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Identification of bioactive peptides released from *in vitro* gastrointestinal digestion of yam proteins (*Dioscorea cayennensis*)

Edilza Silva do Nascimento [a,b], Katya Anaya [c], Julia Mariano Caju de Oliveira [b], José Thalles Jocelino Gomes de Lacerda [d], Michael Edward Miller [e], Meriellen Dias [f], Maria Anita Mendes [f], Juliana de Azevedo Lima Pallone [g], Clarice Weis Arns [e], Maria Aparecida Juliano [d], Tatiane Santi Gadelha [h], Maria Teresa Bertoldo Pacheco [i], Carlos Alberto de Almeida Gadelha [a,b,*]

[a] *Department of Food Engineering, Post-Graduate Program in Food Science and Technology, Technology Center, Federal University of Paraiba, João Pessoa, PB, Brazil*
[b] *Department of Molecular Biology, Laboratory of Structural Proteomics, Federal University of Paraíba, João Pessoa, PB, Brazil*
[c] *Faculty of Health Sciences of Trari, Federal University of Rio Grande do Norte, Santa Cruz, RN, Brazil*
[d] *Department of Biophysics, Federal University of São Paulo, São Paulo, SP, Brazil*
[e] *Department of Genetics, Evolution and Bioagents, Institute of Biology, State University of Campinas, Campinas, SP, Brazil*
[f] *Department of Chemical Engineering, University of São Paulo, São Paulo, SP, Brazil*
[g] *Department of Food Science, School of Food Engineering, State University of Campinas, Campinas, SP, Brazil*
[h] *Department of Molecular Biology, Laboratory of Genetic Biochemistry and Radiology, Federal University of Paraíba, João Pessoa, PB, Brazil*
[i] *Institute of Food Technology, Center of Food Science and Quality, Campinas, SP, Brazil*

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ABSTRACT

Bioactive peptides have been broadly studied for their contribution to human health. This study aimed to identify bioactive peptides generated by *in vitro* gastrointestinal digestion of yam proteins. Yam protein concentrate (YPC) was submitted to simulated digestion. Gastric phase hydrolysate (GPH) and total gastrointestinal phase hydrolysate (GIPH) had their peptides identified by nanoLC-ESI-MS/MS. Peptide sequences were subjected to a database-driven (BIOPEP) bioactivity search. *In vitro* tests included: Antioxidant activity, DNA damage protection, ACE-inhibitory activity and antibacterial activity against the bacteria *Escherichia coli*, *Salmonella* sp. and *Lysteria monocytogenes*. Simulated digestion generated small peptides (mostly MW < 3500 Da), several of them with potential bioactive sequences predicted *in silico*. In both GPH and GIPH biological activities were detected, although GIPH displayed stronger DNA damage protection and antibacterial activity against *Escherichia coli*. The digestion of yam proteins releases promising biologically active peptides which can contribute to the prevention of bacterial infection and chronic degenerative diseases, with beneficial effects to human health.

1. Introduction

In recent years, the investigation of natural bioactive compounds from common foods, either of animal or plant origin, have been intensified aiming to elucidate the benefits of those molecules to human health. The yam of the genus *Dioscorea* is a tuber widely cultivated and consumed in the tropics and subtropics, being commonly used in African, Brazilian and Oriental cuisine. This tuber has a high carbohydrate content with a low glycemic index, with dioscorin as the major protein, which has been highlighted by the scientific community as a bioactive compound, due to the biological activities shown in *in vitro* and *in vivo* studies (Lu, Chia, Liu, & Hou, 2012; Zhang et al., 2019).

New approaches have been proposed for the enhancement of the biological activities of food proteins, especially by the release of peptides using *in vitro* digestion (Vilcacundo et al., 2018; Zhang et al., 2020). *In vitro* digestion tests represent a useful tool to obtain peptides

* Corresponding author at: Department of Molecular Biology, Laboratory of Structural Proteomic, Federal University of Paraiba, 58051-900 Joao Pessoa, PB, Brazil.
E-mail addresses: edilsa_nat@hotmail.com (E. Silva do Nascimento), katya_jacinto@yahoo.com.br (K. Anaya), juliacaju0@gmail.com (J.M.C. de Oliveira), thalles_lacerda2@hotmail.com (J.T.J.G. de Lacerda), mike@millerpost.com (M.E. Miller), meriellend@gmail.com (M. Dias), mariaanita.mendes@gmail.com (M.A. Mendes), jpallone@unicamp.br (J. de Azevedo Lima Pallone), arns@unicamp.br (C. Weis Arns), ma.juliano@unifesp.br (M.A. Juliano), santi.tatiane@gmail.com (T. Santi Gadelha), mtb@ital.sp.gov.br (M.T. Bertoldo Pacheco), calbgadelha@gmail.com (C.A. de Almeida Gadelha).

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similar to those released in the protein digestion that normally occurs in the human body. Thus, these peptides can be used for predictive studies of biological activities (Lucas-González, Viuda-Martos, Pérez-Alvarez, & Fernández-López, 2018). Bioactive peptides derived from foods have been gaining attention due to their high tissue affinity and specificity, with no side effects, and have potential to be used as natural alternatives to drugs (Daliri, Oh, & Lee, 2017). They can be found encrypted in the protein matrix of food origin and are released after hydrolysis, displaying beneficial effects on the human body (Li-Chan, 2015).

Therefore, recent studies have aimed to elucidate the effects of these low molecular weight compounds, as well as the exposure of amino acid sequences that may be essential for the mechanisms of action and increased bioactivity (Calvo-Lerma et al., 2019). These effects are due to their interaction and participation in different mechanisms of action, which result in physiologically important outcomes such as inhibition of the angiotensin-converting enzyme (ACE), causing reduction of blood pressure; inhibition of dipeptidyl peptidase-IV (DPP-IV), related to the reduction of blood glucose levels; and antioxidative activity, which reduces the oxidative stress and consequently prevents the onset of degenerative diseases (Li et al., 2020; Zhang et al., 2020).

The bioactivity of the peptides is related to their chemical structure, composition and sequence of amino acids, which can act by inhibiting the action of enzymes and also by stabilizing free radicals, among other interactions. Even from different protein sources, peptides with the same key amino acid residues at the active site are likely to have the same bioactive functions (Mojica & Mejia, 2016).

The most recent trend in the prediction of the activity of bioactive peptides involves the use of databases as tools to identify sequences of peptides released from *in vitro* digestion. By comparing the generated peptides with bioactive peptides already described in the literature, it is possible to detect new potentially bioactive peptides (Tu, Cheng, Lu, & Du, 2018). The *in silico* approach has several advantages, as it does not require sample preparation or the use of laboratory techniques that demand time and cost. Computational methods and statistical tools are used for the collection and analysis of biochemical data, as well as mathematical models to predict the behaviour of biochemical systems (Udenigwe, 2014). Thus, the search for similarity between the sequences of newly identified peptides with those of bioactive peptides already described has been proposed as an alternative method of useful application.

Biopep database is one of the bioinformatics tools used to predict or confirm bioactive peptide sequences, which comprises databases of known protein sequences, bioactive peptides and sensory peptides. Therefore, it consists of an integrated program that assists in the prediction of protein hydrolysates (Mooney et al., 2013). In addition to this approach, *in vitro* assays help to confirm the bioactive potential of protein molecules generated by hydrolysis and recognized by sequence matching tools.

Based on the well-established knowledge that protein gastrointestinal digestion generates sequences of smaller molecular weight and considering that several studies have been demonstrating biological activities for peptides from an assortment of food sources, we hypothesized that a great variety of promising bioactive peptides would be released with the digestion of yam proteins. To the best of our knowledge, this is the first study to look into the production of bioactive peptides from *Dioscorea cayenensis*. We aimed to identify peptides generated by *in vitro* gastrointestinal digestion of proteins from *D. cayenensis* and to prospect for biological activities using a database-driven search and *in vitro* assays to examine radical scavenging activity, DNA damage protection, antibacterial activity and ACE inhibitory activity.

