Bioactive compounds from Dioscorea bulbifera L. obtained by combined ultrasound- assisted and solid-liquid extraction

Gabrielly Ribeiro Carneiro
Federal University of Parana, Jandaia do Sul
Leomara Floriano Ribeiro (leomara.ribeiro@ufpr.br)
Federal University of Parana, Jandaia do Sul

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Abstract

The aim was to extract bioactive compounds from the husk and pulp of *Dioscorea bulbifera* L. by the combination of ultrasound and solid-liquid extraction techniques, as well as to evaluate the antioxidant potential. The extraction at 80 °C for 60 minutes showed the highest content of total phenolic compounds (TPC), 715.53 ± 8.00 mg EAG 100 g^{-1}, for the husk, while the extraction at 70°C for 45 minutes showed 235.50 ± 25.30 mg EAG 100 g^{-1}, for the pulp. The extracts with the highest TPC were evaluated for the other bioactive compounds. The husk and pulp extracts showed flavonoid content of 363.63 ± 8.92 and 102.44 ± 1.51 mg EC 100 g^{-1}, respectively. The estimation of the total carotenoid content allowed obtaining 2.13 ± 0.11 µg 100 g^{-1} for the husk and 1.34 ± 0.11 µg 100 g^{-1} for the pulp. The antioxidant potential was evaluated according to the FRAP assay (125.09 ± 8.52 and 32.76 ± 0.65 µM ferrous sulfate g^{-1}), the removal of H_2O_2 (29% and 41%), the ABTS radical assay (66.88 ± 0.93 and 14.93 ± 0.31 µM Trolox g^{-1}) and the β-carotene/linoleic acid system, (84% and 47%) for husk and pulp, respectively. The bioaccessibility was obtained around 25% (pulp) and 16% (husk) accessible for absorption in the intestine. The combination of ultrasound and solid-liquid extraction methods proved to be effective in extracting bioactive compounds, which is a potential source for isolation and purification of bioactive compounds, with possible applications in the food industry.

Keywords: Cara-moela. Unconventional Food Plants. Simulated gastrointestinal digestibility. Antioxidant capacity.

1. Introduction

Dioscorea bulbifera L. is a tuber belonging to the Dioscoreaceae family, of the genus Dioscorea, native to Africa, but is a tuber widely cultivated and consumed in the tropics and subtropics (Achy et al. 2016). It is considered a type of air yam and popularly known as cara-moela, air yam and butterfly yam. Its cultivation and use are not widespread due to lack of knowledge of its nutritional and application potential (Olatoye and Arueya 2019). This tuber belongs to the group of Unconventional Food Plants (UFPs). The UFPs can be described as food species that have one or more edible parts such as roots, tubers, bulbs, rhizomes, leaves, fruits, but without daily use, but with potential to supply the food chain. UFPs can be consumed both fresh and processed. For this reason, interest in UFPs has been growing recently, as these plants can be sources of a wide variety of nutrients, such as proteins, carbohydrates, minerals, vitamins, dietary fiber, and phenolic compounds, but they are still little explored food sources (Milião et al. 2022).

Scientific studies on *Dioscorea bulbifera* L. are still scarce, but what is in the literature has shown that this tuber has potential for introduction in human food. The tuber has a low lipid content, between 0.85 and 1.83 g/100 g, and a high carbohydrate content, around 71.25 g/100 g (Bolaniran et al. 2019; Carneiro et al. 2020), in addition to having bioactive compounds directly related to different biological activities, such as anticancer activity (Gao et al. 2002), anti-inflammatory activity (Chaniad et al. 2020; Nguelefack et al. 2011) and antioxidant (Ghosh, 2015; Odeghe et al., 2020; Olatoye and Arueya, 2019; Song et al., 2010). The bioactive compounds are secondary metabolites via the shikimate or acetate pathway and consist of an aromatic ring and a benzene ring with one or more hydroxyl groups (Melini et al. 2020). Depending on the number of

phenolic rings and the structure of the rings interconnected, phenolic compounds can be classified into two groups: compounds of a flavonoid nature and compounds of a non-flavonoid nature (Yang et al. 2022). In addition to being responsible for the color of food (such as yellow, orange, red and blue pigments), for the taste and flavor (such as vanillin and eugenol) of food. Bioactive compounds have a specific metabolic and physiological action in the human body, modulating antioxidant defense and inflammatory and mutagenic processes (Ozcan et al. 2014).

The functionality of bioactive compounds in foods is related to their availability after reaching the gastrointestinal tract (Miskinis et al. 2023; Salvia-Trujillo et al. 2016). For absorption by enterocytes to occur, the released compounds must be solubilized by gastrointestinal fluids during digestion. These different conditions can cause changes in the physical state and chemistry of bioactive compounds, decreasing or increasing their bioaccessibility. These metabolites can be absorbed in the large intestine and further metabolized in the liver and/or excreted in the stool. Many metabolites are generated during the passage of polyphenols through the gastrointestinal tract, impacting the health benefits generated by polyphenols. Some metabolites are easily absorbed, while others are not (Rein et al. 2013). Thus, in vitro, and in vivo digestibility or bioaccessibility or bioavailability studies are extremely relevant to provide a depth knowledge regarding interactions between nutrients and its food components (Barba and Orlien 2017). The digestibility refers to the amount of nutrients absorbed by the individual and is generally calculated as the amount of nutrient consumed minus the amount of nutrient retained in the feces (Watts et al. 2013). While bioaccessibility is the amount of nutrients or components that are available to be absorbed across the intestinal epithelia (Rein et al. 2013). And bioavailability concept is

the rate and extent to which the active ingredient is absorbed and becomes available at the site of action (Demarco et al. 2022).

