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Yam (*Dioscorea cayennensis*) protein concentrate: Production, characterization and *in vitro* evaluation of digestibility

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ABSTRACT

This study aimed to produce a yam protein concentrate (YPC), *Dioscorea cayennensis,* to evaluate its protein digestibility and the peptide profile generated through simulated gastrointestinal digestion (GID). After concentration, the YPC that resulted in 64.0% protein was obtained. Simulated GID and free amino acids analysis were performed to evaluate its digestibility. For the peptide profile was evaluated by means of structure, molecular weight (MW) (nano-LC-ESI-MS/MS and SEC-FPLC), and hydrophobicity (RP-HPLC). Roughly 71.0% of peptides showed MW < 2 kDa after GID. The peptide sequences KQAVNE**NAINNARPL**QPTN, **GRSDPFL**SDL, and KNEINAG**VVDPN**QLQF were identified in the gastric digestion. These sequences were further digested in the intestinal phase, yielding the **NAINNARPL**, **GRSDPF,** and **VVDPN** peptides**.** The GID process generated molecules with smaller MW, with consequent exposure of ionizable and functional groups, potentializing the yam proteins bioactive capacity. The peptides obtained after the digestion should be explored in future studies with the objective of understanding the bioactive potential of these molecules for human health.

1. Introduction

The yam *(Dioscorea* spp.*)* is considered an important tuber for the human diet. It is cultivated in Africa, Asia, as well as Central and South America (Xue, Miyakawa, Sawano, & Tanokura, 2012). The most important edible species are *D. cayennensis*, *D. alata*, *D. japonica*, *D. batatas* and *D. opposite* (Zhang et al., 2019). In Brazil, the most cultivated species are *Dioscorea cayenensis* Lam. and *Dioscorea alata* L. whose varieties are named locally as "Da Costa" and "São Tomé", respectively. According to the main agricultural research agency in Brazil, the Brazilian Agricultural Research Corporation (EMBRAPA), the "Da Costa" cultivar of the *D. cayennensis* species is the most studied variety. Additionally, this species has been recommended for commercial planting in Brazil, especially in the northeast region, which concentrates its largest national production (Oliveira, Barbosa, Pereira, Silva, & Oliveira, 2007).

Nutritionally, the tubers of these species represent a substantial source of starch but a protein from the *Dioscorea* genus has been gaining prominence - the dioscorin - its main storage protein (Brito, Soares, Furtado, Castro, & Carnelossi, 2011). The *D. cayennensis* yam proteins'

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biochemistry description showed that 85% of the total protein is composed of dioscorin (Conlan et al., 1995). The benefits of this tuber are well known in medicinal and culinary applications. The tuber is also extensively used in traditional Chinese medicine as phytotherapic treatment for diabetes, neurodegenerative diseases, and cancer. Studies from the last decade have shown that the dioscorin of the Diocorea genus tubers, alongside its hydrolysates and peptides, are responsible for a number of biological activities *in silico*, *in vitro,* and *in vivo,* such as antioxidant, antihypertensive, immunomodulatory, and anti-inflammatory effects (Han, Lin, & Hou, 2014; Lu, Chia, Liu, & Hou, 2012; Lin et al., 2014; Zhang et al., 2019).

Various protein concentrates and isolates have been characterized and subjected to simulated gastrointestinal digestion (GID) including soybean seeds, soy milk, bean, quinoa, amaranth, and rice bran (Capriotti et al., 2015; Cho, 2020; Mune, Minka, & Henle, 2018; Piñuel et al., 2019; Rodríguez & Tironi, 2020; Vilcacundo, Miralles, Carrillo, & Hernández-Ledesma, 2018; Vilcacundo, Miralles, Carrillo, & Hernández-Ledesma, 2018). Simulated GID reportedly induces bioactive peptides from several proteins. However, to our knowledge there is no study in the literature concerning the digestibility, nutritional and functional quality of protein concentrate from *Diocorea cayennensis*.

Bioactive peptides are fragments of proteins that have different mechanisms of action with positive effects for the human organism. Given their biological effects, bioactive peptides have increasingly attracted attention as they help prevent degenerative diseases and contribute to human health as a whole (Udenigwe & Aluko, 2012).

In this context, the digestion process is essential to identify the nutritional and functional properties of food proteins because of the release of peptides and amino acids, as well as their bioavailability after absorption (Korhonen & Pihlanto, 2006). The simulated gastrointestinal digestion (GID) is an excellent research resource for understanding the quality of food, such as the behavior of its composition and structure during digestion (Lundin, Golding, & Wooster, 2008). Minekus et al. (2014) emphasizes the use of GID *in vitro* and addresses the digestibility and bioacessibility in several pharmaceutical products and macronutrients, such as proteins. Thus, the GID represents a viable alternative to peptide obtainment with the focus on potential health benefits (Lucas-González, Viuda-Martos, Pérez-Alvarez, & Fernández-López, 2018).