2. Material and methods

2.1. Material

Dioscorea cayennensis tubers were collected from the experimental planting fields of the Federal University of Paraíba, Campus Areia (João Pessoa, PB). Porcine pepsin, porcine pancreatin, bile salts, 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazilyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchromanic-2-carboxylic acid), Abz-Gly-p-Phe(NO2)-Pro-OH, α-aminobutyric acid and Angiotensin I-Converting Enzime (from rabbit lung) were purchased from Sigma-Aldrich® (St. Louis, MO, USA). [Glu1]-Fibrinopeptide and acetonitrile were purchased from Waters (Milford, MA, USA). All solvents and reagents were of analytical grade.

2.2. Preparation and chemical characterization of yam protein concentrate

Yam flour was obtained according to the procedures described by Contado et al. (2009), with modifications. Briefly, yam tubers were sanitized with 200 ppm sodium hypochlorite solution and stored frozen until processing. Once peeled and cut, yam tubers were mixed in distilled water (1:10; m:v; tuber:water) and crushed in a domestic blender. After filtration in polyester fabric, the recovered yam mass was defatted overnight with 70% ethanol. The suspension was centrifuged at 3372 g for 15 min., the supernatant was discarded, and the pellet was left to dry at room temperature in a plastic tray for approximately 24 h, resulting in a dry yam flour.

For protein extraction, the yam flour was dispersed in distilled water (1:10, w/v) and the pH was adjusted to 9.0 with NaOH 1 mol.L^{-1}. The suspension was stirred for 3 h at 25 °C and centrifuged at 3372 × *g* during 30 min at 4 °C. The supernatant was recovered and the precipitate was used to repeat the protein extraction process under the same conditions. The supernatants were combined, subjected to protein precipitation with pH adjustment to 5.7 (using HCl 1 mol.L^{-1}) and centrifuged at 3372 × *g* during 20 min at 4 °C. The supernatant was recovered to repeat the precipitation process, adjusting pH to 4.5 (using HCl 1 mol.L^{-1}). The precipitates were combined and neutralized to pH 7.0 with NaOH 1 mol.L^{-1}. The protein concentrate was dialyzed in Milli-Q water, under magnetic stirring at 25 °C, on a 3.5 kDa MWCO cellulose membrane (Spectrum™ Labs Spectra/Por™, New Brunswick, NJ, USA). Then, the protein concentrate was frozen and freeze-dried (freeze-dryer model LS3000, Terroni Equipamentos, SP, Brazil) to obtain the yam protein concentrate (YPC). The yam protein concentrate used in our study reached 64.0% purity and 84.4% yield (Nascimento et al., 2020). The molecular weight profile of YPC proteins is shown in Fig. S1 (supplementary material).

The proximal chemical characterization of the YPC was performed as described by Latimer (2012). Total carbohydrates were calculated by difference. The total nitrogen was measured by the Kjedahl method and protein content was calculated using the conversion factor 5.75. All analyzes were performed in triplicate. Results are available as supplementary material (Table S1).

2.3. In vitro gastrointestinal digestion and degree of hydrolysis of YPC

The YPC digestion was performed following the internationally accepted digestion protocol developed by the INFOGEST Cost Action (Minekus et al., 2014). Briefly, 500 mg of YPC were dissolved in 5 mL of deionized H_2O at 37 °C and left in an ultrasonic bath for 30 min. The mixture, previously submitted to pH 2.8 adjustment using HCl 1 mol.L^{-1}, was added with porcine pepsin (20 mg.mL^{-1}) and subjected to digestion for 60 min at 37 °C, in a water bath under agitation at 150 rpm (gastric phase). Samples were taken at the starting point and after 60 min of digestion. The gastric phase was interrupted by pH adjustment to 8.0 with NaOH 1 mol.L^{-1}. The suspension from the gastric phase was

mixed with the same volume of simulated intestinal fluid (pH 8.0) containing porcine pancreatin (40 mg.mL^{-1}) and porcine bile extract (24 mg.mL^{-1}) for 60 min at 37 °C, in a water bath under agitation at 150 rpm (total gastrointestinal phase). The digestion was stopped by heating at 85 °C for 15 min, followed by centrifugation at 3700 g for 15 min (centrifuge model RC5C, Sorvall Instruments Dupont, Wilmington, USA). Digestions were performed in duplicate.

The hydrolysates of the gastric phase (GPH) and total gastrointestinal phase (GIPH) were dialyzed in Milli-Q water in a 500 Da membrane (Spectrum™ Labs Spectra/Por™, New Brunswick, NJ, USA), recovered, frozen and freeze-dried (Edwards super Modulyo, West Sussex, UK) for further analysis.

The degree of hydrolysis (DH) was determined according to Nielsen, Pertersen & Dambmann (2001), using 6 mM o-phthaldehyde (OPA) (containing 1% SDS, 5.7 mM DTT and 0.17 mol.L^{-1} disodium tetraborate). The calibration curve was built with serine (0.1 mg.mL^{-1}). Deionized water was used as blank. DH calculation was performed according to the equation

$$DH(\%) = \frac{h}{h_{tot}} \times 100$$

where h_{tot} is a constant (7.8), for which soy protein was used as reference, and h is determined as follow:

$$h = \frac{(SerineNH_2 - \beta)}{\alpha}$$

$$SerineNH_2 = \frac{\left[\left(Abs_{sample} \div Abs_{blank}\right) \div \left(Abs_{serine} \div Abs_{blank}\right)\right] \times [0.9516 \times 10]}{m \times N}$$

where *Abs* are the values for absorbance, *0.9516* is serine-NH_2 meqv/L, *m* is the sample mass and *N* is the nitrogen-to-protein conversion factor (5.75). The values assumed for the constants α and β were those estimated for unknown raw materials (1.00 and 0.40, respectively) (Adler-Nissen, 1986).

2.4. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis

MALDI-TOF-MS analysis was performed to molecular mass profiling assessment of peptides released from *in vitro* gastrointestinal digestion of yam. The dried peptide mixture was diluted in bidistilled water:trifluoroacetic acid (TFA) (99.9:0.1, v:v), filtered in 0.22 μm-nylon filter and 1 μL of peptide solution was mixed (1:1) with a solution of α-cyano-4-hydroxycinnamic acid (α-CHCA matrix, 25 mg.mL^{-1}) in water:acetonitrile:TFA (69.9:30:0.10, v:v:v); 1 μL of peptide mixture in the matrix was placed to an MSP 96 ground steel sample target (Bruker Daltonik, Bremen, Germany) and dried at room temperature. Four spots were prepared for analysis.

MALDI-TOF-MS spectra were acquired with Microflex LT linear mass spectrometer (Bruker Daltonics), using FlexControl software package (version 3.4, Bruker Daltonics). The spectra were recorded in the positive linear mode (laser frequency, 1000 Hz; ion source 1 voltage, 20.05 kV; ion source 2 voltage, 18.35 kV; lens voltage, 6.22 kV; sample rate, 0.50 GS/s; pulsed ion extraction, 230 ns; gain factor, 15.1x) and five independent spectra (1000 shots at random positions on the same target place, for spectrum) were manually collected. Measurements were performed in independent analysis on mass ranges 500–1520 Da, 1500–2600 Da, 2500–3600 Da calibrated externally using Peptide Calibration Standard 2 (Bruker Daltonics) according to mass range specifications.

FlexAnalysis (version 3.4) software package (Bruker Daltonics) was used for the analysis of all MALDI-TOF-MS data, which included spectral mass adjustment, optional smoothing (using the Savitsky – Golay algorithm with a frame size of 25 Da), spectral baseline subtraction, normalization, internal peak alignment, and peak picking. Pretreated data were then subjected to visualization and statistical analysis.

