

Optimization of the extraction of bioactive compounds from *Clitoria ternatea* L and evaluation of encapsulation by ionotropic gelation

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Abstract

The aim of this work was to optimize the extraction of bioactive compounds from *Clitoria ternatea* L and then to encapsulate them by ionic gelation. The extraction of the bioactive compounds, using water as solvent, was carried out the solid-liquid extractions in cold and hot, obtaining a response factor the content of total phenolic compounds (TPC). The bioactive compounds of the extracts were evaluated based on the levels of TPC and total monomeric anthocyanins. Analyzes of physical, chemical, phytochemical and antioxidant potential were performed. Encapsulation was performed by ionic gelation. The capsules were evaluated by characterization and visual aspects. Simulated gastrointestinal digestibility was determined based on TPC bioaccessibility. There was no statistical difference ($p \leq 0.05$) between the extracts. The quadratic mathematical model presented a correction coefficient, $R^2 = 0.997$ and $R^2_{\text{adjust}} = 0.974$. Hot extraction at 80°C/5 minutes at a concentration of 0.008 g/mL showed a TPC of 23.91 ± 0.90 mg (GAE)/g and 0.97 ± 0.50 mg (MVE)/g for monomeric anthocyanins. For antioxidant potential, the values of ABTS, FRAP and β -carotene/linoleic acid were 84.86 ± 1.52 μ M trolox/g, 220.83 ± 3.69 μ M ferrous sulfate/g and $55, 95 \pm 4.51\%$, respectively, for the same assay. Visually, the capsules had regular sizes and preserved color, high solubility (99%), encapsulation efficiency (76%). Bioaccessibility was 71% for lyophilized capsules, with greater intestinal absorption. Therefore, extracts and capsules of *Clitoria ternatea* L. have bioactive compounds with an antioxidant profile.

Introduction

Brazil is one of the countries with the greatest biodiversity in the world [1]. In which there are native and adapted plant species. There is a group of plant species that are known as unconventional food plants (UFP). This term is used for a group of plants that are not cultivated and commercialized conventionally but have one or more edible parts and can be introduced into the diet [2, 3]. Among the various UFP is the *Clitoria ternatea* L., popularly called butterfly bean, butterfly pea, wedge, ismenia or palheteira, which belongs to the Fabaceae family, with characteristics of a fickle vine, a perennial herbaceous plant, in which the flowers have a blue-violet color, due to the presence of anthocyanins [4–6].

Anthocyanins are considered bioactive compounds with antioxidant action, due to their oxide reduction effect. The degenerative oxidative process is delayed, as the molecules compete with the active sites and receptors, being a defense for the organism. These compounds are soluble in water, belong to a subclass of flavonoids, which are phenolic phytochemicals, with structure in the form of a glycoside [7]. As the pH increases, the flavylium cation loses a proton and undergoes hydration, forming a pseudobase or carbitol, and this change in pH influences its color [8, 9]. In addition, factors such as light, temperature, oxygen, metal ions, presence of enzymes and structure affect the stability of anthocyanins, especially when these compounds are commonly used as dyes in foods and drugs[10].

However, to obtain anthocyanins it is important to know the extraction methods, advantages, and disadvantages. In this regard, the extraction of anthocyanins and pigments derived from anthocyanins with the use of solvents is the most common method, using mainly methanol [11, 12], ethanol [13, 14], acetone [15], water [16, 17], or a mixture of these solvents because they are polar molecules [10, 18]. In addition, other extraction methods include those using enzymatic extraction [19] and ultrasound-assisted extraction [20, 21], due to the numerous advantages such as low energy use, fast process, high production yield and ease of operation [22, 23].

To improve the stability of bioactive compounds, preserve them and enhance their application, encapsulation methods can be used. Microencapsulation techniques can be classified into three groups (a) physical methods, which include spray drying or atomization, freeze drying, supercritical fluid precipitation, and solvent evaporation; (b) physicochemical methods, which include coacervation, liposomes, and ionic gelation; and (c) chemical methods, which include interfacial polymerization and molecular inclusion complexation. For the compounds present in *Clitoria ternatea* L. there are studies with encapsulation by spray drying [24], freeze drying [25] or ionic gelation [26, 27]. Ionic gelation encapsulation is a

simple and easy procedure, does not require specialized equipment, high temperature or organic solvent and can be considered low cost [28]. The method uses hydrocolloids and ionic solutions at appropriate concentrations, and can be applied in controlled release processes, for example [29, 30]. Based on this context, the present study aimed to optimize the extraction of bioactive compounds from *Clitoria ternatea* L. and to evaluate the encapsulation process by ionic gelation.

Material and methods

Preparation of the samples

The samples of *Clitoria ternatea* L. were previously dried from the combined process of natural drying in the shade and in an oven at 45°C. The samples were ground and separated in sieves, with a granulometry of 150 µm, stored in low density polyethylene (LDPE) packages, vacuum sealed (GSVAC-GS420), and stored away from light, under refrigeration until they were used in the analyses later.

Optimization of the extraction of bioactive compounds

For the extraction of the bioactive compounds from *Clitoria ternatea* L. water was used as solvent, and it was carried out under different cold and hot conditions. The cold solid-liquid extraction was carried out based on the Box-Behnken 3^2 design at room temperature, while the hot extraction was carried out based on the fractional factorial design $3^{(3-1)}$, with the response factor being the total phenolic compounds (TPC) content of according to Table 1. All solutions were placed in an ultrasonic bath (SOLIDSTEET) for 30 minutes at 25°C. Then, the solutions were shaken in a shaker (TECNAL-TE4200) at 150 rpm with the parameters of the experimental design adjusted. Then, the extracts were centrifuged (SOLAB-SL-700) at 6000 rpm for 20 minutes, filtered and stored in amber bottles under refrigeration to perform the other analyses.

Table 1
– Experimental design for extraction of of total phenolic compounds from *Clitoria ternatea* L.