Therefore, this study aimed to produce a yam protein concentrate (YPC) and investigate the effects of simulated gastrointestinal digestion *in vitro*, including the protein digestibility and peptide characterization.

2. Material and methods

2.1. Material

The "Da Costa" *D. cayennensis* yam cv. tubers were cultivated from February to December and acquired seven months after planting in the experimental planting station of Chã de Jardim at UFPB-Campus II (Areia, PB, Brazil). This condition was essential to ensure the selection of the specific variety of the cultivar. This region sits at 574.62 m above sea level at a longitude of 35° 42 'WGR and latitude 6° 58′12′S. The predominant bioclimate is the 3DTH sub-dry northeastern region with around 1400 mm annual rainfall. According to Köppen's classification the climate of the research area is type As', which is characterized as hot and humid with average annual temperature of 23 and 24 °C. According to the Brazilian System of Soil Classification (SiBCS), the soil of the experimental area is classified as Regolitic Neosol with sandy loam texture (BRASIL, 1972). These edaphoclimatic characteristics, associated with the conditioning and adequate fertilization of the soil, ensured the appropriate conditions recommended for the cultivation of the "Da Costa" yam cv.

Porcine pepsin (≥250 units/mg solid)) and pancreatin (from porcine pancreas, 8 × UPS), bile salts, α-aminobutyric acid, tricine, α-Lactoalbumin (L6010), Insulin (I2643), L-β-4- Dihydroxyphenylanine (D-9628), and Vitamin B12 (Fw13554) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). [Glu1]-Fibrinopeptide, acetonitrile was purchased from Waters (Milford, MA, USA). All other chemicals were of analytical or higher grade.

2.2. Preparation of yam protein concentrate (YPC)

After the tubers were sanitized, shade-dried, and cut, they were mixed in distilled water in a 1:10 ratio and grinded. After filtration in a polyester fabric, the recovered yam mass was left overnight in 70% ethyl alcohol. The mass was then centrifuged at 3700 g for 15 min, which followed with the supernatant being discarded and the dry mass powdered to obtain the 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 (NaOH 1 mol/L). The suspension was stirred for 3 h at 25 °C and centrifuged at 3700 g for 30 min at 4 °C. The supernatant was recovered and subjected to protein precipitation with pH adjustment to 5.7 (HCl 1 mol/L) and centrifuged at 3700 g for 20 min at 4 °C. The precipitation process was repeated, adjusting pH to 4.5 (HCl 1 mol/L), and then the precipitates were combined and neutralized to pH 7.0 (NaOH 1 mol/L). The protein concentrate was dialyzed in Milli-Q water, under magnetic stirring at 25 °C on a 3.5 kDa MWCO cellulose membrane, Sigma-Aldrich Co. (St. Louis, MO, USA). Then, the protein concentrate was freeze-dried to obtain the YPC.

2.3. Protein content

The total protein content was measured by the Kjeldahl method using 5.75 as a nitrogen-protein conversion factor (Latimer, 2012).

2.4. GID in vitro of YPC

The YPC digestion was performed following the internationally accepted digestion protocol developed by the INFOGEST (Minekus et al., 2014). An amount of 1000 mg of YPC was dissolved in 10 mL of deionized H_2O at 37 °C and ultrasonicated in ultrasound bath for 30 min. Porcine pepsin (20 mg/mL) previously adjusted to pH 2.8 using HCl 1 mol/L was added to the mixture and subjected to digestion for 120 min at 37 °C in water bath under agitation at 150 rpm (gastric phase). The gastric phase was interrupted by pH adjustment to 8.0 with NaOH 1 mol/L. In sequence, the enzyme (pancreatin 40 mg/mL) and porcine bile extract (24 mg/mL) were added. The digestion proceeded in water bath under agitation at 150 rpm for 120 min at 37 °C (total gastrointestinal phase). The digestion was stopped when the digest was heated to 85 °C for 15 min, followed by centrifugation at 10,410 g for 15 min. Digestions were performed in duplicate.

The gastric phase (GPH) and total gastrointestinal phase hydrolyzates (GIPH) were dialyzed in Milli-Q water in a 500 Da membrane, Spectrum™ (New Brunswick, NJ, USA), recovered, and then freeze-dried.

2.5. Tricine-SDS-PAGE

The hydrolysis process was accompanied by tricine-sodium dodecyl/sulphate-polyacrylamide-gel electrophoresis (Tricine-SDS-PAGE) (Schagger & Jagow, 1987). Three gels were used: stacking (4% T and 3% C), spacer (10% T and 3% C), and resolving (16% T and 3% C). The samples were diluted in reducing buffer, homogenized in a vortex, then placed in an oven at 100 °C for 10 min. The samples were applied to the gel and run at 25 mA for approximately 6 h. After running time, the gel was fixed in a solution of methanol, acetic acid, and water (5:1:4 v/v/v) and stained in 0.025% Coomassie Brilliant Blue G-250 solution in 10% acetic acid. To compare the samples' molecular weights (MW), a standard low MW marker from GE Healthcare Life Science (Rockford, IL, USA) of 38 to 3.5 kDa was used.

