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# 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 the study was to optimize the extraction of bioactive compounds from *Clitoria ternatea* L. followed by its encapsulation by ionic gelation. The extraction of the bioactive compounds, using water as solvent, was carried out with solid-liquid extractions at hot and room temperature, obtaining as 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 ($p \leqslant 0.05$) between the extracts. The quadratic mathematical model presented a correction coefficient, $R^2 = 0.997$ and $R^2_{adjust} = 0.974$. Hot extraction at 80°C 5 minutes$^{-1}$ at a concentration of 0.008 g mL$^{-1}$ showed a TPC of 23.91 ± 0.90 mg (EGA) g$^{-1}$ and 0.97 ± 0.50 mg (MVE) g$^{-1}$ for monomeric anthocyanins. For antioxidant potential, the values of ABTS, FRAP and β-carotene/linoleic acid were 84.86 ± 1.52 µM trolox g$^{-1}$, 220.83 ± 3.69 µM ferrous sulfate g$^{-1}$ and 55, 95 ± 4.51%, respectively, for the same assay. 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. possess bioactive compounds with an antioxidant profile.

**Keywords:** Butterfly bean; capsules; total phenolic compounds; antioxidant potential.

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## Introduction[1]

Brazil biodiversity is the greatest in the world (Polmann et al., 2021) in which there are native and adapted plant species. There is a group of plant species that are known as non-conventional food plants (NFP). 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 in the diet (Costa et al., 2023; Milião et al., 2022). Among the assorted NFP 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 (Mehmood et al., 2019; Oguis et al., 2019; Singh & Tiwari, 2012).

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 (Welch et al., 2008). As the pH increases, the flavylium cation loses a proton and undergoes hydration, forming a pseudo base or carbitol, and such change in pH influences its color (Khoo et al., 2017; Laleh et al., 2006). 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 (Castañeda-Ovando et al., 2009).

[1] The article has already been shared as a preprint at Research Square [https://doi.org/10.21203/rs.3.rs-3210578/v1].

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 (Dincheva & Badjakov, 2016; Kim et al., 2021), ethanol (Ji et al., 2015; Wu et al., 2016), acetone (Ambigaipalan et al., 2017), water (Forghani et al., 2022; Luna-Vital et al., 2017), or a mixture of these solvents because they are polar molecules (Castañeda-Ovando et al., 2009; Yan et al., 2021).

In addition, other extraction methods include those using enzymatic extraction (Granato et al., 2022) and ultrasound-assisted extraction (He et al., 2016; Ramić et al., 2015), due to the numerous advantages such as low energy consumption, fast process, high production yield and easy operation (Oladzadabbasabadi et al., 2022; Ongkowijoyo et al., 2018).

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 (Surya et al., 2013), freeze drying (Ikrawan et al., 2023) or ionic gelation (Suzihaque & Karim, 2022; Pasukamonset et al., 2016). Ionic gelation encapsulation is a simple and easy procedure, does not require specialize equipment, high temperature or organic solvent and can be considered low cost (Kurozawa & Hubinger, 2017). The method uses hydrocolloids and ionic solutions at appropriate concentrations, and can be applied in controlled release processes, for instance (Copado et al., 2019; Kanokpanont et al., 2018). 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

### Material

Samples of *Clitoria ternatea* L. were obtained from a producer in Mato Grosso do Sul, Brazil. The samples were initially dried using a combined process of natural shade drying followed by oven drying at 45°C for 60 minutes. They were then ground and separated using sieves with a particle size of 150μm. The ground samples were stored in low-density polyethylene (LDPE) packaging, vacuum-sealed (GSVAC-GS420), and kept refrigerated and protected from light for later use.

### Optimization of the extraction of bioactive compounds

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. For the extraction of the bioactive compounds from *Clitoria ternatea* L. water was used as solvent, under different conditions. The solid-liquid extraction was carried out based on the Box-Behnken $3^2$ design, at room temperature (~28°C), 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 as shown in Table 1.

