ULTRASONIC EXTRACTION OF ANTHOCYANINS FROM CLITORIA TERNATEA: EVALUATION OF ANTIOXIDANT CAPACITY AND POTENTIAL FOR APPLICATION IN THE FOOD INDUSTRY

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INTRODUCTION

The use of aromatic and medicinal plants for therapeutic, cosmetic, food, and religious purposes dates back to ancient civilizations, evidencing humanity's long tradition of taking advantage of the properties of these plant species (Jeyaraj et al., 2021; Ez zoubi et al., 2022). In recent times, interest in the exploration of unconventional plants, especially those rich in bioactive compounds, has grown significantly due to their potential to contribute to human health and prevent diseases (Sharma et al., 2020; Jeyaraj et al., 2021).

Among these plants, *Clitoria ternatea*, popularly known as butterfly bean, butterfly pea or blue pea flower, stands out. *Clitoria ternatea*, belonging to the Fabaceae family, is a perennial climbing plant that exhibits intense blue flowers, containing unique anthocyanins called ternatins. Given the stability and intensity of its pigment, in addition to its therapeutic use, this plant has potential application in the food industry, where the blue pigment extracted from its flowers is used as a natural dye in various preparations (Mota et al., 2022; Jeyaraj et al., 2021). Synthetic dyes, while widely used, are often associated with potential adverse health effects, leading to a growing interest in natural dyes (Amchova et al., 2024). Among the prominent natural pigments are anthocyanins, known for their vibrant colors ranging from blue to purple, and for their antioxidant properties (Bendokas et al., 2020).

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Figure 1. The flower of Clitoria ternatea collected by the authors.

Source: Authors (2024).

This plant is a rich source of various bioactive compounds, particularly polyphenols such as anthocyanins and flavonoids, which are also known for their potent anti-inflammatory and anticancer properties (Jeyaraj et al., 2021). Anthocyanins, water-soluble pigments responsible for the vibrant colors of various fruits and flowers, have been widely studied due to their antioxidant properties, which involve the neutralization of free radicals, due to the polyphenolic structure that allows hydrogen donation and free electron stabilization (Li et al., 2023; Kowalczyk et al., 2024). Effective extraction of these bioactive compounds is crucial to unlocking the full potential of C. ternatea (Mota et al., 2022; Li et al., 2023).

Several extraction methods have been explored to obtain the phytochemicals of *Clitoria ternatea*, ranging from conventional techniques, such as maceration, to unconventional techniques, including ultrasound-assisted extraction. Each method offers specific advantages, both in terms of extraction efficiency and the preservation of the antioxidant properties of the compounds. Studies indicate that parameters such as solvent type, pH, temperature and extraction time significantly influence the yield and activity of the extracted compounds. The use of polar solvents, such as ethanol and methanol, has been shown to be effective in the extraction of anthocyanins and other phenolic compounds, while ultrasound-assisted methods have shown greater efficiency, in addition to being considered "green" methods because they require shorter extraction times and lower solvent consumption (Jeyaraj et al., 2021).

OBJECTIVE

The present study aims to investigate the ultrasound-assisted extraction applied to *Clitoria ternatea*, evaluating the potential of the extracts obtained in relation to the anthocyanin composition and antioxidant capacity by the radical scavenging method (DPPH). The exploration of this unconventional method represents an opportunity to optimize extraction processes, offering a sustainable and efficient approach to the incorporation of natural colors in the food industry.

METHODOLOGY

COLLECTION

The flowers of *Clitoria ternatea* were collected in Extremoz, Rio Grande do Norte, Brazil, (Latitude: 5°42'32"S, Longitude: 35°11'48"W) between September and October 2024 and taken to the food engineering laboratory (LEA) of the Federal University of Rio Grande do Norte (UFRN) immediately.

DRYING

The clitoral petals were dried in an oven (Lucadema, São Paulo, Brazil) for 24 hours at a temperature of 50°C.

HUMIDITY

Approximately 2 grams of the sample were weighed in previously dried and tarted weighing capsules. The samples were placed in an incubator (Lucadema, São Paulo, Brazil) at a temperature of 105°C until they reached a constant weight (AOAC, 2000).