Experimental design 3 ² for hot extraction						
Assays	Codes factors and levels			Factors and levels		
	Concentration		Time	Concentration		Time [h]
				[g/mL]		
H1	-1		-1	0.002		1
H2	-1		0	0.002		3.5
H3	-1		+ 1	0.002		6
H4	0		-1	0.008		1
H5	0		0	0.008		3.5
H6	0		+ 1	0.008		6
H7	+ 1		-1	0.014		1
H8	+ 1		0	0.014		3.5
H9	+ 1		+ 1	0.014		6
Experimental design fractional factorial 3 ⁽³⁻¹⁾ for cold extraction						
Assays	Codes factors and levels			Factors and levels		
	Concentration	Time	Temperature	Concentration	Time [min]	Temperature
				[g/mL]		(°C)
C1	-1	-1	-1	0.002	5	40
C2	-1	0	+ 1	0.002	10	80
C3	-1	+ 1	0	0.002	15	60
C4	0	-1	+ 1	0.008	5	80
C5	0	0	0	0.008	10	60
C6	0	+ 1	-1	0.008	15	40
C7	+ 1	-1	0	0.014	5	60
C8	+ 1	0	-1	0.014	10	40
C9	+ 1	+ 1	+ 1	0.014	15	80

Analysis of bioactive compounds

Determination of monomeric anthocyanins

The total anthocyanin content of the extracts was determined by the differential pH method [31]. The solutions used were potassium chloride buffer (0.025 mol/L, pH 1.0) and sodium acetate buffer (0.4 mol/L, pH 4.5). Samples were diluted at a 1:20 ratio, where 200 µL of sample extract were mixed with 3,800 µL of each buffer solution. Absorbance

was measured in a UV/Vis spectrophotometer (Drawell/DU-8800RS) at 510 nm and 700 nm. The concentration of monomeric anthocyanins (MA) was expressed in milligrams of malvidin-3-monoglycoside (EMV)/g of sample.

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-Ciocalteu 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)/g, calculated by fitting the standard curve of gallic acid at concentrations from 25 at 800 mg/L in a spectrophotometer (Drawell/DU-8800RS) at 765 nm [32]. The extract with the highest content of total phenolic compounds was used for the other analyses.

Antioxidant capacity Assays

ABTS radical scavenging activity

The evaluation of antioxidant activity was performed by the ABTS radical scavenging method with modifications [33]. 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.

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

Antioxidant activity was evaluated by the β-carotene/linoleic acid system [34]. 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. The solutions were incubated in a water bath (Nova instruments/NI 1215) at 50°C. For control, Trolox (0.2 mg/mL) 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. (1).

$$AA\% = 100x \left[1 - \left(\frac{A_{Cinitial} - A_{Cfinal}}{A_{Ainitial} - A_{Afinal}} \right) \right]$$

1

Where: AA = percentage of antioxidant activity; $A_{Cinitial}$ = Initial absorbance of the control; A_{Cfinal} = Final absorbance of the control; $A_{Ainitial}$ = Initial extract absorbance; A_{Afinal} = Final extract absorbance.

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, 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 [35].

Encapsulation process

The encapsulation process was carried out using the ionic gelation. 50 g of pure liquid extract was mixed with 2% sodium alginate. The solution was stirred for 30 minutes. Then, a solution containing 6% calcium chloride was prepared and stirred. The extract solution with sodium alginate was dripped onto this mixture, forming the capsules. The capsules were sieved and washed with distilled water. Then, the capsules were frozen and lyophilized (Liotop L101), at 300 µmHg, approximately [30].

Characterization of the capsules

Yield

The powder yield was determined on the ratio between the mass of formed capsules and the mass of extract [36].

Visual aspects

To evaluate the visual appearance, four parameters were considered: color (uniform, brightness, opacity), size (regular, uniform), homogeneity (absence of visible insoluble particles) and presence of air bubbles.

Moisture

The capsules were weighed and kept at 105°C in a drying oven (SolidSteel/SSDcr-110L) until constant mass [37].

Average capsule diameter

The average diameter of the capsules was determined based on the average of ten samples measured at random positions using a digital caliper.

Encapsulation efficiency

The encapsulation efficiency was performed before and after lyophilization, breaking 0.5 g of the capsules in 5 mL of 10% sodium citrate (w/v). Soon after, the pH was adjusted to 8.0 using a 0.1 mol/L sodium hydroxide solution. The mixture was stirred for 2 minutes. Next, 5 mL of 99.5% ethanol were added to the mixture of tubes and agitation was maintained for another 2 minutes. The mixture was centrifuged (Solab scientific/SL-700) at 4000 rpm for 20 minutes and the content of total phenolic compounds (TPC) was determined before and after this procedure [38]. The encapsulation efficiency value was determined using the Eq. (2).

$$Encapsulation\ efficiency(\%) = \left(\frac{TPC - TPCS}{TPC} \right) \times 100$$

2

Where: TPC = Total phenolic compounds (mg GAE/g) after rupture of a known number of microcapsules, TPCS = Total phenolic compounds on the surface (mg GAE/g) contained in the same number of microcapsules.

Solubility

The solubility of the capsules was determined after dispersion in distilled water, at a concentration of 1:10 (m/v). Then, the solution was stirred for 5 minutes and centrifuged (Solab scientific/SL-700) at 3500 rpm for 20 minutes. Then, 2.5 mL aliquots were transferred to previously prepared crucibles and placed in a drying oven (SolidSteel/SSDcr-110L) for 5 hours at 105°C [39].

Hygroscopicity

The hygroscopicity was determined after 0.50 g of the capsules were placed separately in hermetically sealed containers with saturated solutions of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ – 33% RH), sodium bromide (NaBr – 58% RH), sodium chloride (NaCl – 75% RH), potassium chloride (KCl – 84% RH) and potassium sulfate (K_2SO_4 – 97% RH), kept at 25°C in BOD until stability in the mass determination [40].