2.6. Total amino acid analysis

Total amino acid analysis was performed on a liquid chromatograph (Shimadzu Corporation, Tokyo, Japan) using a Luna C18 reverse phase column (250 mm × 4.6 mm, 5 μm, Phenomenex Inc. (Torrence, CA, USA). Amino acids were quantified by comparison with Thermo-Scientific (Rockford, IL, USA) amino acid standards. An internal standard of α-aminobutyric acid from Sigma-Aldrich Co. (St. Louis, MO, USA) was used, (Hagen, Frost, & Augustin, 1989). Tryptophan content was determined after enzymatic analysis with Pronase (40 °C/22–24 h), followed by colorimetric reaction with 4-(dimethylamino) benzaldehyde in H2SO4 at 10.55 mol/L and reading at 590 nm. The tryptophan content was calculated from a standard L-tryptophan curve (Spies, 1967). Amino acid score (AAS) and essential amino acid index (EAAI) were used to measure the proteins' biological quality. These parameters were calculated using the standard amino acid requirement (FAO/WHO/UNU, 2007) where EAAS = mg of EAA (essential amino acid) in 1g of test sample protein/mg of EAA in 1g of standard protein (FAO/WHO) × 100.

2.7. Analysis of free amino acids

Free amino acids were extracted with 0.1 mol/L hydrochloric acid (g/mL) using orbital agitation for 60 min, followed by derivation in a pre-column with phenylisothiocyanate (PITC) (White, Hart, & Fry, 1986) and (Hagen, Frost, & Augustin, 1989). The separation of phenylthiocarbamyl-amino acid derivatives (PTC-aa) was performed using a high-performance liquid chromatographer (Shimadzu Corporation, Tokyo, Japan) in a C18-Luna-Phenomenex reverse phase column (250 mm × 4.6 mm, 5 μm; (Phenomenex Inc., Torrence, CA, USA). The sample was injected automatically (50 μL) and detection occurred at 254 nm. The chromatographic separation was carried out at a constant flow of 1 mL/min, at 35 °C. The run time was 45 min and results were expressed in mg of amino acid per 100 g of sample, in which quantification was performed by adding internal α-aminobutyric acid standard and the identification of amino acids was performed by comparison to external standard amino acids from Thermo Scientific (Rockford, IL, USA).

2.8. Molecular weight distribution

The YPC, GPH and GIPH were characterized according to molecular mass distribution profile by AktaPure FPLC chromatography system, GE Healthcar, (Rockford, IL, USA) with detection at 280 nm in a Superdex 30 Increase 10/300 GL chromatographic column GE Healthcare, (Rockford, IL, USA), with a 100–7000 Da separation range. Chromatograms were monitored and obtained by Unicorn 6.3 Software. The samples (varying concentrations) or standards (0.2 and 1.0 mg/mL) were solubilized in a mobile phase (25 mM sodium phosphate buffer pH 7.4 150 mM NaCl) and sonicated for 10 min. Both the mobile phase and samples/standards were filtered through a hydrophilic polytetrafluoroethylene membrane (PTFE; 0.45 μm). The sample injection volume was 100 μL and the running time was of 65 min. Standards with different MW were used, such as: α-lactoalbumin (14,178 Da), Insulin (5807.6 Da), Vitamin B12 (1355.4 Da), and L-β-4-Dihydroxyphenylanine (197.2 Da) for the analytical curve (log MW x TR) and the calculation of the MW distribution was performed by retention time range (TR) percentage.

2.9. Hydrophobicity profile

The samples hydrophobicity profile was determined by reversed-phase high-performance liquid chromatography (RP-HPLC) on a Shimadzu HPLC system, with photodiode array detector (Shimadzu, Kyoto, LLHA, JPN), C18 Luna 100 Å column (4.6 mm × 250 mm; 5 μm particle) (Phenomenex, Torrence, CA, USA). The solvent composition was: solvent A) Milli-Q water with 0.1% trifluoroacetic acid (TFA); solvent B) acetonitrile with 0.1% TFA filtered on a hydrophilic PTFE membrane (0.45 μm). The column was maintained at room temperature with a flow of 1 mL/min, detection at 214 nm, volume of 50 μL injection, and running time of 55 min. The samples (3 mg protein/mL for hydrolysates and 1 mg/mL for intact YPC) were eluted in a linear gradient of 5–20% of solvent B in 20 min, reaching 40% of solvent B in another 20 min and up to 80% in the following 10 min. In the last 5 min of runtime, the condition was returned to 5% of solvent B.

Table 1
Crude protein content (g/100g) and protein yield: yam tuber (YT), yam protein extract (YPE), yam protein concentrate (YPC).

Samples	Protein content g/100g of sample (dry base)	Protein yield (%)
YT	10.0 ± 0,03[a]	NM
YPE	29.5 ± 0,04[a]	66.1
YPC	64.0 ± 0,03[a]	84.4

NM-not measured.
[a] Crude protein content was determined by the Kjedhal method with measurement in triplicate on a dry basis.