2.5. NanoLC-ESI-MS/MS analysis of yam hydrolysate fractions

Hydrolysates GPH and GIPH were resuspended in water:acetonitrile:formic acid (97.9:2:0.1, v:v:v) and subjected to analysis by nanoflux liquid chromatography coupled to sequential mass spectrometry with Electrospray Ionization (nanoLC-ESI-MS/MS), performed on a NanoLC Dionex Ultimate 3000 system (Thermo Fisher Scientific), coupled to an Impact II quadrupole time-of-flight (Q-TOF) mass spectrometer (Bruker Daltonics). The peptides were retained in the Acclaim Pepmap nano-trap column (Dionex-C18, 100 Å, 75 μm × 2 cm) and separated in line using the Acclaim Pepmap RSLC analytical column (Dionex-C18, 100 Å, 75 μm × 15 cm) under gradient elution from 2 to 98% of acetonitrile:trifluoroacetic acid (99.1:0.1, v:v) for 180 min, and flow adjusted to 300 nL.min^{-1}. Mass spectra of MS precursors were acquired in positive ion mode and MS/MS products acquired were acquired at 2 Hz in the mass range of 50–3000 *m/z* and the branched collision-induced dissociation energy parameters varied from 7 to 70 eV.

2.6. In silico analysis

Raw MS/MS data files were imported into the PEAKS Studio 8.5 software (Bioinformatics Solution Inc., Waterloo, Canada) for *de novo* analysis and database searches. *De novo* analysis was performed with a precursor mass tolerance of 07 ppm, fragment mass tolerance of 0.025 Da, no specific enzymatic cleavage and oxidation in Met (+15.99 Da) and Pyro-Glu from Q (−17.03 Da) were used as dynamic modifications. As the *Dioscorea cayennensis* database presented few protein sequences, peptides were *de novo* sequenced with an average local confidence (ALC) ≥ 50% and submitted to the database search using SPIDER tools (Han et al., 2005), against the Uniprot KB Dioscorea database (71 Swiisprot sequences and 2703 TrEMBL sequences, downloaded on May 3, 2018 from http://www.uniprot.org/). The false discovery rates (FDRs) for proteins and peptides were fixed at a maximum of 1%. Peptides with ALC > 90% unmatched in the homology database were considered as complementary analysis.

2.7. Prediction of bioactive peptides

Occurrence of biological activities was predicted by analyzing the peptide sequences using BIOPEP database (Minkiewicz et al., 2008). The PeptideRanker (Bioware.ucd.ie) was used to rank the predicted sequences according to its potential ACE-inhibition.

2.8. DPPH radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the hydrolysates was estimated according to Picot et al. (2010), with slight modifications. Aliquots of 30 μL of YPC, GPH and GIPH samples, previously diluted in distilled water (10–20 mg.mL^{-1}), were mixed with 1.5 mL of DPPH solution (60 μmol.L^{-1}) diluted in methanol. The mixture was stirred for 60 min, at 25 °C, protected from light. Then, the samples were centrifuged at 14,500 rpm for 5 min and the absorbance of the supernatant was measured at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 500–2000 μmol.L^{-1}, was used as standard. The DPPH scavenging activity (%SA) was calculated according to the equation

$$\%\ Scavenging\ acticity\ (SA) = \left[\left(Abs_{blank} - Abs_{sample}\right) \div Abs_{blank}\right] \times 100$$

Furthermore, the Trolox equivalent antioxidant capacity (TEAC) was calculated dividing the Trolox concentration from the curve by the final sample concentration in mg.mL^{-1}.

2.9. ABTS radical scavenging activity

The ABTS [2,2′-azinobis- (3-ethylbenzothiazoline)-6-sulfonic acid] assay was based on the method described by Wiriyaphan, Chitsomboon & Yongsawadigul (2012). Stock solution of ABTS•+ was prepared mixing ABTS•+ (7 μmol.L^{-1}) and potassium persulfate (140 μmol.L^{-1}), protected from light for 16 h. Fresh working solution was prepared by diluting the stock solution in ethanol to achieve an absorbance of 0.7 ± 0.05 at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), up to 1000 μmol.L^{-1}, was used as standard. A 20 μL aliquot of each sample (YPC, GH and GIH, 10–20 mg.mL^{-1}) was mixed with 3.0 mL of the ABTS•+ radical solution in test tubes and homogenized on a tube shaker. After 6 min, absorbance was measured at 734 nm. The scavenging of the ABTS•+ radical was calculated according to Equation 1. Trolox equivalent antioxidant capacity (TEAC) was calculated as previously described in section 2.8.

2.10. Oxygen radical absorbance capacity (ORAC)

The antioxidant capacity was also determined by using the ORAC method, as described by Huang et al. (2002). Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4). The final assay mixture (200 μL) contained fluorescein (150 μL, 55 nM), radical initiator 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH) (25 μL, 153 mM) and 25 μL of antioxidant Trolox (0.05–4.0 μM) or sample (YPC, GPH or GIPH). Fluorescence was recorded during 50 min, every 60 sec, in an Enspire 2300 plate reader (Perkin Elmer, EUA) with 485 nm excitation and 520 nm emission filters. Phosphate buffer (pH 7.4) was used as negative control. Final ORAC value was expressed as μmol Trolox equivalents per g or mg of sample, according to the area under the curve (AUC).

2.11. Protection against DNA damage

DNA damage protection capacity of YPC, GPH or GIPH was performed according to Huang et al. (2014), with slight modifications. The hydroxyl radical (•OH) was generated by the Fenton's reaction: reaction mixture of 15 μL containing YPC or its hydrolysates (10 and 15 mg.mL^{-1}, respectively), 5 μL of plasmid DNA from *Escherichia coli* (1 mg.mL^{-1}), 2 μL of 18 mM $FeSO_4$ and 3 μL of 60 M H_2O_2 was incubated at 37 °C for 30 min. Then, 2 μL of 1 mM EDTA was added to stop the reaction. The blank was performed with only plasmid DNA and the positive control test was performed with all components of the reaction and water. The mixture was then electrophoresed on 1% agarose gel. To visualize the DNA bands, the gel was stained with Gel-Red ™ (Biotium, Inc., Hayward, USA) and photographed by a Transluminator L.Pix Loccus-Molecular Imaging.

2.12. ACE inhibition assay

In vitro inhibition of ACE was assayed as follow (Farias et al., 2006): the hydrolysis of 10 μM Abz-FRK(Dnp)P-OH was continuously measured at 37 °C in a Shimadzu RF-1501 Spectrofluorophotometer System adjusted to 320 nm (excitation) and 420 nm (emission). Enzyme concentration was 6.8 nM. The tests were carried out in 0.1 mol.L^{-1} Tris-HCl buffer pH 7.0 containing 50 mM NaCl and 10 μM $ZnCl_2$. Increasing concentrations of the samples YPC, GPH or GIPH (0–160 μg/mL) were pre-incubated with the enzyme for 1 min before tests begun. ACE inhibition Activity was calculated from two independent assays and expressed as IC_{50}, which corresponds to the concentration of yam concentrate or hydrolysate capable of inducing 50% inhibition of ACE activity. IC_{50} was determined using the equation

$$y = \frac{100\%}{1 + (x/IC_{50})^S}$$

where y is the enzyme concentration, x is the sample concentration and S is a slope factor. The equation assumes that y decreases with increasing x. The IC_{50} values for the ACE inhibitor were calculated by the tight-binding titration data analysis, with the software GraFit (Erithacus Software, West Sussex, UK).