Furthermore, to better understand the behavior of bioactive compounds in a food matrix, it is important to consider how to obtain them. The extraction of bioactive compounds can be categorized into conventional and non-conventional techniques (Yusoff et al. 2022). The conventional extraction would cause the degradation of thermo-sensitive compounds resulted from long processing time and high extraction temperature. All techniques of solid liquid extraction (SLE) techniques take advantage of the higher solubility that some compounds have in different solvents, but often very time-consuming and can require large quantities of toxic and non-biodegradable organic solvents, as well as using a large amount of energy in the form of heat (Didion et al. 2023). On the other hand, ultrasound-assisted extraction, which is considered as the non-conventional technology requires lesser solvent, shorter extraction time and produces higher extraction yield, in addition to minimizing the degradation of thermo-sensitive compounds (Fu et al. 2021; Siddiqui et al. 2023). After treatment with ultrasound, the cell walls are disrupted, as the physical forces created during acoustic cavitation cause disruption in plant tissue, increasing the diffusion rate and enhancing the mass transfer of cell content, which helps in the release of bioactive compounds (Siddiqui et al. 2023; Wang et al. 2021; Yusoff et al. 2022). This assists in compound bioaccessibility as compounds are easily released from plant matrix in gastrointestine, and thus being available for intestinal absorption (Hadidi et al. 2021; Yusoff et al. 2022).

For this purpose, the extraction of bioactive compounds should also be studied based on the application of different processes, such as acid hydrolysis in different species of Dioscorea (Raina and Misra 2020), hydro-distillation for extracting bioactive

compounds in yam (Co et al. 2012), ultrasound in various plant crops (Wen et al. 2018), solid-liquid extraction with application of different solvents in *Dioscorea bulbifera* L. (Adeosun et al. 2016), in Dioscorea hirtiflora, Dioscorea dumetorum, and *Dioscorea bulbifera* (Adeniran and Sonibare 2017). To optimize the various extraction processes and minimize possible degradation of bioactive compounds, a combination of extraction processes can be applied. The aim was to extract bioactive compounds from the husk and pulp of *Dioscorea bulbifera* L. by the combination of ultrasound and solid-liquid extraction techniques, as well as to evaluate the antioxidant potential.

2. Materials and Methods

2.1. Samples and preparation

The samples used were from *Dioscorea bulbifera* L., obtained in Mandaguari/PR/Brazil. The samples were washed and sanitized with sodium hypochlorite solution (100 ppm) for 15 minutes and the husk and pulp fractions were separated. For enzymatic inactivation, the fractions were submitted to citric acid solution (2.4%) at 80°C for 5 minutes (Carneiro et al. 2020). Subsequently, the samples were dried in an oven (LUCADEMA 82/150) at 40°C until constant mass. The dried samples were ground, stored in low-density polyethylene (LDPE) packages, vacuum sealed (GSVAC/GS420), protected from light and under refrigeration (4°C) until the extractions were carried out.

2.2. Extraction of bioactive compounds

The extractions of bioactive compounds from the husk and pulp from *Dioscorea bulbifera* L. were carried out according to the Box-Behnken 3^2 experimental design, considering the variables temperature and time, as shown in Table 1.

Assays (E) were carried out separately for husk and pulp from *Dioscorea bulbiferal* L. in the proportion of 1:50 (m/v) in distilled water. The samples were submitted to an ultrasonic bath (SolidSteel/SSBu) 40 kHz for 10 minutes at room temperature, and subsequently submitted to a stirring Dubnoff orbital metabolic bath (Matoli/170M013) at temperatures and times according to the experimental design (Table 1). At the end of the extraction times, the samples were centrifuged (Solab/SL-700) at 6000 rpm for 20 minutes, filtered and the contents of total phenolic compounds, response variable, were evaluated in the extracts. Subsequently, the extracts were stored in amber flasks under refrigeration at 4°C until the time of the other analyses.

2.3. Colorimetric analysis for quantification of bioactive compounds

2.3.1. Total phenolic compounds

The total phenolic compounds content was determined in the extracts by mixing 60 µL of extracts with the addition of 3,000 µL of distilled water and 300 µL of Folin-Ciocalteau reagent (Dinamica), waiting for 3 minutes and adding 900 µL of sodium carbonate (15%) and 1740 µL of distilled water. Gallic acid - EGA (Sigma-Aldrich) was used as a standard, and the results were expressed in gallic acid equivalents (mg EGA) 100 g^{-1}, calculated by fitting the standard curve of gallic acid at concentrations from 25 at 650 mg L^{-1} in a spectrophotometer (Drawell/DU-8800RS) at 765 nm (Singleton and Rossi 1965).

2.3.2. Total flavonoid content

The determination of the total flavonoid content was based on the mixture of 500 µL of extracts with 2.5 mL of distilled water, 150 µL of sodium nitrite (5%), after 6 minutes 300 µL of aluminum chloride hexahydrate were added. (10%), and after resting for 5 minutes, 1 mL of 1 M NaOH was added. The analysis took place in a spectrophotometer (KASVI/K37-UVVIS) at 510 nm. Catechin was used, as a standard, in concentrations from 24.5 to 350 mg L^{-1} and the results were expressed in milligrams of catechin equivalent (mg CE) 100 g^{-1} (Meyers et al. 2003).

2.3.3. Content of total carotenoids

The total carotenoid contents were estimated in the extracts at absorbances of 646.8, 663.2 and 470 nm in a spectrophotometer (KASVI/K37-UVVIS). The results were obtained based on Eq.1, 2 and 3, and expressed in µg 100 g^{-1} (Lichtenthaler 1987).

$$Chlorophyll\ a\ (C_a) = 12.25\ x\ A_{663.2}\ - 2.79\ x\ A_{646.8} \quad (1)$$

$$Chlorophyll\ b\ (C_b) = 21.50\ x\ A_{646.8}\ - 5.10\ x\ A_{663.2} \quad (2)$$

$$Carotenoids = \frac{[1000\ x\ A_{470} - (1.82\ x\ C_a - 104.96\ x\ C_b)]}{198} \quad (3)$$

2.4. Antioxidant capacity assays

2.4.1. ABTS radical scavenging activity

The evaluation of antioxidant activity was performed by the ABTS radical scavenging method with modifications (Re et al. 1999). Aliquots of 90 µL of the

samples (husk and pulp extract) were transferred to test tubes with 9,000 µL of the ABTS•+ radical, previously prepared, after 6 minutes of the reaction, the samples were analyzed at 734 nm in a UV-Visible spectrophotometer (Drawell/DU-8800RS). The ethyl alcohol solvent was used as a blank and the calibration curve was performed with the Trolox standard (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich) at a concentration of 100 to 2000 µM and the results were expressed in µM trolox g^{-1}.