2.10. nanoLC-ESI-MS/MS

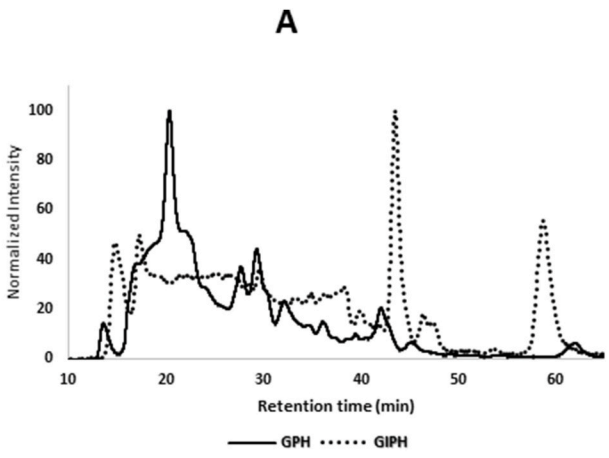
GPH and GIPH were resuspended in water:acetonitrile:formic acid (97.9:2:0.1, v:v:v) and subjected to analysis by nanoflux liquid chromatography, coupled to sequential mass spectrometry, with Electrospray Ionization (nanoLC-ESI-MS/MS) performed on a NanoLCDionex Ultimate 3000 system (Thermo Fisher Scientific), which in turn was coupled to an Impact II quadrupole time-of-flight 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 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 the flow was adjusted to 300 nL min^{-1}. Mass spectra of MS precursors were acquired in positive ion mode and MS/MS products acquired at 2 Hz in a mass range of 50–3000 *m/z* and the branched collision-induced dissociation energy parameters varied from 7 to 70 eV.

2.11. 11Bioinformatics analysis

Raw MS/MS data files were imported into the PEAKS Studio 8.5 software (Bioinformatics Solution Inc., Waterloo, ONT, Canada) for *de novo* analysis and database searches (Zhang et al., 2012). The *de novo* analysis was performed with a precursor mass tolerance of 07 ppm, a 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 database search using SPIDER tools (Han, Ma, & Zhang, 2005) against the Uniprot KB Dioscorea database (71 Swiis-prot sequences and 2703 TrEMBL sequences downloaded on May 3, 2018 from http://www.uniprot.org/). The false discovery rates 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.

2.12. Statistical analysis

For statistical analysis, the GraphPad 6.0 software program was used. Results were expressed as mean ± SD. One-way analysis of variances (ANOVA) was used, followed by Student's t-test. Statistically significant differences were considered when $p < 0.05$.



MW range	Molecular weight distribution (%)		Δ between GPH and GIPH (%)
	GPH	GIPH	
> 7 kDa	38.49	26.57	-30.97
5-7 kDa	12.13	6.63	-45.37
3-5 kDa	9.76	10.01	2.60
2-3 kDa	10.74	7.39	-31.19
< 2 kDa	28.88	49.40	71.05

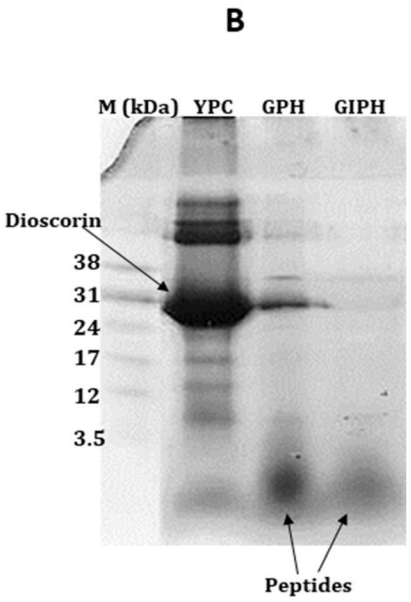


Fig. 1. **(A)** Yam protein hydrolysates molecular weight distribution: size exclusion chromatography –GPH- gastric phase hydrolysate and GIPH- gastrointestinal phase hydrolysate. **(B)**: Tricine-SDS-PAGE: M-molecular weight marker; YPC- yam protein concentrate; GPH- gastric phase hydrolysate; GIPH- gastrointestinal phase hydrolysate.

3. Results and discussion

3.1. Yam protein concentrate (YPC)

The yam, protein extract and YPC protein percentage, as well as the protein extraction yield, are shown in Table 1. The tuber had a protein content of 10.0 g/100g of sample, which is within the range found in other yam species, as in *D. alata* (8.1–15.0 g/100g) (Huang, Chiangb, Chenc, & Wang, 2007). After extraction and precipitation at the proteins isoelectric point, a concentration corresponding to 64.0 g of total protein per 100 g of sample was obtained.