2.13. Antibacterial activity

Antibacterial activity against *Escherichia coli*, *Salmonella* sp and *Lysteria monocytogenes* was tested with YPC, GPH and GIPH by broth microdilution protocol. Each strain, stored at 4 °C, was placed to grow in Brain Heart Infusion (BHI) medium at 35 °C until approximately 1.5 × 10^8 CFU.mL^{-1}, with a McFarland scale of 0.5. Microdilution was performed in 96-well flat-bottom microplates with 90 μL of medium per well. Serial dilutions were made in triplicate, with 1 mg.mL^{-1} of each sample. Then, 10 μL of bacterial suspension (10^6 CFU.mL^{-1}) was added. Negative control was prepared with medium and the positive control, bacteria and medium (with no sample) (NCCLS, 2003). The microplate was incubated at 35 °C in a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and bacterial growth was monitored by measuring the absorbance at 625 nm, every 60 min, for 24 h. Results are presented as minimum inhibitory concentration (MIC).

2.14. Statistical analysis

For statistical analysis, the software program GraphPad Prism version 6.0 (GraphPad Software, CA, USA) was used. The results were expressed as mean and standard deviation (SD). ANOVA test was used, followed by Student's *t* test. Statistically significant differences were considered when $p < 0.05$.

3. Results and discussion

3.1. Peptide profiles of yam hydrolysates

The simulated digestion of YPC resulted in a degree of hydrolysis (DH) of 25.46% in the gastric phase (GPH) and 27.0% in the total gastrointestinal digestion (GIPH). The molecular weight profile of GIPH peptides is shown in Fig. 1. Results suggest that in the first stage of protein digestion, gastric pepsin was quite effective in the ability to hydrolyze a large number of peptide bonds. Similar results were observed by Nguyen, Gathercole, Day and Dalziel (2020), when proteins from yogurt and milk (cow, sheep and goat origin) were submitted to simulated digestion and the greatest change in molecular weight distribution occurred in the first 10 min of gastric digestion. They reported the occurrence of an increase greater than 50% on the number of small polypeptides/peptides (<10 kDa), suggesting that pepsin is capable of hydrolyzing proteins into peptides in the early gastric digestion.

Protein digestion, catalyzed by gastric and intestinal enzymes, has the ability to produce a mixture of peptides of different MW. According to Fig. 1, GIPH peptides have MW ranging from 500 to 3500 Da, most of them below 1500 Da. This MW profile is similar to bioactive peptides identified and described in databases. Peptides of low MW have the ability to interact with various cellular receptors, triggering distinct responses that result in biological activities. For example, Zhang et al. (2020) isolated peptides with MM < 3000 Da identified as antioxidant compounds.

3.2. Peptide identification and in silico bioactive potential prediction

In the gastric hydrolysate (GPH), obtained from the gastric phase digestion, 506 peptides were identified. Total gastrointestinal simulated digestion resulted in 491 peptides, identified by nanoLC-ESI-MS/MS analysis.

The sequences of the peptides generated by digestion of the YPC

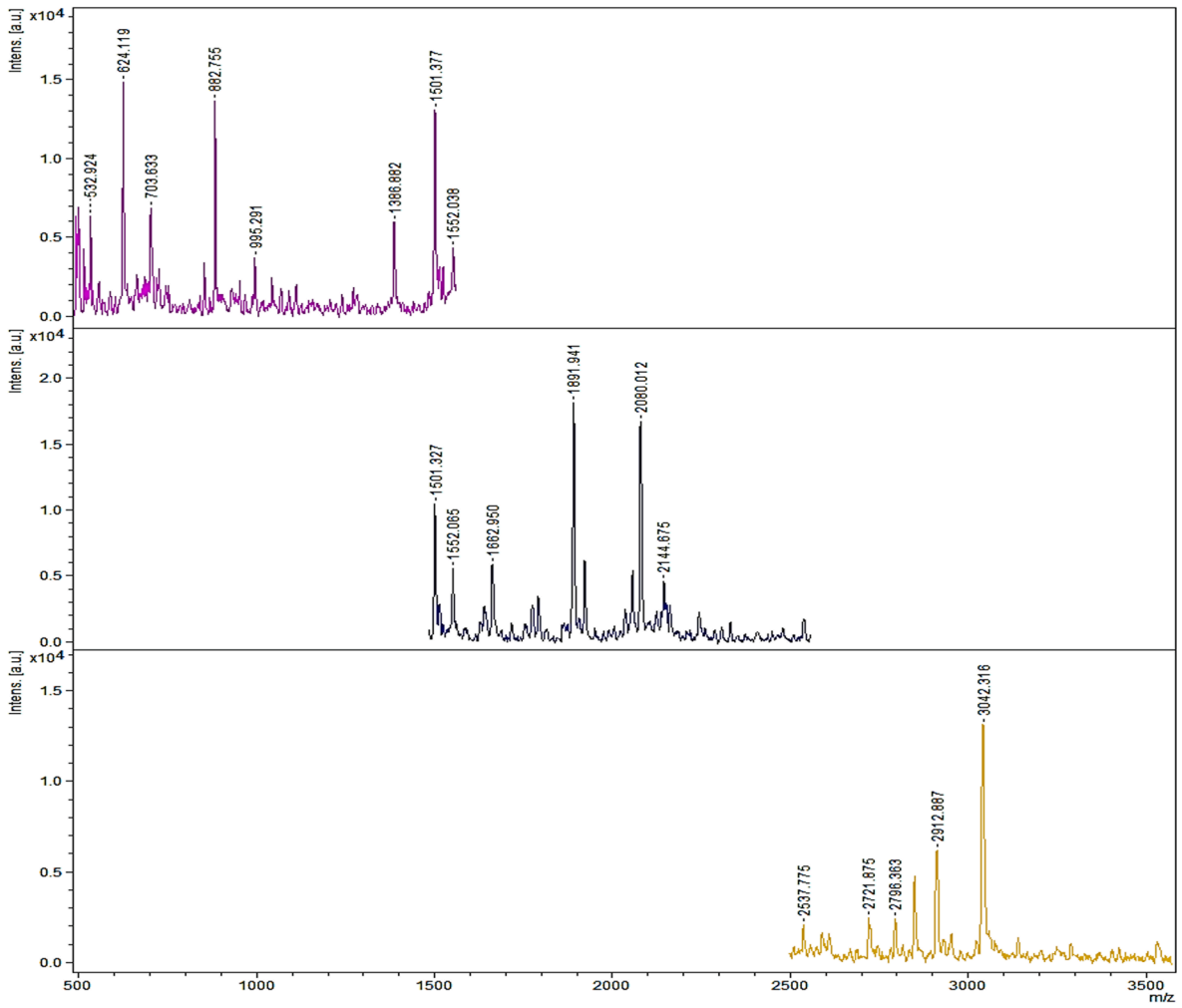

Fig. 1. Representative MALDI-TOF mass spectra of peptides from *in vitro* gastrointestinal digestion hydrolysate (GIHP) of yam (*Dioscorea cayennensis*) protein concentrate.

were analyzed by computational studies, involving the comparison of the sequences with known bioactive peptides from BIOPEP database. Potential bioactive peptides identified from the sequences found in gastric (GPH) and total gastrointestinal hydrolysates (GIPH), their MW (Da), homologous bioactive sequence and its original source, identification number and protein precursor can be seen in Table 1. Yam peptides showed homology with several bioactive peptide sequences from BIOPEP, whose activities include: ACE inhibition, DDP-IV enzyme inhibition, antibacterial and antioxidant effects.

In the first stage of digestion, potential antioxidant peptides were identified (Table 1), such as those containing Y (Tyr), W (Trp) or F (Phe) in C-terminal position: DDC**AY**, SINRV**AY**, EDI**TW**, LLT**W**, KML**FF** and ELD**FF**. Antioxidant peptides from soybeans, wheat, rice and olive proteins, with these amino acid residues in the same position, have already been identified, studied and recorded in the BIOPEP database. According to Elias, Kellerby and Decker (2008), the phenolic structures of aromatic amino acids are highly reactive and participate in the donation of hydrogen, being able to stabilize free radicals such as ABTS.