2.4.2. Auto-oxidation by the β-carotene/linoleic acid system

Antioxidant activity was evaluated by the β-carotene/linoleic acid system (Miller 1971). The β-carotene/linoleic acid emulsion (Sigma-Aldrich) was prepared by mixing 10 mg of β-carotene with 10 mL of chloroform, then 2 mL of this solution was added to 100 mg of linoleic acid (Sigma-Aldrich) and 400 mg of Tween 20 (Dinamica). The chloroform was completely evaporated on a rotary evaporator (MARCONI/442835). Pre-oxygenated distilled water was added for 30 minutes until absorbance remained between 0.6 and 0.7 nm. A 3.5 mL aliquot of the β-carotene/linoleic acid emulsion was mixed with 350 µL of extract at concentrations of 40 mg mL^{-1}. The solutions were incubated in a water bath (Nova instruments/NI 1215) at 50°C. For control, Trolox (0.2 mg mL^{-1}) was used. The initial absorbance was determined immediately after adding the samples to the system for the determination of time zero. The emulsion oxidation was determined by spectrophotometry (Drawell/DU-8800RS) at 470 nm after 120 minutes of incubation. The antioxidant capacity was calculated in terms of percentage of oxidation inhibition, according to Eq. 4.

$$AOA\ (\%) = \frac{(DR_C - DR_S)}{DR_C} x\ 100 \quad (4)$$

Where: AOA is the percentage of oxidation inhibition, DRC is the control degradation rate = ln (a/b)/120, DRS is the sample degradation rate = ln (a/b)/120, a is the initial absorbance at time 0 and b is the absorbance at 120 minutes.

2.4.3. Removal of hydrogen peroxide (H_2O_2)

The removal activity was determined by mixing 0.5 mL of H_2O_2 (0.1 mmol L^{-1}) with 0.5 mL of extract, 0.05 mL of ammonium molybdate (3% w/v), 5 mL of sulfuric acid (2 mol L^{-1}), 3.5 ml of potassium iodide (1.8 mol L^{-1}). For the pulp, concentrations of 40, 20 and 10 mg mL^{-1} were used, while for the husk were 40 and 20 mg mL^{-1}. The titration of negative (glucose) and positive (ascorbic acid) controls at the same concentrations of the extracts was performed with sodium thiosulfate (5 mmol L^{-1}). Removal capacity was calculated according to Eq. 5.

$$Removal\ of\ hydrogen\ peroxide = \left(\frac{V_0 - V_1}{V_0}\right) x\ 100\ \% \quad (5)$$

Where: V_0= volume used in the titration of the control and V_1= volume used in the titration of the samples.

2.4.4. Iron Reduction Method (FRAP)

The determination of the iron reduction activity was carried out in test tubes, to which 150 µL of extract were added at concentrations of 2.5 to 40 mg mL^{-1}, 2850 µL of FRAP reagent and kept in the dark in a water bath at 37°C for 30 minutes. The samples were evaluated in a spectrophotometer (Drawell/DU-8800RS) at 593 nm. The ferrous

sulfate was used as standard in the concentrations of 100 – 2000 μM. Results were expressed as μM ferrous sulfate g^{-1} (Thaipong et al. 2006).

2.5. Simulated gastrointestinal digestibility

The bioaccessibility was performed by simulating the digestibility by *in vitro* model (Bornhorst and Singh 2013; Dantas et al. 2019). Initially, 0.5 g of sample were mixed with 5 mL of distilled water and submitted to digestion simulation in the oral, gastric, and intestinal phases. For the oral phase, 5 mL of saline solution (simulated saliva) was added, and they were kept at 37°C for 10 minutes. To start the gastric phase, the pH was adjusted between 1 and 2, and then 15 mL of simulated gastric fluid was added and kept at 37°C for 120 minutes. In the intestinal phase, the pH was adjusted to 6 and then 5 mL of 120 mmol L^{-1} NaCl, 5 mL of 5 mmol L^{-1} KCl and 30 mL of simulated intestinal fluid were added, kept at 37°C for 60 minutes. As can be seen in Fig. 1. All steps were performed at 37°C in a Dubnoff orbital metabolic bath (MATOLI/170M013) under agitation. To stop the digestion process, the samples were placed in an ice bath for 10 minutes.

In each phase, aliquots were taken to determine total phenolic compounds (TPC). Bioaccessibility was calculated using Eq. 6.

$$Bioacessibility(\%) = \left(\frac{D}{I}\right) x\ 100 \quad (6)$$

Where: D = TPC (mg GAE $100g^{-1}$) content after intestinal digestion; I = TPC (mg GAE $100g^{-1}$) content before digestion.

3. Results and Discussion

3.1. Analysis of bioactive compounds

Bioactive compounds from the husk and pulp of *Dioscorea bulbifera* L. were extracted and quantified based on the levels of total phenolic compounds, expressed in Table 2, according to the Box-Behnken design. It was found that the husk extracts presented contents between 545.30 and 715.53 mg GAE 100 g^{-1}, considerably higher than those obtained for the pulp, which ranged from 166.95 to 235.50 mg GAE 100g^{-1}.

In addition to differences in region, climate, cultivation method, growth conditions, maturation stage, storage conditions that can interfere with the chemical composition of the husk and pulp, the content of total phenolic compounds are also influenced by the extraction method and parameters such as temperature, time and, especially, the solvent used (Damodaran et al. 2007; Tzia and Liadakis 2003).