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

| Experimental design $3^2$ at room temperature extraction | | | | |
|---|---|---|---|---|
| Assays | Codes factors and levels | | Factors and levels | |
| | Concentration | Time | Concentration [g $mL^{-1}$] | Time [h] |
| 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 |

| Assays | Experimental design fractional factorial $3^{(3-1)}$ for hot extraction | | | | | |
|---|---|---|---|---|---|---|
| | Codes factors and levels | | | Factors and levels | | |
| | Concentration | Time | Temperature | Concentration [g $mL^{-1}$] | Time [min] | Temperature (°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 |

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.

### *Analysis of bioactive compounds*

The total content of phenolic compounds was determined in the extracts in a spectrophotometer (Drawell/DU-8800RS) at 765 nm. Gallic acid - EGA (Sigma-Aldrich) was used as a standard, and the results were expressed in gallic acid equivalents (mg EGA) $g^{-1}$ (Singleton & Rossi, 1965). The extract with the highest content of total phenolic compounds was used for the other analyses. And the total anthocyanin content of the extracts was determined by the differential pH method (Giusti & Wrolstad, 2005). 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) $g^{-1}$ of sample.

### Antioxidant capacity Assays

The evaluation of antioxidant activity was performed by the ABTS radical scavenging method with modifications (Re et al., 1999) at 734 nm in a UV-Visible spectrophotometer (Drawell/DU-8800RS). The calibration curve was performed with the Trolox standard (Sigma-Aldrich) at a concentration of 100 to 2000 µM and the results were expressed in µM trolox/g. For determination by the β-carotene/linoleic acid system (Miller, 1971) it was performed using the β-carotene/linoleic acid emulsion mixed with extract at concentrations of 40 mg $mL^{-1}$. The emulsion oxidation was determined by spectrophotometry (Drawell/DU-8800RS) at 470 nm after 120 minutes of incubation. The determination of the activity on iron reduction it was carried out in a spectrophotometer (Drawell/DU-8800RS) at 593 nm and 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).

### Encapsulation process

The encapsulation process was carried out using the ionic gelation. The amount of 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. Subsequently, the capsules were frozen and lyophilized (Liotop L101), at 300 µmHg, approximately (Kanokpanont et al., 2018).

### Characterization of the capsules

The powder yield was determined on the ratio between the mass of formed capsules and the mass of extract (García-Lucas et al., 2017). 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. The moisture content of the capsules was determined using the drying oven (SolidSteel/SSDcr-110L) method at 105 °C until constant mass (Association of Official Analytical Chemists [AOAC], 2010). *The average diameter of the capsules was determined based on the average of ten samples*

*measured at random positions using a digital caliper.* The encapsulation efficiency was performed before and after lyophilization, breaking the capsules with 10 % sodium citrate (w/v). Soon after, the mixture was stirred and 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 (Selamat et al., 2009). The solubility of the capsules was determined after dispersion in distilled water, at a concentration of 1:10 (m/v), stirred for 5 minutes and centrifuged (Solab scientific/SL-700) at 3500 rpm for 20 minutes. Then, aliquots were transferred to previously prepared crucibles and placed in a drying oven (SolidSteel/SSDcr-110L) for 5 hours at 105°C (Cano-Chauca et al., 2005). The hygroscopicity was determined after 0.50 g of the capsules were placed separately in hermetically sealed containers with saturated solutions of magnesium chloride ($MgCl_2$ $6H_20$ – 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 (Goula et al., 2008).

### Simulated gastrointestinal digestibility

The bioaccessibility was performed simulating the digestibility by *in vitro* model (Bornhorst & Singh, 2013; Dantas et al., 2019). 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 $L^{-1}$ 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. 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).

### Statistics analyses

The results were expressed as mean ± standard deviation. Data were analyzed using analysis of variance (ANOVA). Significant differences between the means were analyzed by Tukey's test, with $p \leqslant 0.05$. The statistical software Origin Pro 8.0 (Northampton, MA 01060, U.S.A.) and Minitab v. 19 (Minitab, Inc. USA) trial version were used for the analyses.

## 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 extraction process at room temperature, the variables concentration and time did not statistically affect ($p \leqslant 0.05$) the TPC results.