Simulated gastrointestinal digestibility

The bioaccessibility was performed by simulating the digestibility by in vitro model [41, 42]. Initially, 0.5 g of sample (*Clitoria ternatea* L. extract and freeze-dried capsules) 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 NaCl, 5 mL of 5 mmol/L KCl and 30 mL of simulated intestinal fluid were added, kept at 37°C for 60 minutes. 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 all phases (oral, gastric, and intestinal) aliquots were collected to determine the content of total phenolic compounds (TPC).

Statistical analysis

Results were expressed as mean $\pm$ standard deviation. Data were evaluated by analysis of variance (ANOVA). Significant differences between means were analyzed using the Tukey test, with $p \leq 0.05$.

Results and discussion

Optimization of extraction processes

For the optimization of the extraction processes, the content of total phenolic compounds (TPC) was used as a response factor. Furthermore, the content of monomeric anthocyanins was analyzed for both processes (Table 2). In the case of the cold extraction process, the variables concentration and time did not statistically affect ($p \leq 0.05$) the TPC results. Regarding the monomeric anthocyanin content, it can be observed that the C2 assay, at a concentration of 0.002 g/mL and with 3.5 h of extraction, had the highest value of monomeric anthocyanins 1.22 ± 0.17 mg MVE/g, being superior and statistically different ($p \leq 0.05$) from C3, C8 and C9 assays. Although all assays showed significant results for TPC (17.91 ± 1.23 to 22.25 ± 0.31 mg GAE/g) and monomeric anthocyanins (0.91 ± 0.05 to 1.22 ± 0.17 mg MVE/g), it was not possible to obtain a predictive model for cold extraction.

Table 2
– Content of total phenolic compounds and total monomeric anthocyanins for each experimental design

Experimental design fractional factorial $3^{(3-1)}$ for cold extraction					
Assays	Concentration [g/mL]	Time [min]	Total phenolic compounds (mg GAE/g)	Total monomeric anthocyanins (mg MVE/g)	
C1	0.002	1	17.99 ^a ± 2.40	1.06 ^{ab} ± 0.10	
C2	0.002	3.5	18.60 ^a ± 3.09	1.22 ^a ± 0.17	
C3	0.002	6	19.37 ^a ± 4.85	0.91 ^b ± 0.10	
C4	0.008	1	20.86 ^a ± 2.05	1.04 ^{ab} ± 0.11	
C5	0.008	3.5	19.35 ^a ± 1.19	1.02 ^{ab} ± 0.07	
C6	0.008	6	17.91 ^a ± 1.23	1.00 ^{ab} ± 0.09	
C7	0.014	1	19.32 ^a ± 2.85	1.11 ^{ab} ± 0.04	
C8	0.014	3.5	18.99 ^a ± 0.97	0.94 ^b ± 0.08	
C9	0.014	6	22.25 ^a ± 0.31	0.91 ^b ± 0.05	
Experimental design 3^2 for hot extraction					
Assays	Concentration [g/mL]	Time [min]	Temperature [°C]	Total phenolic compounds (mg GAE/g)	Total monomeric anthocyanins (mg MVE/g)
H1	0.002	5	40	21.75 ^a ± 3.43	3.43 ^a ± 0.83
H2	0.002	10	80	23.31 ^a ± 0.96	0.21 ^c ± 0.15
H3	0.002	15	60	21.90 ^a ± 0.82	2.95 ^a ± 0.10
H4	0.008	5	80	23.91 ^a ± 0.90	0.97 ^{bc} ± 0.50
H5	0.008	10	60	22.92 ^a ± 3.49	1.66 ^b ± 0.55
H6	0.008	15	40	21.85 ^a ± 1.18	1.16 ^{bc} ± 0.52
H7	0.014	5	60	20.58 ^a ± 0.88	1.04 ^{bc} ± 0.32
H8	0.014	10	40	20.39 ^a ± 1.17	1.21 ^{bc} ± 0.04
H9	0.014	15	80	22.12 ^a ± 0.62	1.15 ^{bc} ± 0.03

* Results expressed as mean ± standard deviation. Different lowercase letters in the same column indicate statistical difference between assays by *Tukey's* test ($p \leq 0.05$).

In relation to the hot extraction process, the results were expressed in terms of TPC and monomeric anthocyanins (Table 2). The monomeric anthocyanin content ranged from 0.21 ± 0.15 to 3.13 ± 0.83 mg MVE/g. All assays showed

significant values for the hot extraction of *Clitoria ternatea* L. But for all assays of TPC there was no statistical difference ($p \leq 0.05$). And it was possible to obtain a quadratic mathematical model to determine the total phenolic compounds as a function of the variables, concentration, time, and temperature, according to Eq. (3).

$$CFT \left(\text{mg g}^{-1} \right) = 20,35 + 340,90C + 0,0066t + 0,001T - 33795C^2 - 0,00761t^2 + 0,000362T^2 + 9,24Ct$$

3

$$R^2 = 0.997 \text{ e } R^2_{\text{adjust}} = 0.974$$

Where: C = concentration (mg/L), t = time (min.) e T = temperature (°C).

Based on the extraction data, the interaction and influence of the parameters of the experimental design can be evaluated. By analyzing the response surface methodology fixing the concentration and evaluating how time and temperature influenced the TPC content (Fig. 1), it can be observed that for the three concentrations (0.002; 0.008 and 0.014 mg/L) at as the temperature increased from 40 to 80°C, there was a progressive increase in the TPC content (20.39 ± 1.17 to 23.91 ± 0.90 mg GAE/g). At the concentration of 0.002 mg/L, the highest TPC content was obtained in the shortest time, 5 minutes. As well as for the concentration of 0.008 mg/L. As for the highest concentration 0.014 mg/L, the highest TPC value was obtained at the highest temperature (80°C) and the longest time (15 minutes) applied.