Solid-liquid extraction is a process that involves the transfer of solute from a solid matrix to a solvent. It is an operation often used to extract important food components (Tzia and Liadakis 2003). The efficiency of this extraction method varies with the size of the solid particles, due to the contact surface of the solute with the solvent, with the temperature, increasing permeability of cell walls and membranes with the application of heat, with the choice of solvent, influencing the solubility of the compound by the interaction with the solute, contact time between solvent and solid particles, among other factors such as surface tension, viscosity and agitation, which help in the mass transfer mechanism.

In this study, the solid-liquid extraction was preceded by ultrasound (UAE) which helped to break the cell walls, releasing the intracellular compounds into the

283 solvent. In this case, only water was used, as in addition to helping mass transfer, it is
284 not an aggressive solvent, generating less environmental impact and facilitating future
285 applications (Damodaran et al. 2007). In Fig. 2 are (a) the results of the response surface
286 methodology and (b) the contour curves. It can be observed that the bark extract
287 submitted to a higher temperature (80°C) and a longer time (60 min.), resulted in the
288 highest CFT content, around 715 mg EAG 100 g^{-1}.

289 The results may be associated with the presence of fibers, since there are
290 compounds of phenolic origin that are structurally bonded to the fibers and, with the
291 action of temperature, become more malleable, being released directly into the
292 extraction solvent (Tzia and Liadakis 2003). Knowing that the husk of *Dioscorea*
293 *bulbifera* L. has around 4.90 g 100 g^{-1} of fiber, significant levels of phenolic compounds
294 were expected when subjected to higher temperatures (Carneiro et al. 2020), as occurred
295 in the test E9. From the data obtained for extracting the husk, a complete second-order
296 quadratic mathematical model was obtained to determine the TPC content (dependent
297 variable) of the husk extracts as a function of temperature and time (independent
298 variables) which can be expressed by Eq. 7.
299
300 $$TPC = 2268 - 50.70T - 5.94t + 0.38T^2 - 0.02t^2 + 0.10Tt \quad (7)$$
301 $$R^2 = 0.93 \text{ e } R^2_{adjusted} = 0.81$$
302 Where T = temperature (°C); t = time (min.)
303
304 By maximizing the mathematical model, 699.31 ± 14.30 mg EAG 100 g^{-1} were
305 obtained for the content of total phenolic compounds in the condition of 80°C and 60
306 minutes, as extraction parameters for the husk extract. The time factor was not

307 significant while the temperature factor had an influence on the variations of the total
308 phenolic compound's contents of the husk extracts of *Dioscorea bulbifera* L.

309 In Fig. 3 are the results of the TPC content of the pulp extract, being (a) the
310 results of the response surface methodology and (b) the contour curves. Extraction at a
311 temperature of 70°C and 45 minutes resulted in the highest TPC content, around 235 mg
312 EAG 100 g^{-1}.

313 In the pulp extract, it can be observed that the increase in temperature resulted in
314 lower levels of TPC. This fact is due to the heat treatment applied because it can
315 degrade the bioactive compounds, as well as it can interfere with the ability to
316 hydrolyze bonds between phenolics associated with proteins and/or carbohydrates,
317 which can negatively or positively influence the content of total phenolic compounds
318 (López et al. 2013).

319 From the data obtained for pulp extraction, a second order quadratic
320 mathematical model was obtained to determine the TPC content (dependent variable) of
321 extracts from the pulp of *Dioscorea bulbifera* L. as a function of temperature
322 (independent variable), the variable time did not have a significant influence, being
323 disregarded from the model, expressed by Eq. 8.

324

325 $$TPC = -0.26T^2 + 34.31T - 874 \quad (8)$$

326 $$R^2 = 0.96 \text{ e } R^2_{adjusted} = 0.95$$

327 Where T = temperature (°C)

328

329 The maximization of the mathematical model allowed to obtain, at the optimal
330 point, 235.98 ± 6.00 mg GAE 100 g^{-1} for the content of total phenolic compounds in the
331 condition of 64.4 °C, for temperature, and 50.3 minutes of time extraction method for

the pulp extract of *Dioscorea bulbifera* L. The time and temperature factors showed different behavior, and the time was not significant while the temperature factor had an influence on the variations of the phenolic compound's contents of the pulp extracts, as can be seen in the main effects graph.

There are interactions between phenolic compounds and the porous structure of carbohydrates (Jakobek 2015). In this sense, as the pulp of *Dioscorea bulbifera* L. contains about 75.70 g 100 g^{-1} of carbohydrates, the results of the contents of total phenolic compounds for the pulp extracts may indicate such associations (Carneiro et al. 2020). Therefore, the differences between the results for the pulp and husk extracts are justified due to the structural differences of these fractions.

The analysis of the bioactive potential also took place in relation to the levels of total flavonoids and total carotenoids, as shown in Table 3, but only with the extracts that presented the best results according to the experimental design, that is, for the husk at 80°C for 60 minutes (E9) and for the pulp at 70°C for 45 minutes (E5).

The husk and pulp fractions showed considerable levels of total flavonoids, with the skin having the highest result, 363.63 mg CE $100g^{-1}$, when compared to the pulp. Flavonoids are the main active compounds corresponding to about 39.6% (Guan et al. 2017), they are bioactive compounds synthesized by plants through the shikimic acid and mevalonate pathways and act as antioxidants, neutralizing the effects of reactive oxygen and nitrogen species, acting as well as protective agents against diseases (Dzomba and Musekiwa 2014).