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

| Experimental design $3^2$ at room temperature extraction | | | | |
|---|---|---|---|---|
| Assays | Concentration [g $mL^{-1}$] | Time [min] | Total phenolic compounds (mg GAE $g^{-1}$) | Total monomeric anthocyanins (mg MVE $g^{-1}$) |
| C1 | 0.002 | 1 | $17.99^{a} \pm 2.40$ | $1.06^{ab} \pm 0.10$ |
| C2 | 0.002 | 3.5 | $18.60^{a} \pm 3.09$ | $1.22^{a} \pm 0.17$ |
| C3 | 0.002 | 6 | $19.37^{a} \pm 4.85$ | $0.91^{b} \pm 0.10$ |
| C4 | 0.008 | 1 | $20.86^{a} \pm 2.05$ | $1.04^{ab} \pm 0.11$ |
| C5 | 0.008 | 3.5 | $19.35^{a} \pm 1.19$ | $1.02^{ab} \pm 0.07$ |
| C6 | 0.008 | 6 | $17.91^{a} \pm 1.23$ | $1.00^{ab} \pm 0.09$ |
| C7 | 0.014 | 1 | $19.32^{a} \pm 2.85$ | $1.11^{ab} \pm 0.04$ |
| C8 | 0.014 | 3.5 | $18.99^{a} \pm 0.97$ | $0.94^{b} \pm 0.08$ |
| C9 | 0.014 | 6 | $22.25^{a} \pm 0.31$ | $0.91^{b} \pm 0.05$ |

| Experimental design fractional factorial $3^{(3-1)}$ for hot extraction | | | | | |
|---|---|---|---|---|---|
| Assays | Concentration [g $mL^{-1}$] | Time [min] | Temperature [°C] | Total phenolic compounds (mg GAE $g^{-1}$) | Total monomeric anthocyanins (mg MVE $g^{-1}$) |
| H1 | 0.002 | 5 | 40 | $21.75^{a} \pm 3.43$ | $3.43^{a} \pm 0.83$ |
| H2 | 0.002 | 10 | 80 | $23.31^{a} \pm 0.96$ | $0.21^{c} \pm 0.15$ |
| H3 | 0.002 | 15 | 60 | $21.90^{a} \pm 0.82$ | $2.95^{a} \pm 0.10$ |
| H4 | 0.008 | 5 | 80 | $23.91^{a} \pm 0.90$ | $0.97^{bc} \pm 0.50$ |
| H5 | 0.008 | 10 | 60 | $22.92^{a} \pm 3.49$ | $1.66^{b} \pm 0.55$ |
| H6 | 0.008 | 15 | 40 | $21.85^{a} \pm 1.18$ | $1.16^{bc} \pm 0.52$ |
| H7 | 0.014 | 5 | 60 | $20.58^{a} \pm 0.88$ | $1.04^{bc} \pm 0.32$ |
| H8 | 0.014 | 10 | 40 | $20.39^{a} \pm 1.17$ | $1.21^{bc} \pm 0.04$ |
| H9 | 0.014 | 15 | 80 | $22.12^{a} \pm 0.62$ | $1.15^{bc} \pm 0.03$ |

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

Regarding the monomeric anthocyanin content, it can be observed that the C2 assay, at a concentration of 0.002 g $mL^{-1}$ and with 3.5 h of extraction, had the highest value of monomeric anthocyanins 1.22 ± 0.17 mg MVE $g^{-1}$, being superior and statistically different ($p \leqslant 0.05$) 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 $g^{-1}$) and monomeric anthocyanins (0.91 ± 0.05 to 1.22 ± 0.17 mg MVE $g^{-1}$), it was not possible to obtain a predictive model at room temperature extraction.

Regarding 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 $g^{-1}$. All assays showed significant values for the hot extraction of *Clitoria ternatea* L. But for all assays of TPC there was no statistical difference ($p \leqslant 0.05$). 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 Equation 1.

$$TPC\ (mg\ g^{-1}) = 20.35 + 340.90C + 0.0066t + 0.001T - 33795C^2 - 0.00761t^2 + 0.000362T^2 + 9.24Ct \quad (1)$$

Where: C = concentration (mg $L^{-1}$), t = time (min.) and T = temperature (°C).