WATER ACTIVITY

The analysis was performed using a direct water activity meter, equipped with a digital sensor (Aqualab S3TE, Decagon, USA). The samples were placed in small sampling capsules provided by the equipment, ensuring that their surface was completely exposed to the sensor for an accurate reading. The capsules were inserted into the equipment's reading chamber, which was closed and allowed to stabilize until it reached equilibrium with the internal environment.

EXTRACTION

The dried flowers of *Clitoria ternatea* were extracted using water as a solvent in an ultrasonic bath (ALTSONIC Clean 3IA, 3 L, São Paulo, Brazil) at a frequency of 40 kHz and 100 W, without agitation and without temperature, for 75 minutes. The ratio between petals and water was 2 g per 100 mL (1:50 g/mL). The pH of the extract was adjusted to 2 with hydrochloric acid (HCl).

ANTHOCYANIN ANALYSIS

After extraction, the extracts were filtered to remove the residual solid material. The anthocyanins present in the extracts were quantified by UV-Vis spectrophotometry at 535 nm. The method was described by Mauludifia et al. (2019) where 1 mL of the extract was diluted in 10 mL of a buffer solution at pH 1 and another dilution in a buffer solution pH 4.5. The amount of anthocyanin is given by the following equations:

$$A = ((A510 - A700) \times pH1) - ((A510 - A700) \times pH4{,}5) \quad (1)$$

$$C\ (mg/L) = (A \times FD \times MM \times 1000) / (e \times b) \quad (2)$$

Where A510 is the absorbance measured at 535 nm and A700 the absorbance measured at 700 nm. A is the resulting absorbance of equation 1, FD is the dilution factor, MM is the molar mass of the pattern in g/mol, *and* is the coefficient of molar absorptivity of the pattern, and *b* is the optical path given by the thickness of the spectrophotometer.

ANTIOXIDANT ACTIVITY (DPPH)

The antioxidant activity by scavenging the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined following the method described by Bobo-García et al. (2014). A 150 μL aliquot of a methanolic solution of DPPH (150 μM) was added to 20 μL of the samples in 96-well microplates. The mixtures were then incubated at room temperature, in the dark, for 40 minutes, and absorbance was measured at 515 nm using a microplate reader. The results are expressed by equation 3 below.

$$DPPH\ (\%) = ((A\ controle - A\ amostra) \div A\ controle)\ x\ 100 \quad (3)$$

STATISTICAL ANALYSIS

All analyses were performed in at least triplicate and are presented as mean ± standard deviation.

DEVELOPMENT

The drying process promotes the removal of moisture, making it possible to increase the useful life and the concentration of important components in the materials. Also, making the products submitted to this process available even in the off-season periods (Martins et al., 2020). According to ANVISA (2015), dried or dehydrated vegetable products must have a water content (moisture) of a maximum of 12%.

Table 1. Physicochemical parameters for dried Clitoria petals.

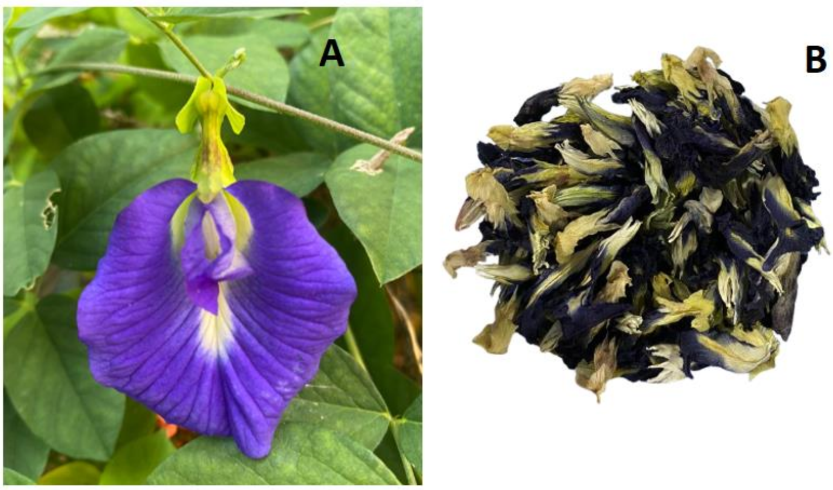
Physicochemical parameters	Average value
Humidity (%)	8.32 ± 1.05
Water Activity (aw)	0.58 ± 0.01

Source: Authors (2024).