In relation to total carotenoids, the available content in the extracts was estimated, and the husk extract of *Dioscorea bulbifera* L. had the highest content, about 2.13 µg $100g^{-1}$, when compared to the pulp extract. Carotenoids are fat-soluble pigments with a polyene chemical structure consisting of conjugated double bonds,

357 enabling these compounds to absorb excess energy from other molecules and eliminate
358 reactive oxygen species (ROS) and free radicals. Thus, through the results of the
359 analysis of total phenolic compounds, total flavonoids and total carotenoids, there is an
360 indication of bioactivity of the husk and pulp fractions of *Dioscorea bulbifera* L.
361
362 *3.2. Antioxidant profile of bioactive compounds*
363
364 The antioxidant capacity can be identified from different mechanisms of
365 antioxidant action; thus, it is important that its evaluation is done by more than one
366 methodology (Soriano Sancho et al. 2015). In this sense, the evaluation was carried out
367 through the mechanism of hydrogen atom transfer (HAT) by the β-carotene/linoleic acid
368 auto-oxidation system, and the mechanism by electron transfer (SET), through the
369 FRAP and hydrogen peroxide removal (H_2O_2) assays, in addition to the ABTS assay
370 that contemplates the HAT and SET mechanisms simultaneously (Prior et al. 2005).
371 In the present study, the hydrogen peroxide removal, ABTS, FRAP and β-
372 carotene/linoleic acid assays were used to estimate the antioxidant capacity of the husk
373 and pulp extracts of *Dioscorea bulbifera* L. (Table 4). For all tests of antioxidant
374 potential, the husk extract showed the highest values when compared to the pulp extract.
375 It is noteworthy that the ABTS assay allows the evaluation of the antioxidant capacity
376 from the removal of the free radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic
377 acid), a stable compound generated from a chemical or enzymatic reaction, which
378 allows estimating the activity of compounds of a hydrophilic nature, such as phenolic
379 compounds, and lipophilic, such as carotenoids, for example (Arnao 2000; Pérez-
380 Jiménez and Saura-Calixto 2006; Sucupira et al. 2012). While, in the β-carotene/linoleic
381 acid autoxidation system, the antioxidant activity is determined from the inhibition of

382 the peroxyl radical (LOO•) formed by linoleic acid in reaction with oxygen, which,
383 when reacting with β-carotene, causes loss of coloring. If the sample contains
384 antioxidants that react with peroxyl, the decrease in β-carotene absorbance will be
385 delayed (Marco 1968; Miller 1971).

386 The FRAP method evaluates the antioxidant activity by reducing the ferric-
387 tripyridyltriazine complex (Fe^{3+}-TPZ) to the ferrous complex (Fe^{2+}-TPZ), a complex
388 that has an intense blue-violet color, being considered an assay with high reproducibility
389 and high correlation with compounds phenolics. In view of the above, the results
390 obtained for the analysis of antioxidant capacity allowed us to assume that both
391 fractions, husk, and pulp, of *Dioscorea bulbifera* L. have an expressive antioxidant
392 profile, mainly linked to compounds such as carotenoids and total phenolic compounds
393 (Apak et al. 2004; Benzie and Strain 1996).

394

395 *3.3. Study of simulated gastrointestinal digestibility*

396

397 The simulation of gastrointestinal digestion contributes to the understanding of
398 how the bioactive compounds present in a food matrix behave during the phases of
399 digestion in relation to their bioaccessibility, providing information of interest to
400 researchers and the food industry (Barba and Orlien 2017). Thus, the contents of total
401 phenolic compounds (TPC) were evaluated for the husk and pulp fraction of *Dioscorea*
402 *bulbifera* L. for each phase of the simulated digestion, as shown in Fig. 4.

403 From the simulated gastrointestinal digestion process, it can be seen that the
404 TPC content decreased considerably when comparing the results at the beginning of the
405 digestion process, during the process until the final phase for both the husk (Fig. 4a) and
406 the pulp (Fig. 4b) of *Dioscorea bulbifera* L. TPC levels ranged from 715 to 82 mg EAG

407 $100g^{-1}$, and in all phases there was a statistical difference ($p \leq 0.05$) between the values
408 of these bioactive compounds.

409 The TPC contents of the pulp fraction of *Dioscorea bulbifera* L. showed similar
410 behavior to that of the husk fraction, with a decrease in values from 235 to 37 mg EAG
411 $100g^{-1}$, however, between the gastric and intestinal phases there was no significant
412 difference by the Tukey test ($p \leq 0.05$). It can be observed that in the husk and pulp
413 fractions there was a greater loss of oral digestion in relation to the other phases,
414 however, there was no difference in the profile of the percentage of bioavailability.

415 In the husk and pulp fractions, there was a greater loss in oral digestion, with no
416 differences in the profile of the percentage of bioavailability, showing a decrease at each
417 stage of the simulated digestion. A factor that may be directly related to the nature of
418 the phenolic compounds, about their interactions with simulated fluids, mainly in
419 relation to their instability when subjected to pH variations.

420 Regarding bioaccessibility, it is known that it is a parameter related to the ability
421 of a compound extracted from the food matrix to be absorbed by intestinal cells after the
422 gastrointestinal digestion process (Barba and Orlien 2017; Heaney 2001). In this sense,
423 from the TPC results, it can be observed that approximately 16% of the total phenolic
424 compounds in the husk and 25% of the pulp were considered bioaccessible to be
425 absorbed in the intestine, and that despite the husk having a higher TPC content, the
426 pulp fraction had a higher bioaccessibility (Fig. 5).

427 The results of the husk and pulp fraction may have been influenced by the
428 interaction of phenolic compounds with the structure of the food matrix. In this sense,
429 the higher the fiber content, the lower the TPC content, as the fibrous structure makes it
430 difficult to release phenolic compounds that are associated with it. In addition, it is
431 possible that the phenolic compounds present in the samples have had interactions with

other components of the sample, such as proteins, polysaccharides or even with components of the simulated digestive system, which can cause changes in their stability (Quan et al. 2018). Some phenolic compounds present in human food are negatively influenced when exposed to high pH due to their structural characteristics and studies on such characteristics can help predict the stability of specific phenolic compounds when subjected to different pH ranges (Friedman and Jürgens 2000). Some phenolic compounds may have undergone transformations when subjected to acidic conditions, making their detection difficult by the Folin-Ciocalteu method (Barba and Orlien 2017; Carbonell-Capella et al. 2014; Sengul et al. 2014).