The process used to concentrate the proteins was able to increase the protein content almost fivefold, constituting the greatest and most interesting component for the research of a proteome. This technique showed a yield of 84.4%, which is advantageous considering other isolation methods, based on the use of ultrafiltration membranes and columns since the chemical reagents used were cheap and the procedure simple to perform. This means that economic and nutritional value can be easily added to a common by-product in the agro-industry, which in this case is the recovered YPC.

3.2. In vitro simulated GID of yam proteins

Yam proteins contain various bioactive activities according to Lu et al. (2012). Thereby, further research on gastrointestinal protein digestion is important to elucidate the protein quality after digestion, as well as the characterization and functionality of the peptides produced. Thus, the YPC was submitted to simulated GID *in vitro* for peptide release in two phases: gastric and intestinal. According to Wang et al. (2017), proteins subjected to simulated gastric and intestinal digestion can generate different biological characteristics.

The digestion process includes mechanical, chemical, and enzymatic steps to release nutrients and facilitate their absorption. Given the complexity of this biological process, an international consensus on digestion in adulthood has been standardized to simulate this process *in vitro*. For this, a static protocol based on physiologically relevant conditions obtained from human beings was elaborated. The sequence of events that occur during this simulation is very similar to gastrointestinal digestion *in vivo* (Minekus et al., 2014).

The undigested protein (YPC) and digestion phases: gastric (GPH) and intestinal (GIPH) patters are shown in Fig. 1 B. The YPC showed protein bands greater than 38 kDa and lower than 3.5 kDa. Among which, the most intense band (~31 kDa) represents the main storage protein, dioscorin (Hou, Chen, & Lin, 2000). Differences between the GPH and GIPH phases are presented in Fig. 1 (A and B). The efficiency of pepsin in initial protein hydrolysis is verified through molecular mass distribution (Fig. 1A), as approximately 39.0% of molecules present a mass of >7 kDa. After gastric digestion, the dioscorin band, although reduced, can still be visualized (Fig. 1B). This is due to the pepsin enzyme's ability to nonspecifically digest macroproteins in polypeptides in the gastric phase. Hence, in the first phase, the acidic environment of the stomach provides the denaturation of yam proteins with consequent unfolding of molecules and exposure of peptide bonds, followed by hydrolysis and generation of these polypeptides for the continued hydrolysis in the intestinal phase of the digestive process. Whereas, polypeptides are digested into smaller fragments only in the intestinal digestion phase (MacFarlane, 2018).

With the progression of hydrolysis in GIPH, the gastric phase polypeptides are fragmented by pancreatin into oligopeptides. As a result, as shown in Fig. 1A, larger polypeptides (MW 5–7 kDa) were converted into smaller oligopeptides (MW < 3 kDa) after complete GID. In accordance with this result, a decrease of higher molecular weight proteins and an increase of diffuse bands of low molecular weight peptides (<3.5 kDa) can also be seen in Fig. 1B.

Finally, the *in vitro* GID process showed that proteins were fragmented into low molecular weight molecules, according to the MW distribution profile, in which approximately 71.0% of peptides had MW < 2 kDa. Therefore, it is suggested that yam proteins are easily digested by gastrointestinal system enzymes. Thus, their ionizable groups, amino acid sequence, and functional groups are exposed with greater capacity to perform different biological activities (Moller & Scholz-Ahrens, 2008).

Table 2
Composition of amino acids and chemical score of yam protein concentrate (YPC) and gastrointestinal phase hydrolysate (GIPH).

Amino acids (AA)	Samples			
	YPC		GIPH	
Essential	(g/100g protein)	Score	(g/100g protein)	Score
Lys	5.77 ± 0.06	1.28	4.97 ± 0.06	1.10
Trp	1.06 ± 0.02	1.77	2.01 ± 0.11	3.35
Phe	6.95 ± 0.03	2.86*	6.76 ± 0.05	2.77*
Tyr	3.93 ± 0.02		3.76 ± 0.23	
Met	1.90 ± 0.08	0.94**	2.18 ± 0.10	0.94**
Cys	0.16 ± 0.02		0.11 ± 0.01	
Thr	4.35 ± 0.04	1.89	4.20 ± 0.03	1.70
Leu	7.98 ± 0.03	1.35	7.33 ± 0.09	1.24
Ile	4.23 ± 0.03	1.41	3.98 ± 0.08	1.32
Val	5.16 ± 0.05	1.32	4.80 ± 0.10	1.23
His	0.60 ± 0.02	0.40	2.48 ± 0.04	1.65
Non-essential				
Asp	13.29 ± 0.1	NE	13.80 ± 0.23	NE
Glu	17.09 ± 0.1	NE	17.37 ± 0.10	NE
Ser	5.91 ± 0.03	NE	5.88 ± 0.01	NE
Arg	9.87 ± 0.04	NE	9.39 ± 0.03	NE
Ala	4.56 ± 0.03	NE	4.39 ± 0.09	NE
Pro	4.19 ± 0.02	NE	4.11 ± 0.08	NE
Gly	3.80 ± 0.01	NE	4.47 ± 0.10	NE
AA distribution (%)				
Hydrophobic	36.0		35.6	
Hydrophilic	46.6		48.0	
Neutral	17.4		16.4	

Asp = Aspartic acid, Ala = Alanine, Arg = Arginine, Gln = Glutamine, Gly = Glycine, His = Histidine, Cys = Cysteine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Ser = Serine, Thr = Threonine, Glu = glutamic acid, Tyr = Tyrosine, Val = Valine.
Hydrophobic (Ala, Val, Met, Phe, Leu, Ile, Pro,Trp) Hydrophilic (Arg, Asp, His, Lys, Glu) Neutral (Ser, Gly, Thr,Tyr, Cys).
*Fenilalanina + Tirosina; **Metionina + Cisteine. NE-not estimated. Chemical score performed for adults >18 years (FAO, 2007).