The peptides generated from *in vitro* gastrointestinal hydrolysis (GIPH) presented potential as antihypertensive compounds, once they showed similarities with the sequence of ACE inhibitors (Table 1). Peptides capable of inhibiting ACE are a key target for the treatment of hypertension, since the angiotensin I-converting enzyme controls blood pressure. According to Nongonierma and FitzGerald (2015), the presence of P (Pro) residues in peptide structure is essential, as it promotes a conformation related to hypotensive effects linked to ACE inhibition. LA**PLPL** for example, a GIPH peptide, presents close similarity to the LHL**PLPL** peptide, a well-known milk ACE inhibitor. Other BIOPEP sequences of analogous peptides have also been identified from wheat and fish proteins as well as synthetic peptides.

After simulated digestion of yam proteins, several inhibitors of the enzyme dipetidyl-peptidase IV (DPP-IV) were also identified, mainly after the final phase, in GIPH. DPP-IV is an enzyme known to inactivate glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These two gut hormones play an important role in regulating glucose, stimulating pancreatic glucose-dependent insulin and suppressing the release of glucagon. In this way, they promote β cell proliferation and survival, delay gastric emptying and modulate appetite (Lim & Brubaker, 2006). Thus, DPP-IV inhibiting peptides can be considered as an important approach for the treatment of type 2 diabetes due to its hypoglycemic effect. According to Lacroix and Li-Chan (2014), peptides presenting P (Pro) residues up to the third position are more resistant to hydrolysis and, therefore, are believed to be more potent DPP-IV inhibitors. As shown in Table 1, the FV**VDPN** and TTT**VDPN** peptides from TH have proline residues (Pro) and similar sequences to other DDP-IV inhibitory peptides.

Structural homologies between ACE inhibitors and DPP-IV are also reported, due to the presence of P (Pro) residues (Nongonierma & FitzGerald, 2015). Yam peptides such as LREP**W** and **F**VVDPN, with residues of W (Trp) F (Phe) in the C-terminal and N-terminal positions, respectively, are expected to be good enzyme inhibitors, considering that the presence of aromatic amino acids (W-Trp, Y-Tyr and F-Phe)

Table 1
Database-driven prediction of bioactive peptides generated from *in vitro* gastrointestinal digestion of yam (*Dioscorea cayennensis*) protein concentrate.

Biological Activity	Peptide sequence	Molecular Weight (Da)	Number of residues	Bioactive sequence (BIOPEP)	BIOPEP ID	Protein source
Gastric Phase Hydrolysate (GPH)						
Antioxidant	**DDCAY**	586,18	5	**DD**LPR	8429	*Olea europaea*
	LSINRV**AY**	934,52	8	**AY**	7866	Okara residue
	SINRV**AY**	821,43	7	**YAY**	7953	Synthetic peptide
	ELD**FF**	670,31	5	TRTGDP**FF**	8755	Rice
	KML**FF**	685,37	5	V**PW**	8188	Buckwheat
	TFH**PW**	687,32	5	VF**PW**	8189	Buckwheat
				TFE	8220	Synthetic peptide
	LED**PW**	659,30	5	VF**PW**	8220	Synthetic peptide
	WGGPW	602,27	5	**WG**	9082	Synthetic peptide
				VF**PW**	8220	Synthetic peptide
	LLTW	532,31	4	**LL**PHH	3772	*Glycine max*
	EDI**TW**	662,29	5	G**TW**	9165	Synthetic peptide
	TTFFQ	643,30	5	**TT**YY	7871	*Glycine max*
ACE-inhibitory	NAINNA**RP**	868,45	8	YANPAVV**RP**	3404	Not identified
	VMAG**GPPS**	714,34	8	**GPP**	7820	Gliadin (wheat)
	LA**PLP**L	622,41	6	**PLP**	2664	NI
	LA**PL**LP	622,41	6	**PL**	7513	Alaskan fish skin
	NNARL**PL**	796,46	7			
	NAINNAR**PL**	981,54	9			
	A**VV**SIL	600,38	6	PA**VV**LP	2621	NI
	F**VV**DPN	690,00	6	I**VV**E	3260	Synthetic peptide
	VVDGGP	543,00	6	**VVPP**A	7664	NI
DPP-IV inhibitory	LA**PLP**L	622,41	6	L**PLPL**	8618	Synthetic peptide
	LSDS**PL**	631,33	6	PQNIP**PL**	9058	Synthetic peptide
	LSSYAVTL**PL**	932,30	10			
	PNGPENW	812,37	7	**PN**	8860	Synthetic peptide
Antimicrobial	LNQV**YR**	396,72	6	TSK**YR**	8192	NI
	QLVHESQDQ**KR**	1366,69	11	KKFHIR**KR**	3684	NI
	AQLVHESQDQ**KR**	1437,73	12			
	QQ**LK**	499,28	4	K**LK**LLLLLK**LK**	3648	NI
				LKKKKK**LK**KK**LL**K**L**	3652	NI
Gatrointestinal Phase Hydrolysate (GIPH)						
Antioxidant	**FLSWT**	652,32	5	**FL**PE	8998	Synthetic peptide
	DIT**WT**	634,29	5	P**WT**	8050	NI
				R**WT**	8079	NI
				L**WT**	8018	NI
	FD**EL**	522,23	4	**YF**YP**EL**	7887	Casein
	YFQELK	826,42	6	**LK**	8217	NI
				L**ELK**	8724	*Phaseolus vulgaris*
	LGTVS**PK**	700,41	7	**LG**FEYY	8240	NI
	PFCS**PK**	677,32	6	WEG**PK**	8986	NI
				DWD**PK**	9335	NI
	VAGLG**DW**	716,34	7	KLS**DW**	9222	NI
	SW**PWQ**	702,31	5	**PWQ**	8047	NI
	LRE**PW**	699,37	5	**PW**	8190	Buckwheat
	WGGPW	601,26	5	VF**PW**	8188	Buckwheat
				WG	9082	NI
	GIRNPEEI**PW**	805,81	10	V**PW**	8189	Buckwheat
	TVFFY	676,33	5	Y**FY**	7963	NI
				TVYQ	8483	Casein
	TWGG**HQ**	684,29	6	**D**H**HQ**	8135	Marine bivalve
	DYLELQ	739,36	6	**TW**	8459	Rice (*Oryza sativa* L.)
DPP-IV inhibitory	DYLEL**Q**	779,37	6	**KL**LLRR**LQ**	8716	*Phasoulus vulgaris*
	SDLEDFI**RQ**	876,52	9	RLLLKL**RQ**	8732	*Phaseolus vulgaris*
	LREPW	656,80	6	**LRE**NN**KL**MLLELK	8726	*Phaseolus vulgaris*
				VIA**PW**	9517	NI
	KLGTVSPKQ**VL**	968,90	10	**VL**GP	8593	NI
	LAPLPL	622,40	6	**LPLPL**	8820	NI
				LPL	8618	NI
				LAPPG	8616	NI
				L**LAP**	8788	NI
	L**RP**EW	350,69	5	M**RP**VDPNIE	8689	NI
	M**VP**AML	660,33	6	W**VP**VDPNIE	8550	NI
	E**FPAVP**	658,33	6	I**AVP**TGVA	8649	NI
	YE**VP**R	662,33	5	**VP**	8579	NI
	TTTVDPN	746,33	7	M**FPVDP**NIE	8581	NI
	VVDPN	543,27	5	M**FPVDP**NIE	9023	NI
	G**VVDPN**	600,29	6	FRAEH**PL**	8562	Synthetic peptide
ACE-inhibitory	AR**PLQPL**	796,45	8	G**PL**	8670	NI

(*continued on next page*)