Studies reveal that the stability of phenolic compounds is significantly influenced by their chemical structure. Phenolic acids are resistant to the digestion procedure, while flavonols are mainly degraded when they are subjected to digestion and, during the digestion process, TPC can be degraded, released from the food matrix, or attached to other compounds or constituents of the digestive juice (Goulas and Hadjisolomou 2019). In addition, only 5 to 10% of the total phenolic compound content of a food matrix can reach the small intestine (Clifford 2004), thus the TPC content present in both *Dioscorea bulbífera* L. fractions showed significant bioaccessibility for be absorbed in the intestine.

4. Conclusions

In this context, it is concluded that the combination of the ultrasound-assisted technique with the solid-liquid extraction method, provided the extraction of bioactive compounds from *Dioscorea bulbifera* L. The fractions of husk and pulp showed bioactive potential, according to the antioxidant activity methods (hydrogen peroxide, ABTS, FRAP and β-carotene/linoleic acid removal) as well as demonstrated

bioaccessibility through simulated gastrointestinal digestion. Therefore, *Dioscorea bulbifera* L. husk and pulp fractions may be potential sources for isolation and purification of bioactive compounds, as well as applications in the food industry.

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Compliance with Ethical Standards

The authors declare that there is no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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.

Authors' Contribution:

Gabrielly Ribeiro Carneiro: Conceptualization, Data curation, Methodology.

Leomara Floriano Ribeiro: Conceptualization, Writing Original draft preparation and Writing Reviewing, Supervision.

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Tables

Table 1 Factors and levels of the Box-Behnken 3^2 experimental design for extraction of bioactive compounds from husk and pulp from *Dioscorea bulbifera* L.

Assays	Coded factors and levels		Factors and original levels	
	Temperature [°C]	Time [min.]	Temperature [°C]	Time [min.]
E1	-1	-1	60	30
E2	-1	0	60	45
E3	-1	+1	60	60
E4	0	-1	70	30
E5	0	0	70	45
E6	0	+1	70	60
E7	+1	-1	80	30
E8	+1	0	80	45
E9	+1	+1	80	60

Table 2 Content of total phenolic compounds for each husk and pulp extract of *Dioscorea bulbifera* L.

Assays [Temperature (°C)/ time (min)]	**Husk (mg GAE 100g^{-1})**	**Pulp (mg GAE 100g^{-1})**
E1 (60 °C /30 min)	$570.28^{cd} \pm 1.69$	$223.56^{a} \pm 3.16$
E2 (60 °C /45 min)	$550.30^{cd} \pm 14.41$	$228.36^{a} \pm 5.28$
E3 (60 °C /60 min)	$545.30^{e} \pm 8.42$	$233.19^{a} \pm 5.08$
E4 (70 °C /30 min)	$585.06^{cd} \pm 4.58$	$225.43^{a} \pm 19.59$
E5 (70 °C /45 min)	$614.00^{bc} \pm 22.19$	$235.50^{a} \pm 25.30$
E6 (70 °C /60 min)	$553.58^{cd} \pm 7.14$	$218.03^{ab} \pm 34.60$
E7 (80 °C /30 min)	$679.33^{a} \pm 56.69$	$172.50^{bc} \pm 7.63$
E8 (80 °C /45 min)	$670.90^{ab} \pm 20.41$	$166.95^{d} \pm 7.84$
E9 (80 °C /60 min)	$715.53^{a} \pm 8.00$	$174.05^{bc} \pm 10.29$

*Results expressed as mean ± standard deviation (n=3). Different letters in the same column indicate statistical difference between tests (*Tukey* Test, $p \leq 0.05$).

Table 3 Content of total flavonoids and total carotenoids for husk and pulp of *Dioscorea bulbifera* L.

Fraction	**Flavonoids (mg CE 100 g^{-1})**	**Carotenoids (µg 100 g^{-1})**
Husk	363.63 ± 8.92	2.13 ± 0.10
Pulp	102.44 ± 1.51	1.34 ± 0.11

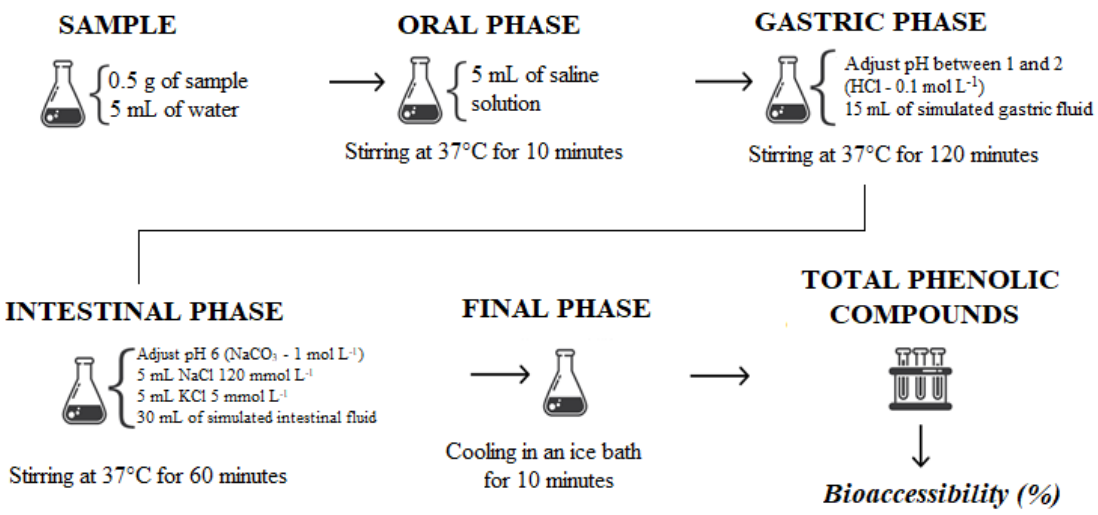
*Results expressed as mean ± standard deviation (n=3). Where CE = Catechin equivalent.

Table 4 Antioxidant capacity of husk and pulp extracts of *Dioscorea bulbifera* L.