Table 3
Free amino acid profile of YPC and GIPH (*Dioscorea cayennensis*).

Free amino acids (g/100g of protein)	YPC[c]	GIPH[b]
Asp	0.01 ± 0.00	0.26 ± 0.00
Glu	0.10 ± 0.00	0.29 ± 0.01
Ser	0.00 ± 0.00	0.47 ± 0.00
Gly	0.01 ± 0.00	0.05 ± 0.02
His	0.05 ± 0.59	0.18 ± 0.00
Arg	0.04 ± 0.00	3.82 ± 0.01
Thr	0.02 ± 0.00	0.13 ± 0.00
Ala	0.02 ± 0.00	0.24 ± 0.01
Pro	0.01 ± 0.40	0.12 ± 0.00
Tyr	0.08 ± 0.00	1.65 ± 0.00
Val	0.01 ± 0.00	0.48 ± 0.00
Met	0.03 ± 0.01	0.32 ± 0.02
Cys	0.10 ± 0.03	0.05 ± 0.00
Ile	0.00 ± 0.00	0.30 ± 0.01
Leu	0.02 ± 0.01	1.88 ± 0.00
Phe	0.43 ± 0.04	2.70 ± 0.00
Lys	0.06 ± 0.00	1.47 ± 0.01
Trp	NM[a]	NM[a]
Total	0.99 ± 0,04[a]	14.41 ± 0,01[b]

Asp = Aspartic acid, Ala = Alanine, Arg = Arginine, Gln = Glutamine, Gly = Glycine, His = Histidine, Cys = Cysteine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Ser = Serine, Thr = Threonine, Glu = glutamic acid, Tyr = Tyrosine, Val = Valine. Different letters: significant difference $p < 0.5$.
[a] Not measured.
[b] Gastrointestinal phase hydrolysate.
[c] Yam protein concentrate.

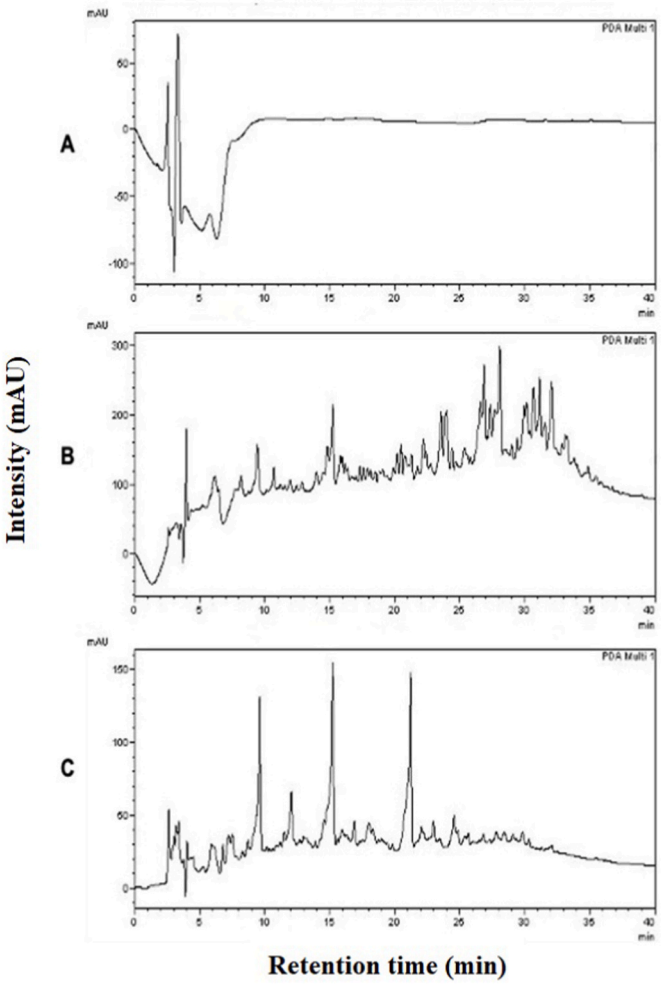


Fig. 2. Hydrophobicity profile by RP-HPLC. Samples (A) YPC- yam protein concentrate, (B) GPH- gastric phase hydrolysate and (C) GIPH- gastrointestinal phase hydrolysate.