Table 1 (*continued*)

Biological Activity	Peptide sequence	Molecular Weight (Da)	Number of residues	Bioactive sequence (BIOPEP)	BIOPEP ID	Protein source
	INNAR**PL**	687,50	7	LHL**PLPL**	7506	NI
	LA**PLLP**	692,60	6	PAVV**LP**	7565	NI
	LA**PLPL**	594,70	6	PQEV**LP**	7586	NI
	DALNNAR**LP**	843,56	8	ENLHL**PLP**	7765	NI
Antimicrobial	QVLM**LK**	730,44	6	K**LK**LLLLL**KLK**	3648	NI
	QLLQFDDPSY**YR**	1543,73	12	TSK**YR**	3684	NI
	VLLLK	1159,59	6	**LKKKKKLKKKLLKL**	3652	NI
	FYFEQ**LK**	695,45	7	**KK**FHIRK**R**	8192	NI
	YFEQ**LK**	973,49	6			
	Q**VLLLK**	826,42	6			
	KALEDF**LKK**	712,48	9			
	YFQE**LK**	1090,63	6			
	KALEDF**LKK**	826,42	9			

DPP-IV inhibitory: dipeptidyl peptidase-IV inhibitory activity.
ACE-inhibitory: Angiotensin I-converting enzyme inhibitory activity.
NI: Not identified.

participate in the structure of enzyme inhibitors (Lacroix & Li-Chan, 2012).

Regarding antibacterial activity, some structural and amino acid composition parameters are decisive for the occurrence of this biological activity. Antibacterial peptides generally have positively charged amino acid residues, which interact with the anionic surface of the bacterial membrane (Ciumac, 2019). Hydrophobic and aromatic amino acids (Ala, Val, Leu, Tyr, Phe and Ile) are also alleged contributors to the antibacterial activity of some peptides (Song et al., 2020). Yam gastrointestinal hydrolysate (GIPH) has peptides with cationic and amphipathic sequences, such as **VLLLK**, QVL**LLK**, **K**ALEDF**LKK**, showing similarities with antibiotic peptides of the BIOPEP database, with lysine and leucine sequences. Béven et al. (2003), when studying helical cationic and amphipathic models of peptides with antimicrobial activities, verified that simple structures composed of two types of amino acids, leucines and lysines (**KLLKLLLKLLLKLLK**), have high antimicrobial activity.

To elucidate the predicted bioactive potential through the BIOPEP database, yam hydrolysates generated during simulated digestion of YPC were tested *in vitro* to confirm their bioactivity. An investigation of antioxidant (ORAC, DPPH, ABTS and protection from DNA damage), ACE inhibition and antibacterial activities was performed.

3.3. Antioxidant activity

Scavenging activity (SA) of yam hydrolysates was significantly higher than that of intact protein (YPC), confirming that bioactive fragments are released during the progress of digestion hydrolysis

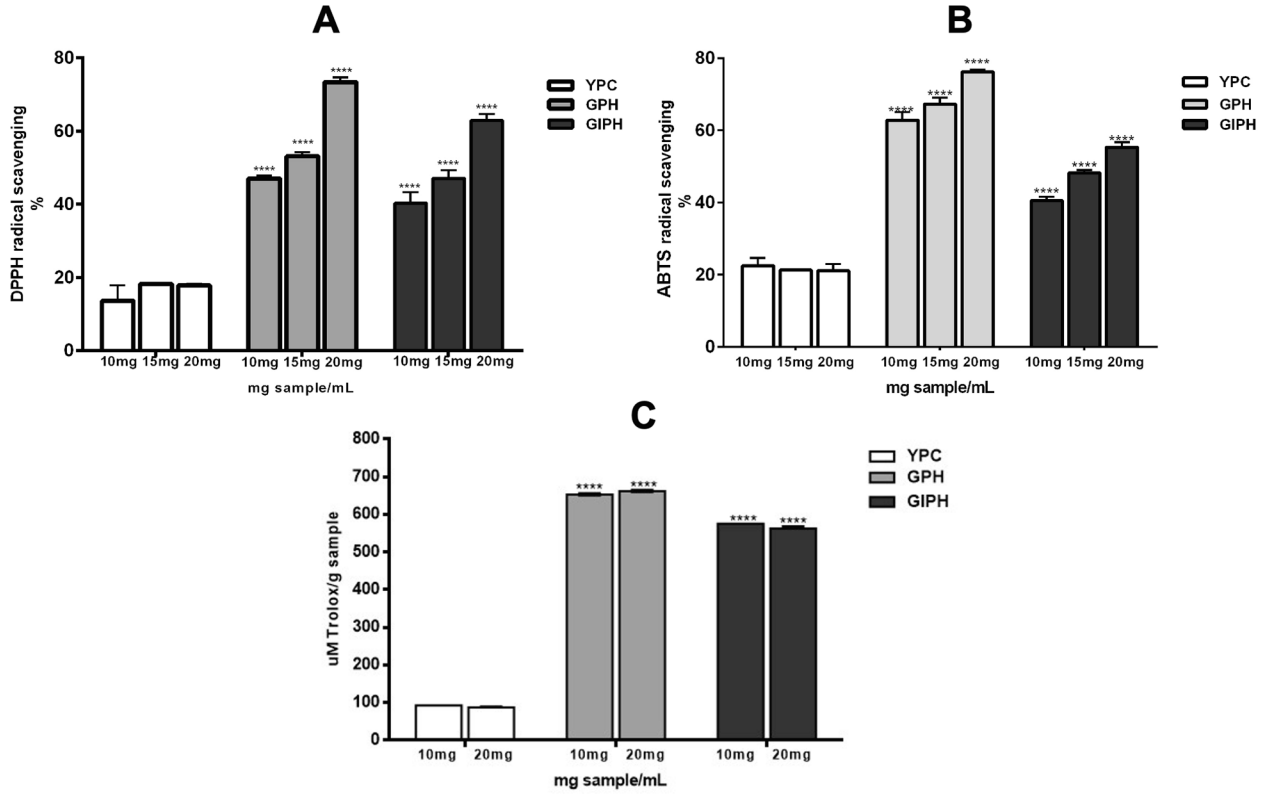

Fig. 2. Antioxidant activity of yam (*Dioscorea cayennensis*) protein concentrate (YPC) and hydrolysates obtained from *in vitro* gastrointestinal digestion phases assessed by (A) DPPH radical scavenging, (B) ABTS radical scavenging and (C) ORAC method. **Legend:** GPH (Gastric phase hydrolysate), GIPH (Gastrointestinal phase hydrolysate). Results are expressed as mean SD (n = 3). **** means extremely significant ($p < 0.0001$) difference between the hydrolysate and the YPC.

(Fig. 2). GPH (Fig. 2-B) showed SA above 80%, while SA of GIPH was 60%. Liu and Lin (2009) performed the simulated digestion of dioscorin using the enzyme pepsin and found that the DPPH elimination activity of the peptic hydrolysates increased with the hydrolysis time.

The major yam protein, dioscorin, is recognized by its antioxidant activity *in vitro*, and it is known that the precursor protein structure, as well as the hydrolytic process can affect the bioactivity of the peptides. Bioactive peptides released after hydrolysis processes expose amino acids responsible for the scavenging of free radicals, highlighting that the chemical and structural characteristics of the peptides generated by hydrolysis are important for the antioxidant capacity. Aromatic residues Phe (F) Tyr (Y) and Trp (W) enhance the antioxidant activity, which may occur due to the presence of the phenyl radical, an excellent hydrogen donor for free radicals (Zou et al., 2016; Zhang et al., 2020).

