good compared to the ones reported for fish muscle hydrolysates (5–90 m^2/g) and chickpea protein hydrolysates (85–213 m^2/g) [12, 35]. The elevated EAI reflects the solubility of the fraction, since the protein must be soluble to migrate to the interface and be located in it to allow a correct diffusion of the peptides towards the water/oil interface [12] (Table 3). The stability of the emulsion was relatively null, over the course of 38 min the phases in the emulsion separated completely. This is attributed to the fact that the peptides is not amphipathic enough to generate good emulsion stability (Table 3).

Conclusion

The maize that was grown in the state of Sinaloa has adequate protein contents (zeins and non-zeins), and when hydrolyzed, it becomes a good source of peptides with biological activity (ACE inhibitor, DPP4 inhibitor, α-glucosidase inhibitor, antioxidant, and anti-inflammatory). The process of hydrolysis of maize proteins with alcalase and fractionation by ultrafiltration, produced bioactive fractions with the ability to inhibit free radicals (ABTS and DPPH) and enzymes involved in the metabolism of carbohydrates (α-amylase, α-glucosidase). These activities showed good stability against digestive enzymes (pepsin and pancreatin) and sudden changes in temperature and pH. In addition, the bioactive fractions of low molecular weight (< 3 kDa) showed good techno-functional properties, which makes them suitable to be used as a supplements or food additives in the development of new products.

Author contributions JVFM: investigation, methodology, data curation, writing—original draft. AGSH: methodology, data curation. MFQS: conceptualization, supervision, investigation, methodology, data curation, writing—review & editing.

Funding This work was supported by Universidad Politécnica del Mar y la Sierra Grant No. UPMYS/2021.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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