$R^2 = 0.997$ and $R^2_{adjust.} = 0.974$

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 (Figure 1A, 1B, 1C), it can be observed that for the three concentrations (0.002; 0.008 and 0.014 mg $L^{-1}$) 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 $g^{-1}$). At the concentration of 0.002 mg $L^{-1}$, the highest TPC content was obtained in the shortest time of 5 minutes. The same happened on the concentration of 0.008 mg $L^{-1}$. As for the highest concentration 0.014 mg $L^{-1}$, 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 at 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 (Figure 1D, 1E, 1F). As the temperature increased, there was an increase in the TPC content in the *Clitoria ternatea* L. extracts up to a certain concentration followed by a reduction in the content. In this way, the optimal point was obtained at a concentration of 0.008 mg $L^{-1}$ 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 (Figure 1G, 1H, 1I). The data showed an optimal point at the concentration of 0.008 mg L-$^{1}$ 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 $L^{-1}$, time = 8.03 minutes and at the temperature of 80°C, obtaining TPC = 24.07 mg GAE $g^{-1}$. As for the optimal point, the parameters obtained were C = 0.008 mg $L^{-1}$, time = 10 minutes and at a temperature of 79.2°C, obtaining TPC = 23.91 mg GAE $g^{-1}$, being the content value of TPC like that obtained experimentally in assay H4 (C = 0.008 mg $L^{-1}$, 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.