When evaluating the methodology graphics of response surfaces fixing time, it was noticed that the data presented a quadratic behavior evaluating the TPC content as a function of the interaction between temperature and concentration (Fig. 2). As the temperature increased, there was an increase in the TPC content in the *Clitoria ternatea* L. extracts up to a certain concentration and then there was a reduction in the content. In this way, the optimal point was obtained at a concentration of 0.008 mg/L close to the curvature, regardless of the times evaluated (5, 10 and 15 min.).

When evaluating the response surfaces fixing the temperature, a similar behavior was noticed when fixing the time (Fig. 3). The data showed an optimal point at the concentration of 0.008 mg/L in a time of 10 minutes, independent of the applied temperature. From the results of the optimization in relation to the analysis of the response surface methodology and the mathematical model, it was obtained, for the maximization of the model, the parameters C = 0.006 mg/L, time = 8.03 minutes and at the temperature of 80°C, obtaining TPC = 24.07 mg GAE/g. While for the optimal point, the parameters obtained were C = 0.008 mg/L, time = 10 minutes and at a temperature of 79.2°C, obtaining TPC = 23.91 mg GAE/g, being the content value of TPC like that obtained experimentally in assay H4 (C = 0.008 mg/L, t = 5 minutes, and T = 80°C). Therefore, the other analyzes for antioxidant potential and encapsulation process were performed only with the extract from this assay.

Antioxidant potential and encapsulation process

Phenolic compounds present in edible flowers may act both as primary antioxidants and secondary antioxidants. For the quantification of antioxidant activity of edible flowers' extracts as *Clitoria ternatea* L., there are several methods available that may be categorized based on chemical reactions, such as hydrogen atom transfer (HAT) and single electron transfer (SET) reaction-based methods. The HAT measures the ability of an antioxidant to eliminate free radicals by donating hydrogen, among tests of this mechanism, for example, the β -carotene/linoleic acid autooxidation system. While SET detects the ability of an antioxidant to reduce a compound (including metals, carbonyl groups and free radicals), through the transfer of an electron, for example, the FRAP assay (Ferric Reducing Antioxidant Power). However, there are assays in which both HAT and SET mechanisms occur, for example, the ABTS assay [2,2'-azino-bis(3-ethyl-benzoline-6-sulfonated)] [43–45].

Thus, the mechanisms of antioxidant activity were evaluated using the FRAP, β -carotene/linoleic acid and ABTS methods, Table 3. The H4 test showed considerable values for each method. In the case of the ABTS assay, the observed value of $84.86 \pm 1.52 \mu\text{M trolox/g}$ was important because this mechanism allowed estimating the antioxidant activity of hydrophilic and lipophilic compounds through chemical or enzymatic reaction. The FRAP assay evaluates the presence of iron, which, if present, forms a TPTZ ferrous complex, and when the reaction occurs, a complex is formed that is intensely bluish in color, indicating the presence of antioxidants, which can be observed experimentally and based on the value obtained from $220.83 \pm 3.69 \mu\text{M ferrous sulfate/g}$. While the β -carotene/linoleic acid assay ($53.95 \pm 4.51\%$) determines antioxidant activity through the presence of antioxidant compounds that protect linoleic acid, discoloration occurs, which is due to the free radical structure formed by the oxidation of linoleic acid, which attacks the double bond of β -carotene and loses its chromophore, resulting in discoloration of the orange pigment, delaying the inhibition of free radicals. Some factors can influence the determination of the antioxidant capacity because they can affect the results regardless of the mechanism of action (HAT and/or SET), among them, there is the type of solvent used in the extraction of the compounds, its polarity, the temperature and the extraction time, the mass/volume ratio and the plant matrix evaluated. Thus, it can be assumed that the sample of *Clitoria ternatea* L. may contain interesting antioxidants that can be preserved in an encapsulation process such as ionic gelation.

Table 3
 – Antioxidant potential of the extract and physical characteristics of the capsules of *Clitoria ternatea* L.

Antioxidant potential of the extract			
H4	ABTS	FRAP	β -carotene/linoleic acid (%)
	($\mu\text{M trolox/g}$)	($\mu\text{M sulfato ferroso/g}$)	
	84.86 ± 1.52	220.83 ± 3.69	53.95 ± 4.51
Physical characteristics of the capsules			
<i>Wet capsules</i>		<i>Freeze-dried capsules</i>	
Encapsulation efficiency (%)	$75.77^a \pm 10.30$	Encapsulation efficiency (%)	$56.41^b \pm 0.00$
Solubility (%)	$99.79^a \pm 0.04$	Solubility (%)	$99.31^a \pm 0.15$
Moisture (%)	$99.04^a \pm 0.28$	Moisture (%)	$9.28^b \pm 1.17$

The capsules formed from the H4 assay maintained a pattern in their light blue color, with regular sizes maintaining homogeneity and little presence of bubbles. Regarding the yield, approximately 64% was obtained, with an average diameter of $2.70 \pm 0.26 \text{ mm}$ of the capsules. Next, the results of encapsulation efficiency were in relation to wet and freeze-dried capsules, as well as solubility and moisture (Table 3).

It can be noted that the wet capsules had a higher encapsulation efficiency, about 76%, when compared to the freeze-drier capsules, 56%. As well as they had a higher moisture content, 99%. However, in terms of solubility there was no significant difference ($p \leq 0.05$) between both processes. And the results are like studies of encapsulated flowers of *Clitoria ternatea* L. using the ionic gelation method, which obtained values from 74.97 ± 0.84 to $84.83 \pm 0.40\%$ for wet encapsulation efficiency [27]. Different parameters influence the encapsulation efficiency, such as the homogenization, stability, viscosity, and temperature of solutions, as well as the operating conditions implemented during process. Thus, for the application of encapsulated *Clitoria ternatea* L. it is recommended to use freeze-drier capsules that have low moisture, that is, less possibility of occurrence of microbiological development, and will still have excellent solubility and good encapsulation efficiency.