3.3. Total amino acid composition

The protein's amino acid profile is of great importance in assessing its nutritional quality. The YPC and GIPH total amino acid composition are shown in Table 2.

In order to know the nutritional value of yam proteins, as well as their hydrolysates, the amino acid content was compared with the FAO/WHO (2007) recommendation standard for adults. All essential amino acids reached a chemical score of >1, except for sulfur amino acids (methionine and cysteine), with a 0.94 chemical score. Low content of sulfur amino acids is common for most vegetal protein sources, such as sweet potato varieties (Pęksa, Miedzianka, & Nemś, 2018) and tubers of the same genus, such as the species *D. alata* (Boye, Wijesinha-Bettoni, & Burlingame, 2012). Nevertheless, these limiting amino acids can be complemented with other protein sources in the diet without compromising the balance of nutrients, while still maintaining nutritional quality.

3.4. Free amino acid composition

In addition to the amino acid composition, digestibility also plays a significant role in the protein quality (Dallas et al., 2017). Therefore, the release of free amino acids represents an important parameter to indicate a protein's bioavailability. Table 3 shows the combination of enzymatic hydrolysis with pepsin and pancreatin in the *in vitro* GID of

Table 4
Identification of peptides released in GPH and GIPH related to the sequence of the protein dioscorin of the genus Dioscorea from the database –Uniprot.

Peptide sequences GPH	Mass (Da)	Number of residues	Peptide sequences GIPH	Mass (Da)	Number of residues	Protein source	Number access
NAINNARPLQPTNY	1584.80	14	**NAINNARPLQ**	1109.59	10	*D.cayennensis*	Q39695
KQAVNE**NAINNARPLQPT**NY	2254.14	20	**NAINNARPLQPT**	1307.69	12	*D.cayennensis*	Q39695
KQAVNE**NAINNARPL**QPTN	2091.08	19	**NAINNARPL**	981.53	9	*D.cayennensis*	Q39695
A**INNARPLQPT**NY	1470.75	13	**INNARPLQPT**	1122.61	10	*D.cayennensis*	Q39695
FSSSQKNEINA**GVVDPN**QLQF	2321.12	21	**GVVDPN**	599.29	6	*D.cayennensis*	Q39695
KNEINAG**VVDPN**QLQF	1784.90	16	**VVDPN**	542.27	5	*D.cayennensis*	Q39695
EDITWT	763.33	6	**DITWT**	634.29	5	*Dio 1=D. alata*	A0A1P8PPN9
GRSDPFLSDL	1105.54	10	**GRSDPF**	677.31	5	*D.polystachya*	Q75N35
AINN**ARPLQPL**KF	1480.85	13	**ARPLQPL**	793.48	7	*D.polystachya*	Q75N35
L**SDLEDF**	837.37	7	**SDLEDF**	724.29	6	*D.cayennensis*	Q39695
IKQ**FSSSQ**KNEINAG	1649.83	15	**FSSSQ**	554.23	5	*D.cayennensis*	Q39695
YFEQLK	826.40	6	**YFEQLK**	826.42	6	*D.cayennensis*	Q39695
SINRVAY	949.49	8	**SINRVAY**	821.43	8	*D.cayennensis*	Q39695

GPH- Gastric phase hydrolysate. **GIPH-** Gastrointestinal phase hydrolysate.

yam proteins through the release of free amino acids (FAAs) in the final hydrolysate (GIPH).

The FAAs concentration after gastrointestinal digestion shows values of Phe (18.8%), Leu (13.1%), and Tyr (11.4%), where the first phase of digestion may have contributed to these results. In the gastric phase, the enzyme pepsin mainly cleaves the peptide bonds of macromolecules between hydrophobic and aromatic amino acids, such as Phe, Leu, and Tyr. Despite generating larger fragments (polypeptides), aromatic amino acids are exposed at the extremities, which can be cleaved and released in a next step. The trypsin released in pancreatic juice during intestinal digestion, in addition to hydrolyzing peptide chains mainly on the carboxylic side, produces more free Arg (26.5%) and Lys (10.2%). These values were presumably due to the higher hydrolytic activity of trypsin among the residues of these amino acids, during the intestinal phase (You, Zhao, Regenstein, & Ren, 2010).

The proteolytic activity, catalyzed by GIT enzymes, was observed to promote an increase in FAAs concentration. Compared with the intact protein (YPC), higher values are found for most amino acids after hydrolysis with a significant impact ($p > 0.05$) on the release of FAAs during simulated GID. Although protein digestion results in peptides of different sizes, di- and tri-peptides, as well as FAAs at the end of digestion, indicate an increase in protein digestibility and absorption at the end of the digestive process.

There is a considerable increase in essential amino acid (Tyr, Leu, Phe, and Lys) concentration, released at the end of digestion, which may indicate their bioaccessibility *in vitro* (Lorieau et al., 2018). Ribeiro et al. (2017) state that some small potentially bioactive peptides are able to resist gastrointestinal digestion and reach the mucosa to only then be absorbed. Thus, one of the important factors to determine the peptides' biological activity includes the digestion products because of the influence on their absorption through enterocytes and bioavailability in target tissues.