Antioxidant activity may be related to peptide sequences containing tyrosine found in the hydrolysates **(DDCAY** and SINR**VAY**). They were, probably, released from dioscorin, since they have homology with parts of the amino acid sequence of the main yam protein. These peptides also present sequences similar to the antioxidant peptides registered in the BIOPEP database (Table 1). Amino acid residues at N and C-terminal regions are very important factors for the ability to neutralize free radicals. Li and Li (2013) studied the relationship between structure and antioxidant activity of peptides with models using quantitative structure activity relationships (QSAR) and found that negative charges at the N1 and N2 positions contributed to results of antioxidant activity, using the ORAC assay. **DDCAY** peptide in GPH, with residues of Asp (D) in positions N1 and N2, may have contributed to the expressive result for antioxidative activity (Fig. 2 C) in the ORAC assay, with 691 μM of Trolox per mg of sample. Peptides **F**LS**W**T and **D**IT**W**T may also be related to the antioxidative activity, due to their aromatic residue and negative charge at N-terminal, respectively.

Thus, the results suggest that the hydrolysis of yam proteins releases peptides capable of scavenging free radicals. Gastrointestinal digestion produces a plethora of antioxidative peptides which remain intact for intestinal absorption and bioaccessibility and may contribute as exogenous antioxidants to the metabolism.

3.4. Protection of DNA oxidative damage

In addition to traditional techniques for assessing the antioxidant capacity of protein molecules, several other procedures, based on electrochemical methods, can be used to evaluate antioxidative activity. Among those, the most important are methods based on protection against oxidation of DNA bases and methods based on the ability to reduce Fe^{3+}. In this study, DNA protection capacity of YPC, GPH and GIPH was also tested, and results are shown in Fig. 3.

DNA bands resulted from tests with samples and negative control can be observed on Fig. 3. The protective activity against DNA damage is shown in lanes 1–3, referring to the different concentrations of GIPH (the sharper the bands, stronger the DNA protection conferred by the sample). On the other hand, for all YPC concentrations there is degradation of the DNA bands, whereas opaque bands in the GPH indicate low protective action or the destruction of the DNA structure, through the reaction of the radicals •OH produced in the Fenton reaction. Free radicals are one of the main causes of DNA damage, being related as causes of the appearance or spread of degenerative diseases (Duracková, 2010).

According to Saiga, Tanabe and Nishimura (2003), peptides presenting Glu and Asp amino acids at the C-terminal contribute to the protection of DNA, inhibiting metal-mediated oxidation processes. Therefore, the peptides **DD**CAY and **ED**ITW, released from *in vitro* gastric digestion (Table 1), are apparently involved with the yam hydrolysate ability of eliminating free radicals and protect DNA from damages induced by the •OH radical. Furthermore, GIPH also presents peptides such as **D**IT**WT** and **FLSWT**, to which antioxidant activity demonstrated by the ORAC method was linked. Therefore, these results

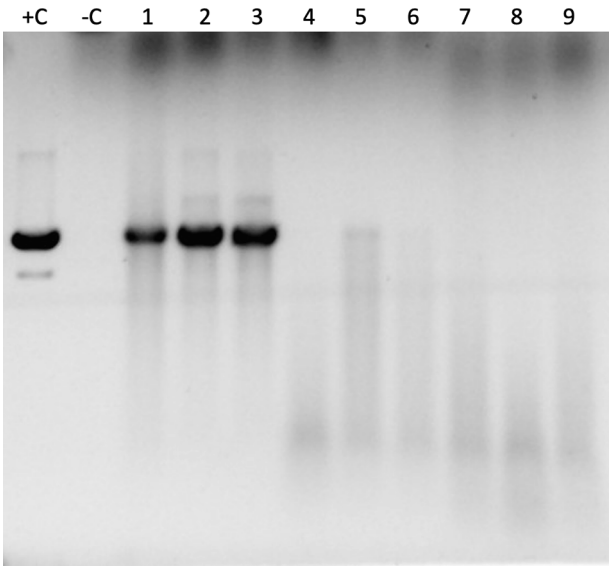

Fig. 3. Representative agarose gel electrophoresis of DNA damage protective effect of yam (*Dioscorea cayennensis*) protein concentrate (YPC) and hydrolysates obtained from *in vitro* gastrointestinal digestion phases. GPH (Gastric phase hydrolysate), GIPH (Gastrointestinal phase hydrolysate). **Legend:** +C (positive control: plasmid DNA only); -C (negative control: plasmid DNA + Fenton's reagent); 1 (GIPH 10 $mg.mL^{-1}$); 2 (GIPH 15 $mg.mL^{-1}$); 3 (GIPH 20 $mg.mL^{-1}$); 4 (GPH 10 $mg.mL^{-1}$); 5 (GPH 15 $mg.mL^{-1}$); 6 (GPH 20 $mg.mL^{-1}$); 7 (YPC 10 $mg.mL^{-1}$); 8 (YPC 15 $mg.mL^{-1}$); 9 (YPC 20 $mg.mL^{-1}$).

suggest that the protection of DNA molecules against free radicals by GIPH can be effective in protecting biomolecules in the human body.

3.5. ACE inhibition activity

Our findings suggest that *in vitro* gastrointestinal digestion can contribute to the ACE- inhibitory activity of yam proteins. Five of the peptides generated from *in vitro* gastrointestinal digestion and further identified, exhibited a high score for antihypertensive potential. The PeptideRanker scores, predicted for the peptides most likely to be bioactive, are shown in Table 2. These results were confirmed by *in vitro* tests, whose outcomes strongly suggest that the digestion of yam proteins produces peptides that may be directly linked to the ACE inhibition presented in this study (Fig. 4).

PeptideRank is useful for tracking a large set of data for sequences of bioactive peptides, since it classifies them according to their biological potential, through records in databases. Results above a score of 0.5 mean that there is an enhanced probability of a peptide displays a bioactivity. The closer the predicted probability of score 1, the greater the reliability of bioactivity. This experimental screening allows focusing on peptides in a subset, leading to a better understanding of biological activities and also to the synthesis of bioactive peptides (Mooney et al., 2013).

Among the selected peptides with bioactive potential (Table 2), the

Table 2
PeptideRanker prediction of Angiotensin I-converting enzyme inhibition for peptides released from *in vitro* gastrointestinal digestion of yam (*Dioscorea cayennensis*) protein concentrate.

Ranking	Peptide sequence	Digestion phase	*Score*
1	LAPLPL	GPH/GIPH	0.79
2	LAPLLP	GPH/GIPH	0.76
3	ARPLQPL	GIPH	0.71
4	LREPW	GIPH	0.62
5	NNARLPL	GIPH	0.50

GPH- gastric phase hydrolysate; GIPH- gastrointestinal phase hydrolysate.

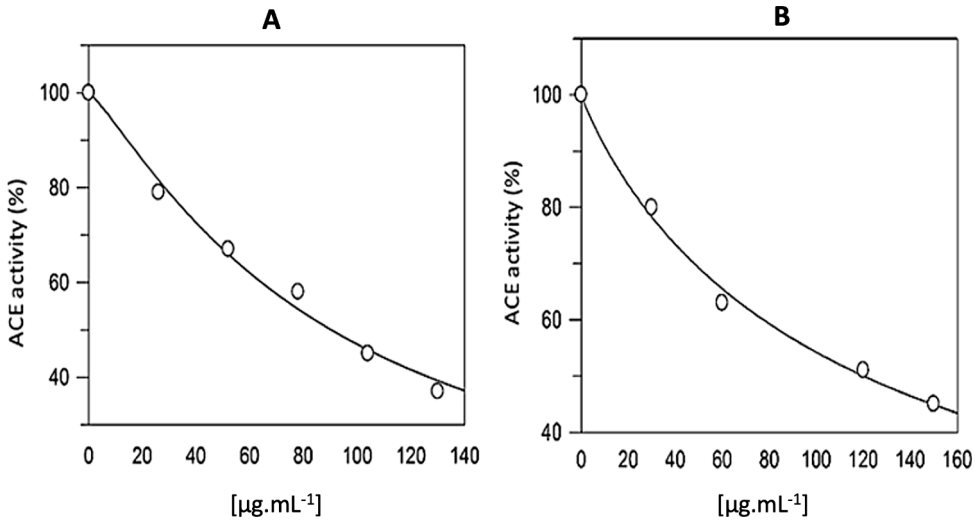

Fig. 4. Angiotensin-converting enzyme inhibitory activity of yam (*Dioscorea cayennensis*) protein concentrate hydrolysates obtained from *in vitro* gastrointestinal digestion. (A) GPH-Gastric phase hydrolysate; (B) GIPH-Gastrointestinal phase hydrolysate.