Regarding the evaluation of hygroscopicity, this analysis was performed with saturated solutions with different concentrations of relative humidity from 33 to 97%, as shown in Fig. 4. As the hygroscopicity of the food is an important parameter, as it interferes with the quality of the powder and its ability to absorb water, the freeze-drier capsules of *Clitoria ternatea* L. obtained an efficient result of hygroscopicity, because it was noticed that the capsules in a with high humidity, they absorbed approximately 50% of water, thus being resistant. In addition, when subjected to a humidity of 33%, it absorbed around 3%, therefore, the lyophilized capsules had a good protection capacity, keeping the compounds present in it protected and being considered resistant.

Simulated gastrointestinal digestibility

Bioaccessibility is defined as the amount of polyphenols contained in the water-soluble fraction of each digestion stage, which, in vivo, may be considered as potentially absorbable. The bioaccessibility of *Clitoria ternatea* L. extract and freeze-dried capsules was evaluated using a simulated in vitro gastrointestinal digestion. In this context, the total phenolic content (TPC) was evaluated by the Folin-Ciocalteu method. Figure 5 shows the mean values (mg GAE/g) of TPC for initial, gastric, and intestinal phases.

Analyzing the results, the freeze-dried capsules of *Clitoria ternatea* L. had a gradual release of bioactive compounds from the initial to the intestinal phase. In addition, it can be noted that in the initial phase the extract of *Clitoria ternatea* L. had a higher TPC content (205.40 ± 25.06 mg GAE/g) when compared to the content of the freeze-dried capsules (70.40 ± 6.42 mg GAE/g). This occurs because the bioactive compounds are initially protected by the physical-chemical bond related to the encapsulation process by ionic gelation.

In the gastric phase, the use of the capsule allowed to avoid the effects of gastric conditions on the bioactive compounds. With the alteration of the pH in this phase, the degradation of the compounds present in the extract may have occurred, for this reason the TPC (118.26 ± 72.96 mg GAE/g) content of the extract decreased rapidly in relation to the oral phase. On the other hand, the bioactive compounds of the capsules were preserved initially, and released following the simulation of the gastric process, which justifies the increase in the TPC value (195.51 ± 37.54 mg GAE/g) for this sample. The capsules for the formulation of products represents a useful strategy to move bioactive compounds to the intestine, where they can be absorbed or can exert their activities in their active form. During the digestion process, the bioactive compounds released from food matrix in the gastrointestinal tract and becomes available for absorption and presystemic metabolism. In the intestinal phase, the released bioactive compounds are mainly absorbed in the duodenum. The non-absorbed compounds move towards the large intestine, wherein they are exposed to intestinal peristalsis, neutral to slightly acidic pH [46]. In the simulation of the intestinal phase, the highest levels of TPC (377.86 ± 161.00 and 645.28 ± 77.45 mg GAE/g) were released for extract and freeze-dried capsules of *Clitoria ternatea* L., respectively.

Although the TPC profile may vary due to the in vitro digestion method used, to environmental conditions, harvest time, exposure to light, among others. Although these results simulate a physiological process on the bioavailability of phenolic compounds [47], the in vitro digestion phases could result in biochemical transformations in the plant matrix and lead to changes by the way of degradation of bioactive compounds, formation of new compounds antioxidants and new pro-oxidants [48].

Conclusion

It was possible to extract and characterize bioactive compounds in the *Clitoria ternatea* L. extract. Hot extraction was more efficient when compared to cold extraction. The H4 assay at 80°C/5 minutes, at a concentration of 0.008 g/mL, obtained the highest TPC content. From the presence of bioactive compounds, it was possible to carry out the

encapsulation by the ionic gelation method, which proved to be efficient, presenting capsules with regular sizes, maintaining the color and little presence of bubbles, being resistant, with good encapsulation efficiency, solubility and hygroscopicity. Therefore, the extracts of *Clitoria ternatea* L. have bioactive compounds with an antioxidant profile and can be encapsulated with potential application in the food industry due to their functional properties.

Declarations

Author contributions

Bruna Rodrigues Santana: methodology, formal analysis, writing – original draft and visualization.

Leomara Floriano Ribeiro: conceptualization, methodology, investigation, resources, data curation, writing – review and editing, visualization, and supervision.

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Data availability

All data included in this manuscript are available upon request by contacting with the corresponding authors.

Declarations Conflict of interest

The authors declare no conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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Figures