3.5. Hydrophobicity/hydrophilicity profile of YPC and digested GPH and GIPH

Fig. 2 showed chromatograms obtained from the RP-HPLC YPC, GPH, and GIPH profiles. In the YPC sample, no peaks were observed during the chromatographic run, revealing its insolubility under analysis conditions. This is probably due to the hydrophobic character of the protein concentrated by the isoelectric point, with greater protein-protein interaction and less interaction with the environment solvent. In the GPH digest, there are more significant peaks between 25 and 30 min, whereas the GIPH digest showed peak distribution in retention times around 10, 15, and 20 min. The chromatograms of protein hydrolysates (Fig. 2 B and C) show an increase in solubility regarding YPC. The decrease in peptide peaks at the end of the GIPH probably results from a more intense proteolysis, caused by enzymatic activity and greater release of free amino acids.

The specific bioactivity of food-derived peptides in acting by different mechanisms in the organism protection depends on its structure, physicochemical characteristics, amino acid residues, as well as hydrophobicity/hydrophilicity, and side chain load (Pripp, Isaksson, Stepaniak, Sorhaug, & Ardo, 2005). As an example, hydrophilic peptides, with repeated proline sequences (IPP, VPP), participate in the angiotensin-converting enzyme (ACE) inhibition mechanism and consequent hypotensive activity modulation (Danish, Vozza, Byrne, Frias, & Ryan, 2017). These sequences also show hypoglycemic activity as they act in the dipeptidyl peptidase IV (DPP-IV) inhibition (Wang et al., 2017).

Antibacterial and antioxidant peptides have hydrophobic amino acid residues in their structure that are capable of scavenging DPPH radicals and inhibiting lipid peroxidation. Hydrophobic peptides can also cause the rupture of negatively charged bacterial membrane. Studies indicate that charge and hydrophobicity are important for the activity of antimicrobial peptides (Bahar & Ren, 2013).

3.6. Identification of peptides from digested

The mass spectrometry analysis performed on digestion products (GPH and GIPH) enabled the identification of several peptides found within the sequences of the reserve proteins in the Dioscorea genus. Table 4 summarizes the peptides released in GPH and that had reduced molecular weight in GIPH, as well as those that did not change during digestion.

From the set of peptides obtained in GID and identified by nanoLC-ESI-MS/MS, an important finding was that most belonged to the *D. cayennensis* protein dioscorin. The analysis was limited to this protein, as it is the only protein sequence deposited in a database (Conlan et al., 1995). The dioscorin isoforms known in the literature, such as *D. alata*: DIO 1 and DIO5; *D. Japonica*: Dj-DIOA1; *D. Pseudojaponi:* Dp-DIOA1; and *D. Polystachya:* D1 (Huang et al., 2007) were also included.

Table 4 demonstrates that peptides from GPH, such as **GRSDPF**LSDL and KNEINAG**VVDPN**QLQF, had their molecular mass decreased with hydrolysis progression in GIPH, such as: **GRSDPF** and **VVDPN,** but maintained part of the amino acid sequence. These findings indicate that enzymes (trypsin and chymotrypsin) from the intestinal phase were able to cleave peptide bonds of larger molecules, with a reduction in their molecular mass at the end of the digestion process. However, other peptides generated in GPH, **YFEQLK,** and **SINRVAY** did not change and remained intact in GIPH, indicating that peptides generated in the stomach can also cross the intestinal barrier without changes.

4. Conclusion

This study contributes to the knowledge on the profile of peptides from simulated GID and attempts to predict their potential to modulate biological activities generated by the digestion process in the human

organism. In this context, the results in this study show that proteins from this tuber are easily digested, with the release of molecules of lower molecular weight. Thus, the digestion process facilitates the intestinal absorption and subsequent use for the target organs. However, the peptides obtained after digestion should be explored in future studies in order to understand the bioactive potential and benefits of these molecules for human health.

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CRediT authorship contribution statement

Edilza Silva do Nascimento: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Preparation, Writing – review & editing, Visualization. **Julia Mariano Caju de Oliveira:** Investigation. **José Thalles Jocelino Gomes de Lacerda:** Formal analysis, Investigation, Writing – review & editing. **Samara Batista Montenegro:** Investigation. **Maria Elisa Caetano-Silva:** Formal analysis, Investigation, Writing – review & editing. **Meriellen Dias:** Investigation. **Maria Anita Mendes:** Investigation. **Maria Aparecida Juliano:** Resources, Investigation. **Tatiane Santi Gadelha:** Resources, Writing – review & editing. **Maria Teresa Bertoldo Pacheco:** Resources, Conceptualization, Methodology, Supervision. **Carlos Alberto de Almeida Gadelha:** Conceptualization, Methodology, Supervision.

Declaration of competing interest

The authors declare that there is no conflict of interest in this study.

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