amino acid Pro (P) is present in all sequences. Four of them have Leu (L) and Pro (P) sequences, such as LA**PLPL**, LA**PLLP**, AR**PLQPL**, NNARL**PL**. ACE inhibition activity is expected to be found for peptides with greater frequency of these two amino acids in their composition, as well as lysine. In fact, ACE inhibitory peptides are characteristically short and carry hydrophobic residues, including proline. Previous studies have shown that the peptides Isoleucine-Proline-Proline (IPP) and Leucine-Lysine-Proline (LKP), are ACE inhibitors, therefore, they have the potential to maintain normal blood pressure (Danish et al. 2017). According to Girgih et al. (2014), ACE inhibition is influenced by the presence of hydrophobic and branched-chain amino acids in the peptide structure, as well as the presence of aromatic amino acids at the C-terminal. This feature can be observed in the yam peptide LRE**PW**, from GIPH.

The LA**PLPL** and LA**PLLP** peptides, with a score of 0.79 and 0.76, respectively, were generated in the gastric phase of the digestion, but remained intact in GIPH. Intact peptides, resistant to intestinal enzymes, can reach the brush border membrane and may be transported across it to bloodstream. These peptides are of low molecular weight, which facilitates their access to active sites, such as the ACE inhibition site (Salim & Gan, 2020). However, brush border hydrolysis before the flux across the epithelial barrier must not be neglected. The breakdown fragments could, potentially, display biological activities as well, as reported by Quirós et al. (2008). In this study, the milk-derived peptide LHLPLP underwent to partial hydrolysis by Caco-2 brush border peptidases, however its hydrolysis fragment had a greater flux than the precursor peptide. The authors suggest that the fragment from LHLPLP was responsible for the antihypertensive effects of in animal models.

As shown in Fig. 4, the inhibition of ACE reached IC_{50} of 90 µg.mL^{-1} for GPH and 120 µg.mL^{-1} for GIPH. In another study, the action of the IPP peptide on ACE inhibition was evaluated, finding an IC_{50} of 2,819 µg.mL^{-1} (Danish et al., 2017). The intact protein (YCP) did not exhibited activity (data not shown), probably due to its low solubility and high molecular weight structure, where the bioactive peptides are encrypted inside the molecule. Other authors also reported direct relation between the increase of solubility and the enhancement of antihypertensive effects when proteins are submitted to simulated digestion (Liu et al., 2020). This may occur due to the specificity of trypsin, which cleaves peptide bonds containing lysine and arginine, leading to the exposure of these amino acids. Toopcham et al. (2017) found that positively charged amino acids, such as lysine and arginine, have the ability to increase the effectiveness of ACE-inhibiting peptides, due to the high affinity for the ACE active site.

3.6. Antibacterial activity

Simulated digestion produced antimicrobial peptides (AMPs) against *E. coli*. Most expressive antibacterial activity was observed after the total gastrointestinal digestion of yam proteins: GIPH had MIC of 0.094 mg.mL^{-1} while GPH and YPC had MIC of 0.188 and 1.5 mg.mL^{-1}, respectively. Statistical differences ($p < 0.05$) were found between the results. This can be explained by the cleavage of peptide bonds and the consequent decrease of the molecular weight of protein molecules, since smaller molecules can easily penetrate the bacterial cell membranes, creating pores, causing cell leakage and damage to the bacteria (Epand & Vogel, 1999). Moreover, hydrolysis generates a mixture of peptides which expose different electrical charges and polarity. Our findings agree with Hwang and Gums (2016), who found the best results for inhibition of Gram-negative bacteria, such as *E. coli*, for linseed peptide fraction with lower molecular weight.

According to Ciumac et al. (2019) the selective antibacterial activity displayed by peptides depends on the result of a delicate interplay different parameters, such as: sequence and conformation, charge (most AMPs are positively charged), amphipathicity and polar angle. Fractions of cottonseed peptides with a higher number of positively charged amino acids were found to be essential for the inhibition of *E. coli* strains (Song et al., 2020). It is likely that the GPH peptide LNQV**YR** contributed to the inhibition of the *E. coli*, due to the presence of arginine (R). Furthermore, the peptides QQ**LK**, QVL**LLK** and V**LLLK**, with sequences of aliphatic and cationic amino acids, such as Leu (L) and Lys (K), may also be involved with the antibacterial activity of the yam hydrolysates. Those features are also present in several peptides from GIPH, such as VIFDQTLG**KLR, KL**GTVSP**K**, VIQTPALG**KL,** DQTLG**KL**, FDQTLG**KL** and SVFYFEQ**LK** (sequences not shown in Table 1). Cationic peptides interact with bacteria membrane, which tend to be highly negatively charged, leading to membrane permeabilization and structural disruption (Ciumac et al., 2019).

Yam concentrate and hydrolysates did not display inhibition activity against *Salmonella* and *L. monocytogenes* growth (Supplementary material, Table S2).

4. Conclusions

After *in vitro* gastrointestinal digestion of yam tubers proteins several peptides with bioactive potential were identified. Using *in silico* tools in the search for homologous sequences, we found peptides sharing strong similarity with known sequences capable of acting as antioxidative,

ACE-inhibitor, DPP-IV inhibitor and antibacterial. *In vitro* assays confirmed the digestion of yam proteins, including dioscorin, releases fragments of peptides with natural bioactive sequences during the hydrolysis process.

Further studies should focus on the synthesis of the sequences of interest, in order to confirm the specific activities displayed by the hydrolysates in our work. Moreover, *in vivo* studies and cell culture experiments with the yam hydrolysates will be essential to better understand their biological activities in living tissues. Other aspects that should be addressed by additional studies include elucidation on peptides' partial hydrolysis by intestinal brush border peptidases prior to the flux across the cell layer, the secretion rate at the basolateral membrane and the possible proteolysis by bloodstream enzymes before reaching the target organs. Once present in gut lumen, yam bioactive peptides could also interact with either the intestinal microbiota or receptors on the surface of the intestinal epithelium, triggering beneficial effects.

Therefore, apart from the fact that yam is a rich source of nutrients, our results show that proteins from *Dioscorea cayennensis* are also sources of bioactive peptides. These promising molecules may contribute to human health either reducing the risk of development of chronic degenerative diseases or preventing/fighting bacterial infections.

CRediT authorship contribution statement

Edilza Silva Nascimento: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Katya Anaya:** Writing - original draft, Writing - review & editing, Visualization. **Julia Mariano Caju Oliveira:** Investigation. **José Thalles Jocelino Gomes Lacerda:** Formal analysis, Investigation, Writing - review & editing. **Michael Edward Miller:** Investigation. **Meriellen Dias:** Investigation. **Maria Anita Mendes:** Investigation. **Juliana Azevedo Lima Pallone:** Resources, Investigation. **Clarice Weis Arns:** Resources, Investigation. **Maria Aparecida Juliano:** Resources, Investigation. **Tatiane Santi Gadelha:** Writing - review & editing. **Maria Teresa Bertoldo Pacheco:** Resources, Conceptualization, Methodology, Writing - review & editing, Supervision. **Carlos Alberto Almeida Gadelha:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing 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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110286.

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