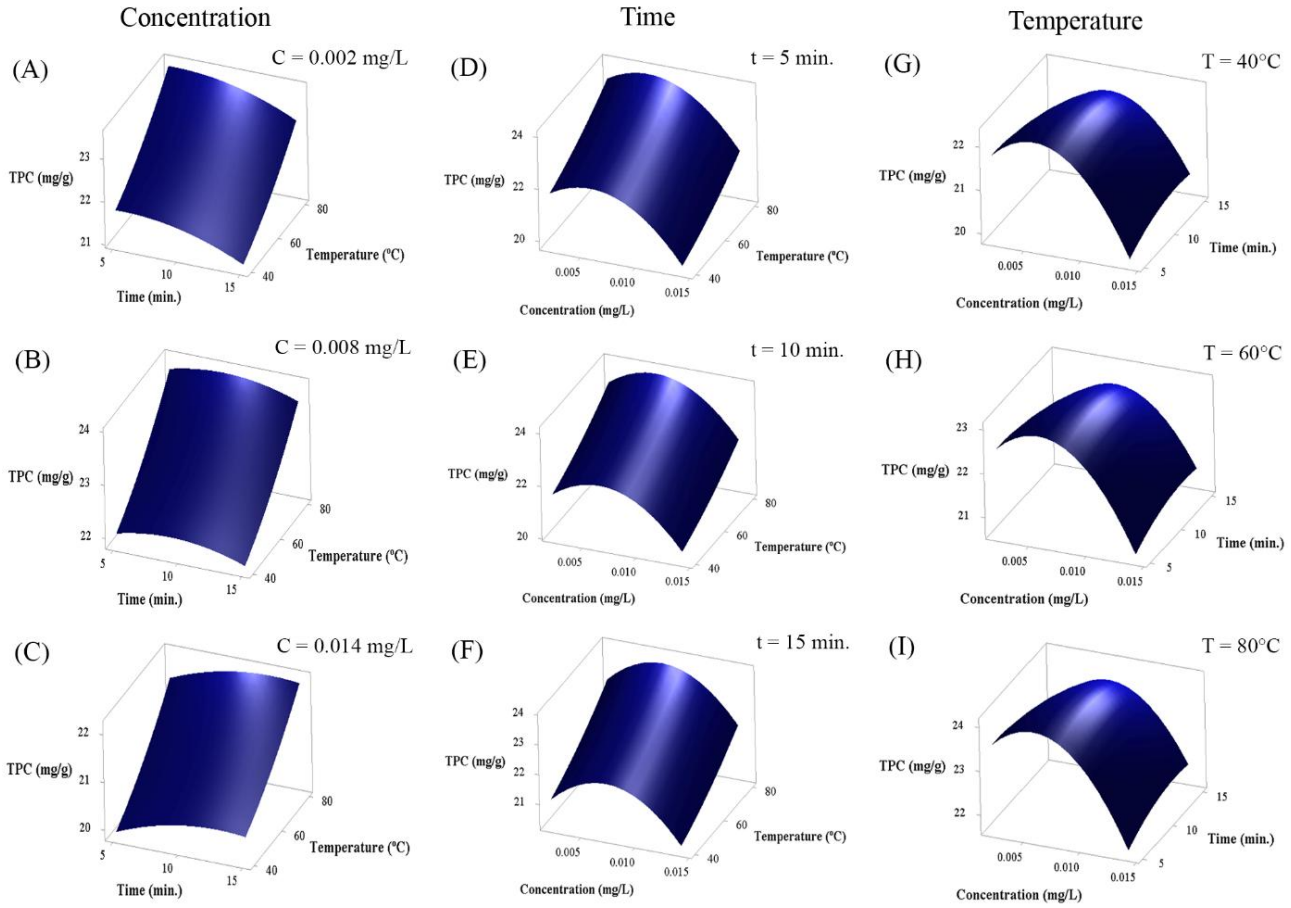


**Figure 1**. Methodology of response surface for analysis of the content of total phenolic compounds of extracts of *Clitoria ternatea* L. fixing the concentration (A, B, C), fixing the time (D, E, F) and fixing the temperature (G, H, I).

## 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 the test of the mechanism, for example, obtaining the β-carotene/linoleic acid autooxidation system. 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. However, there are assays in which both HAT and SET mechanisms occur, for example, the ABTS assay (Kandylis, 2022; Munteanu & Apetrei, 2021; Sadowska-Bartosz & Bartosz, 2022). Thus, the mechanisms of antioxidant activity were evaluated using the FRAP, β-carotene/linoleic acid and ABTS methods, (Table 3).

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

| Antioxidant potential of the extract | | | | |
|---|---|---|---|---|
| H4 | ABTS (μM trolox $g^{-1}$) | FRAP (μM ferrous sulfate $g^{-1}$) | β-carotene/linoleic acid (%) | |
| | 84.86 ± 1.52 | 220.83 ± 3.69 | 53.95 ± 4.51 | |
| Physical characteristics of the capsules | | | | |
| Wet capsules | | | Freeze-dried capsules | |
| Encapsulation efficiency (%) | 75.77$^{a}$ ± 10.30 | | Encapsulation efficiency (%) | 56.41$^{b}$ ± 0.00 |
| Solubility (%) | 99.79$^{a}$ ± 0.04 | | Solubility (%) | 99.31$^{a}$ ± 0.15 |
| Moisture (%) | 99.04$^{a}$ ± 0.28 | | Moisture (%) | 9.28$^{b}$ ± 1.17 |

The H4 assay showed considerable values for each method. In the case of the ABTS assay, the observed value of 84.86 ± 1.52 μM trolox $g^{-1}$ 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 visually observed and also based on the value obtained from 220.83 ± 3.69 µM ferrous sulfate $g^{-1}$. While the β-carotene/linoleic acid assay (53.95 ± 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.

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 ± 0.26 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 showed higher encapsulation efficiency, about 76%. when compared to the freeze-drier capsules, 56%. They also presented a 99% moisture content. However, in terms of solubility there was no significant difference ($p \leqslant 0.05$) between both processes. And the results are similar to the studies of encapsulated flowers of *Clitoria ternatea* L. using the ionic gelation method which obtained values from 74.97 ± 0.84 to 84.83 ± 0. 40% for wet encapsulation efficiency (Pasukamonset et al., 2016). 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 treatment 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, 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 Figure 2.