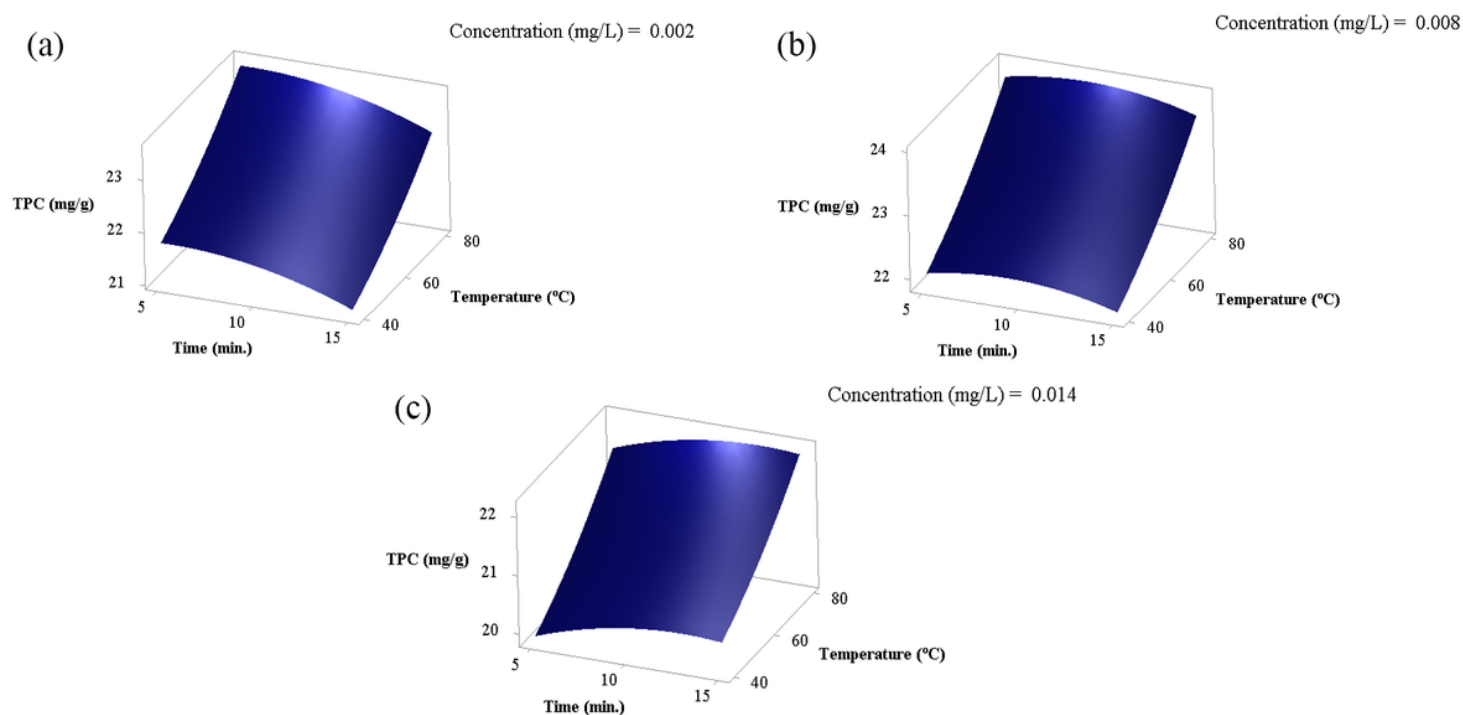


Figure 1

Response surface methodology for analysis of the content of total phenolic compounds of extracts of *Clitoria ternatea* L. fixing the concentration

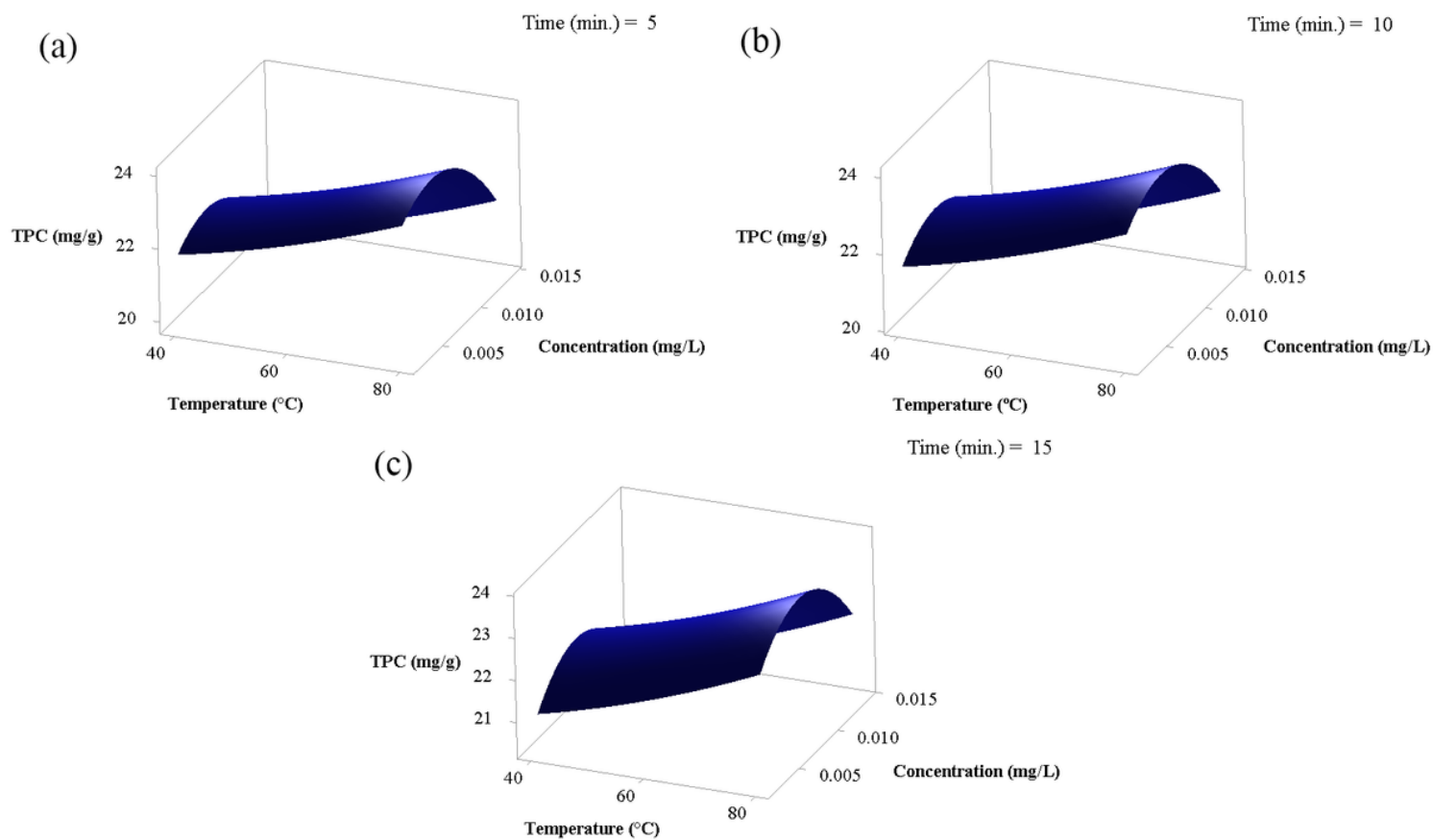


Figure 2

Response surface methodology for analysis of the content of total phenolic compounds of extracts of *Clitoria ternatea* L. fixing the time