Assay	**Fraction**	
	Husk	**Pulp**
ABTS (µM Trolox g^{-1})	66.88 ± 0.93	14.93 ± 0.31
FRAP (µM sulfato ferroso g^{-1})	125.09 ± 8.52	32.76 ± 0.65
β-carotene/linoleic acid (%AOA)	84.50 ± 0.34	46.91 ± 4.66
Hydrogen peroxide removal (%)	41.35 ± 5.85	28.91 ± 5.62

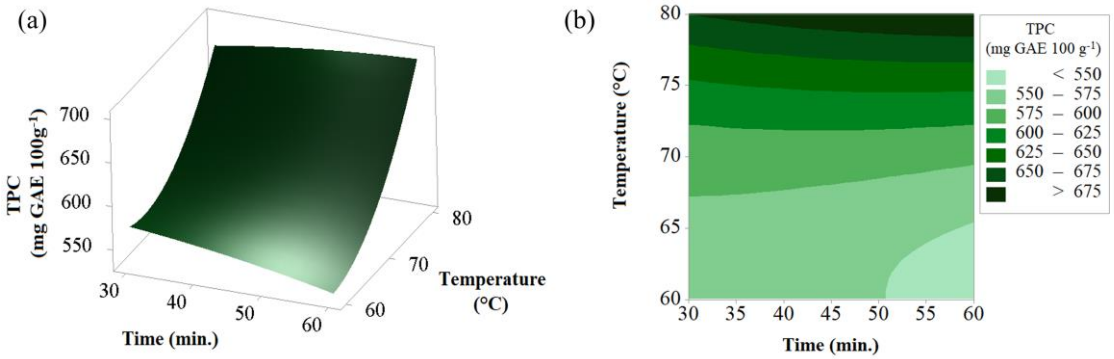
*Results expressed as mean ± standard deviation (n=3).

746 **Figures**



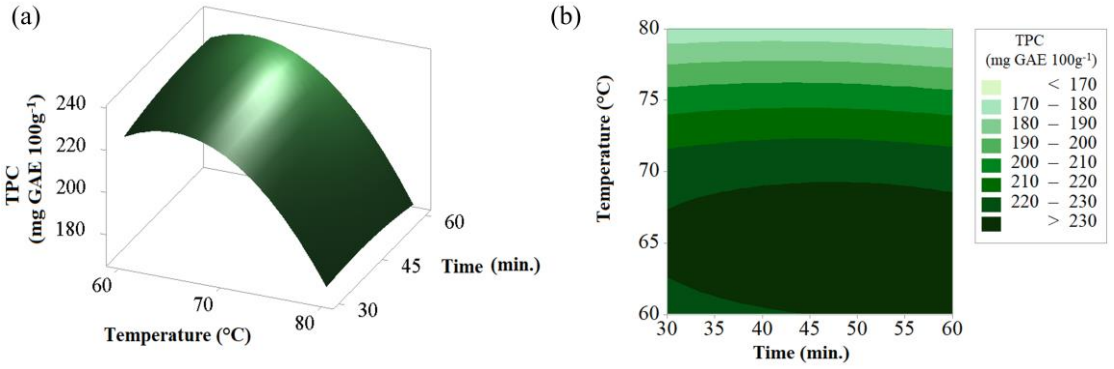
747

748 **Fig 1**



749

750 **Fig 2**



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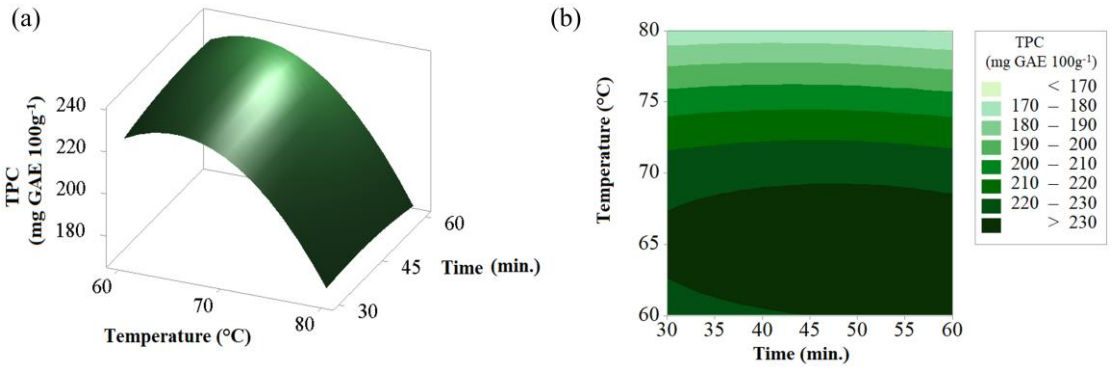
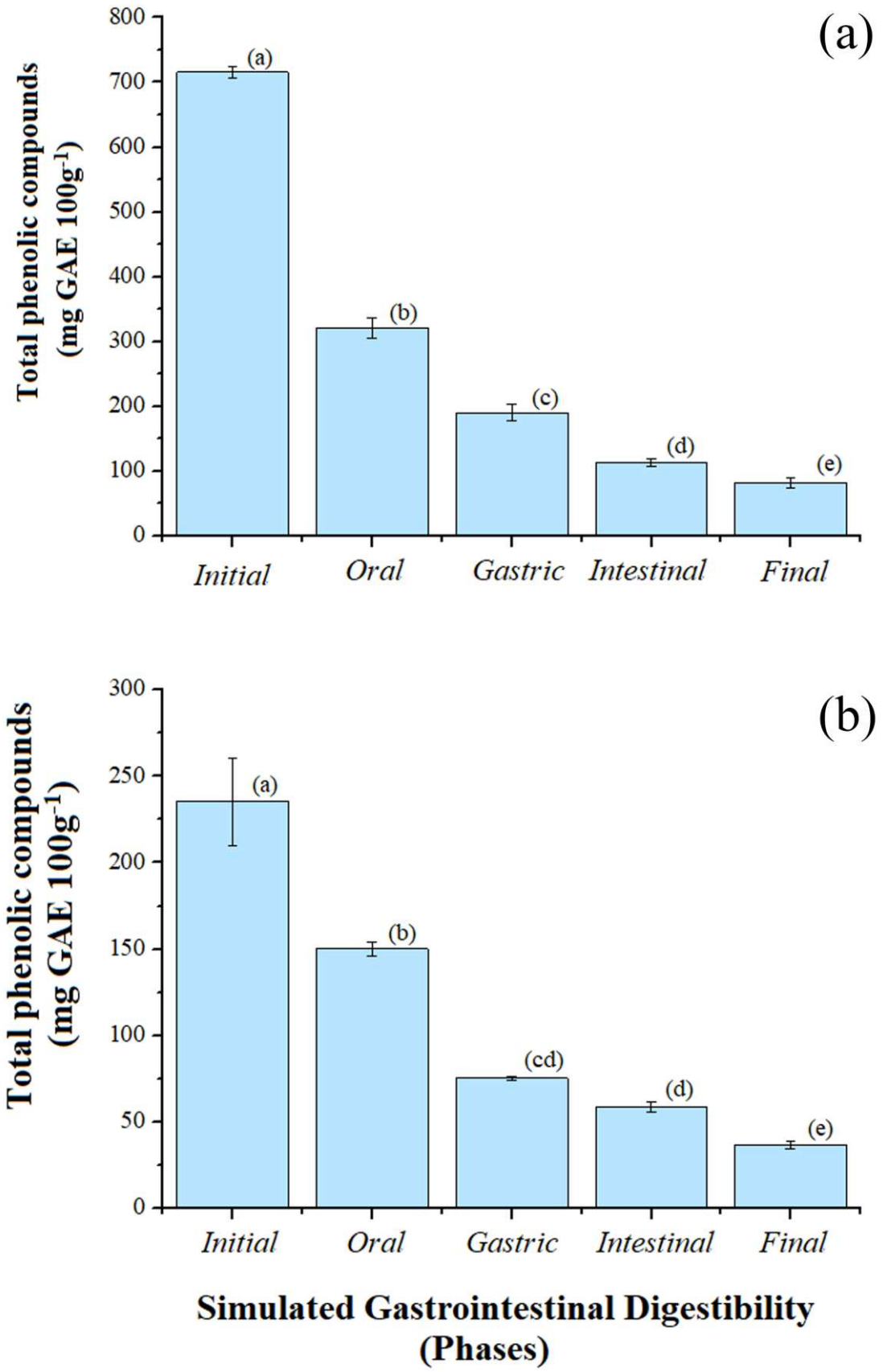