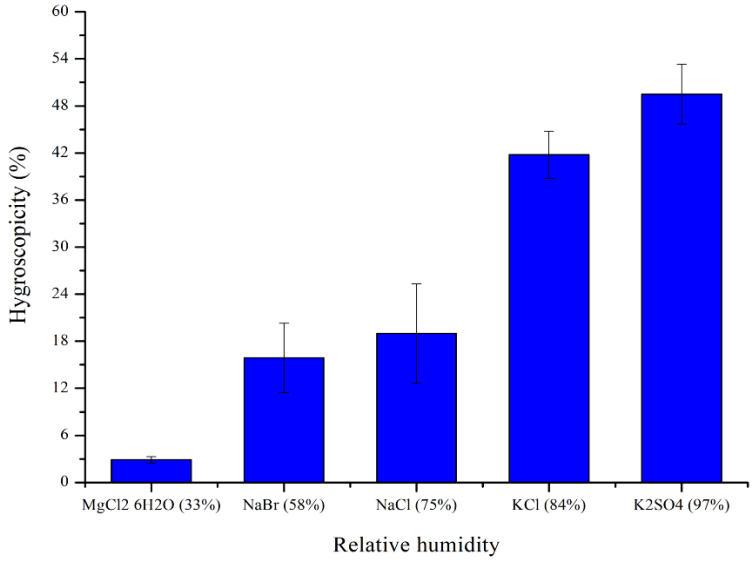


**Figure 2.** Relative humidity versus hygroscopicity of freeze-dried capsules.

At the same times the hygroscopicity of the food is an important parameter, 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 high humidity, 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 endured a good protection capacity, keeping the compounds present 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 3 shows the mean values (mg GAE $100g^{-1}$) of TPC for initial, gastric, and intestinal phases.

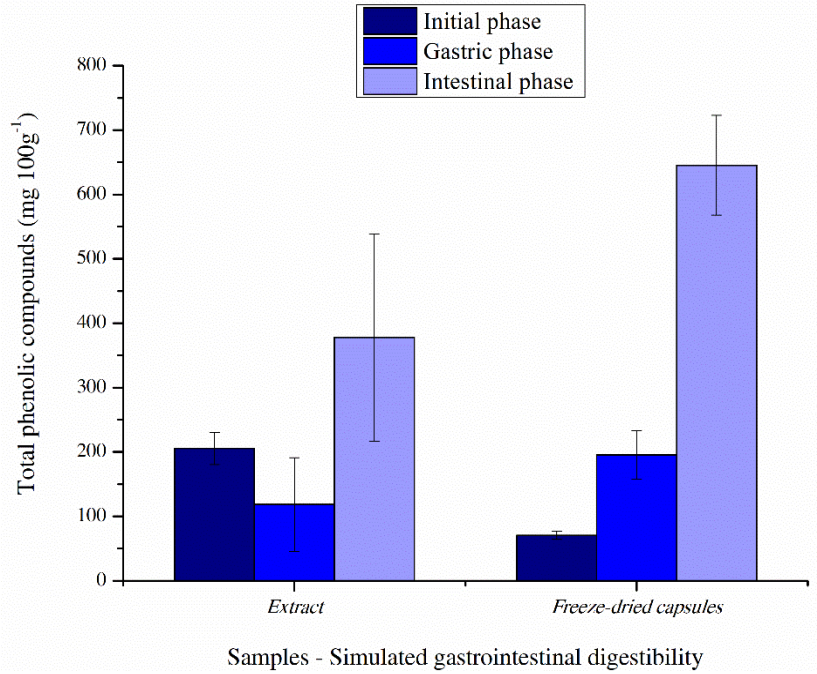


**Figure 3.** Total phenolic compounds for each phase of the simulated gastrointestinal digestibility process for extract and freeze-dried capsules of *Clitoria ternatea* L.

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. presented a higher TPC content (205.40 ± 25.06 mg GAE $g^{-1}$) when compared to the content of the freeze-dried capsules (70.40 ± 6.42 mg GAE $g^{-1}$). 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 $g^{-1}$) 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 $g^{-1}$) 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 (Gonçalves et al., 2021). In the simulation of the intestinal phase, the highest levels of TPC (377.86 ± 161.00 and 645.28 ± 77.45 mg GAE $g^{-1}$) were released for extract and freeze-dried capsules of *Clitoria ternatea* L., respectively.

The TPC profile may vary according to the *in vitro* digestion method used, environmental conditions, harvest time, exposure to light, among other factors. Although these results simulate a physiological process on the bioavailability of phenolic compounds (Peña-Vázquez et al., 2022), 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 (Zhou et al., 2022).

## Conclusion

It was possible to extract and characterize bioactive compounds in the *Clitoria ternatea* L. extract. Hot extraction was more efficient when compared to extraction at room temperature. 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 perform 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.

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