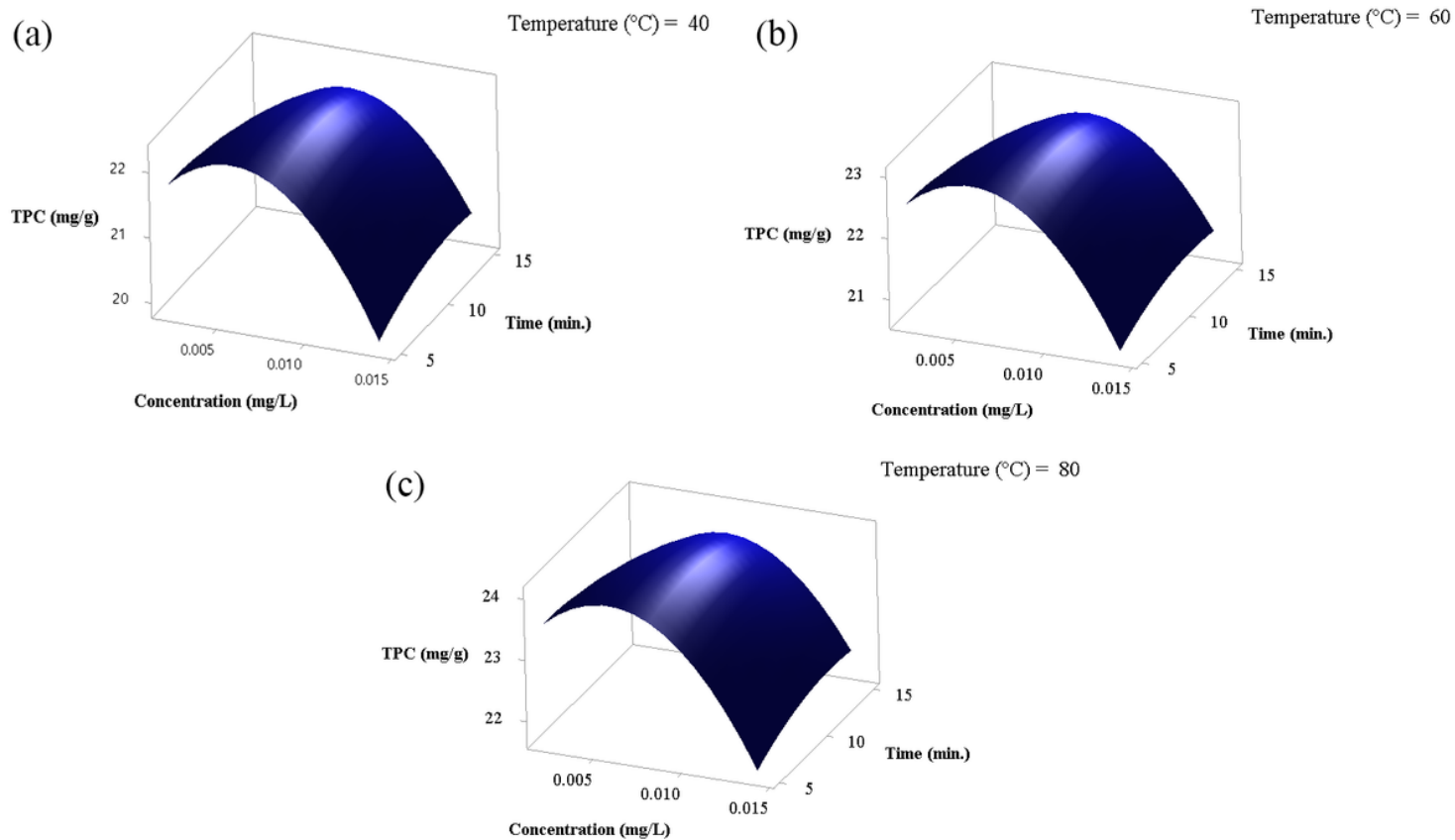


Figure 3

Response surface methodology for analysis of the content of total phenolic compounds of extracts of *Clitoria ternatea* L. fixing the temperature