752

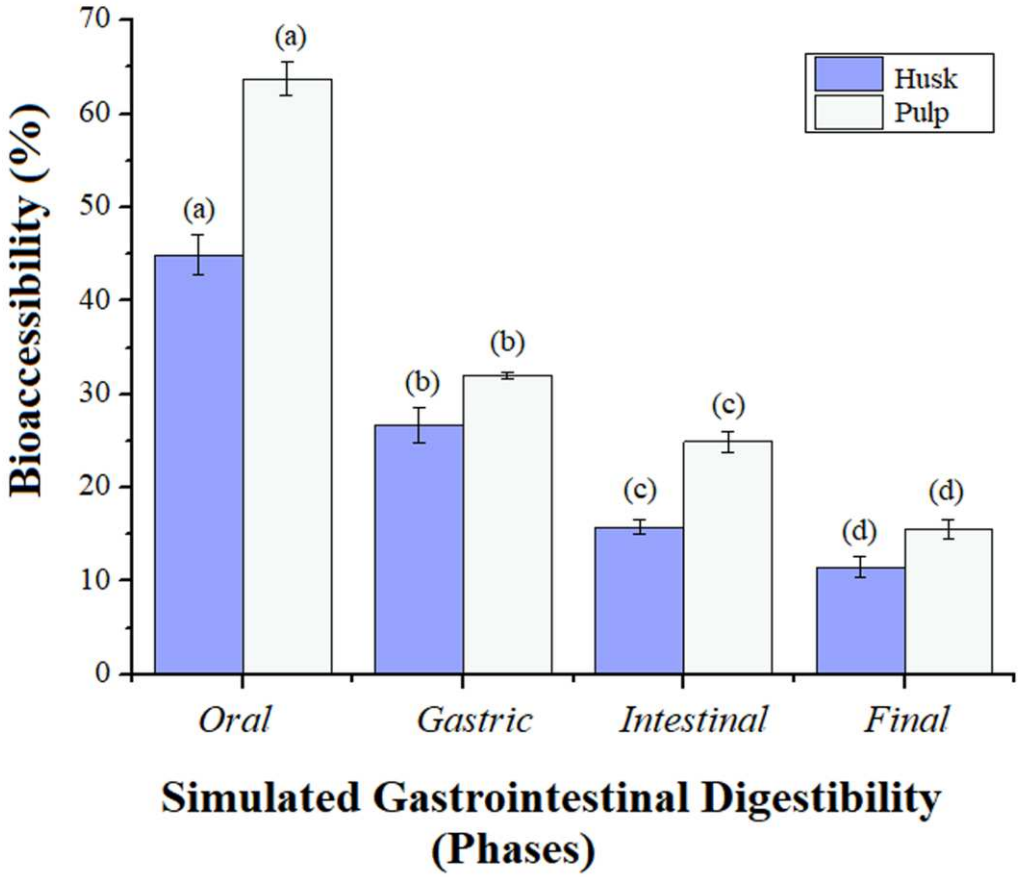
753 **Fig 3**

754


(a)
Total phenolic compounds (mg GAE 100g-1)
800
700
600
500
400
300
200
100
0
(a)
(b)
(c)
(d)
(e)
Initial
Oral
Gastric
Intestinal
Final
(b)
Total phenolic compounds (mg GAE 100g-1)
300
250
200
150
100
50
0
(a)
(b)
(cd)
(d)
(e)
Initial
Oral
Gastric
Intestinal
Final
Simulated Gastrointestinal Digestibility (Phases)

755

756 **Fig 4**



757

758 **Fig 5**

759