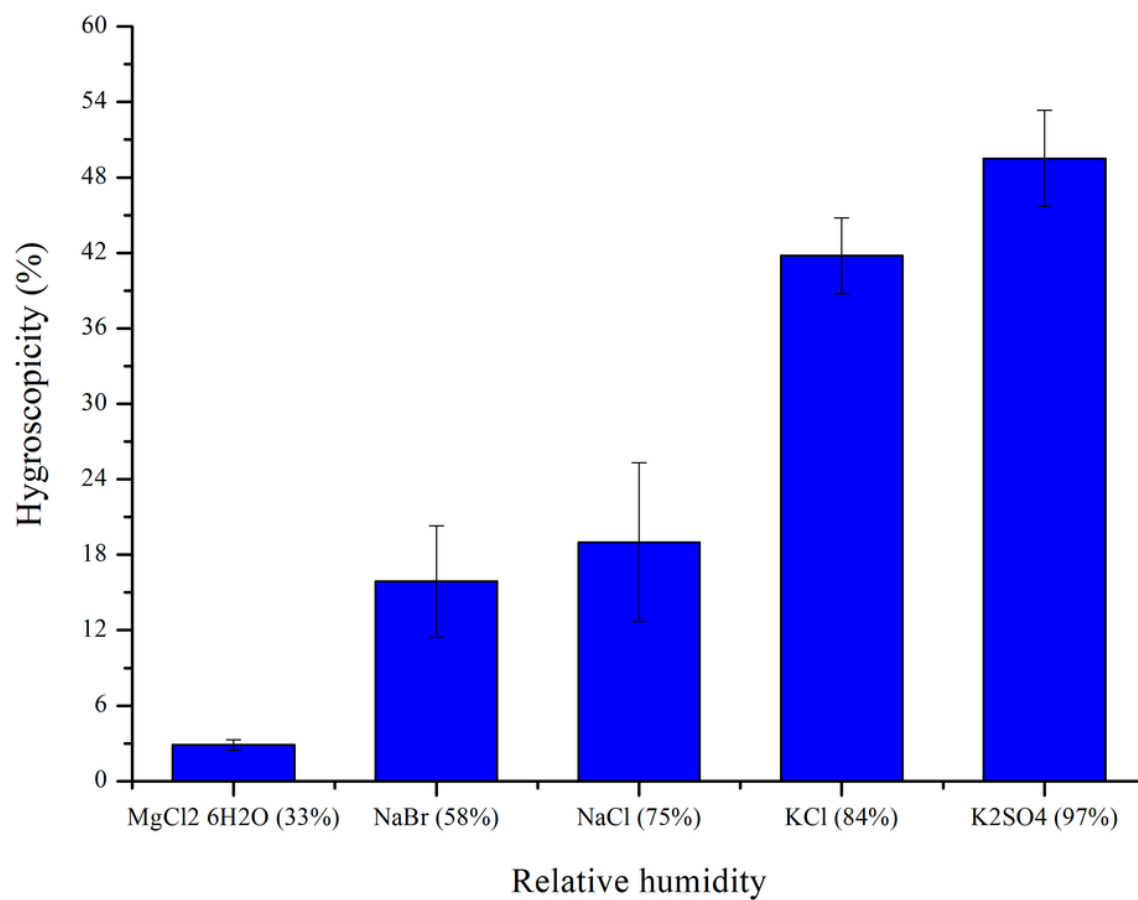


Figure 4

Relative humidity versus hygroscopicity of freeze-dried capsules

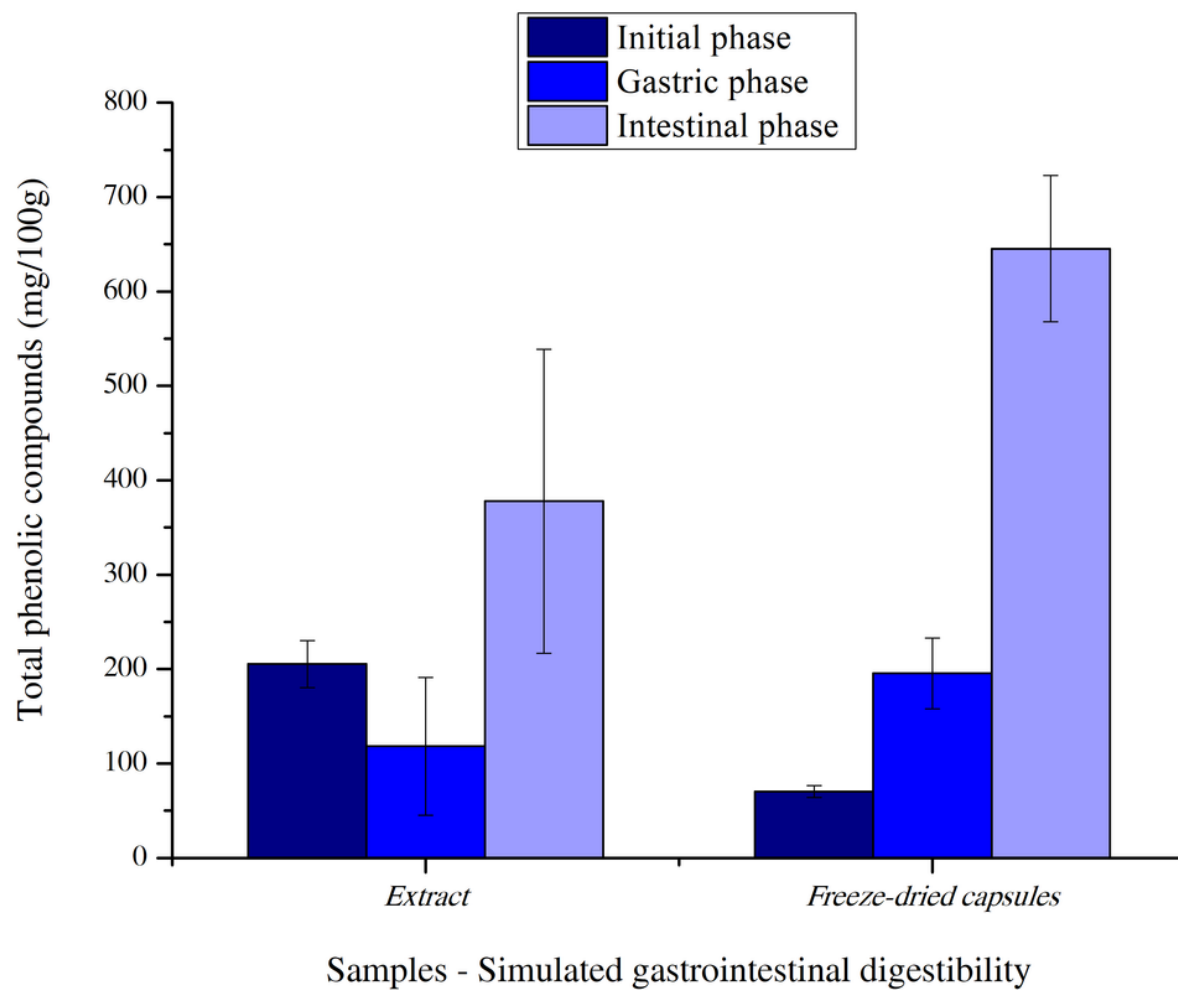


Figure 5

Total phenolic compounds for each phase of the simulated gastrointestinal digestibility process for extract and freeze-dried capsules of *Clitoria ternatea* L.