Table 1 shows the value obtained for the moisture content of the dried Clitoria petals, ranging from 8.32 ± 1.05, being within the indicated range. Mota et al. (2022) found a very similar value, of 8.39% for the Clitoria flower dehydrated by hot air. This humidity control favors the conservation of the flower, facilitates transport, storage and handling. With this, the flower becomes more practical both for consumption and for use in food production.

According to Table 1, it is possible to verify a value of 0.58 ± 0.01 for water activity (aw) of the dehydrated Clitoria petals. Water activity is one of the main elements that controls the development of microbial cells and influences the spoilage process of food (Rifna et al., 2022). Castoldi (2012) points out that although in values of aw > 0.3 there is a reduction in quality for most dry foods, in general, the minimum value of water activity to prevent the growth of practically all microorganisms is less than 0.6. Thus, the value obtained in the present work for water activity is in the range that indicates microbiological safety (Alp and Bulantekin, 2022). The powder obtained in the present work is compatible for storage and applications.

Figure 2. Fresh (A) and kiln-dried Clitoria ternatea leaves (B)



Source: Authors (2024).

Regarding the extraction of anthocyanins from *Clitoria ternatea* flowers , they have great potential for application as a functional ingredient and natural colorant, which has led to the search for optimization of this process (Gomez et al., 2022). In this study, ultrasound-assisted extraction of *Clitoria ternatea* flowers resulted in a concentration of 125.2 mg/100g of anthocyanins, as shown in Table 2. Factors such as temperature, extraction time, solvent type and substrate:solvent ratio are important process parameters, directly influencing the extraction yield of compounds of interest. In recent studies, Gamage and Choo (2023) obtained a significantly higher concentration of anthocyanins (700 ± 1.68 mg CGE/g) when employing aqueous solvent and ultrasound, with ideal conditions of 50 ºC, time of 30 minutes, and substrate ratio of 1:15 (m/v). These differences in results can be attributed to the specific experimental conditions, such as higher temperature and shorter extraction time, which highlights the importance of optimizing these parameters to maximize extraction efficiency.

Table 2. Anthocyanin concentration and antioxidant activity against the DPPH radical of Clitoria ternatea extract obtained by ultrasound

Analysis	Results
Anthocyanins (mg/100g)	125.2 ± 0.07
DPPH (%)	25.16 ± 0.83

Source: Authors (2024).

Gonçalves et al. (2024) applied a multivariate design to study the effect of variables such as temperature, type of solvent and extraction time on lyophilized leaves of *Clitoria ternatea*, finding the best conditions at 45 ºC, 16 minutes and 22.5 mL of acidified aqueous solution, reaching a concentration of 487.25 mg/100g of anthocyanins. These results, although positive, contrast with those of the present study, in which the extraction was performed at room temperature and for 75 minutes, suggesting again the need to optimize the conditions of the extraction process. In conventional extraction processes of *Clitoria ternatea* leaves, maceration is a commonly applied technique. Although this process occurs in mild conditions, the use of long extraction periods and large amounts of solvent is reported. Matos et al. (2024) determined 392.85 mg of anthocyanins/100g in dehydrated flowers of *Clitoria ternatea* after four cycles of maceration in 70% acidified alcoholic solution at pH 2, at room temperature, with a total time of 96 hours. While studies emphasize the effectiveness of various extraction methods, it is essential to consider the environmental impact and sustainability of these processes, taking into account energy consumption and solvent use. In this context, ultrasound has been highlighted as a sustainable alternative, with the potential to reduce these impacts (DA SILVA et al., 2023).

Clitoria ternatea is a source of phenolic compounds, a class of compounds associated with its biological activities. Among them, the antioxidant effect stands out, which is associated with the ability to sequester free radicals, contributing to the prevention of chronic and degenerative diseases (Jeyaraj et al., 2022). As shown in Table 2, the extract showed an antioxidant capacity of 25.16% against the DPPH radical. In a similar study, Gonçalves et al. (2024) reported an antioxidant activity of 17.95% for the same radical, using a concentration of 4.44 mg/mL of C. ternatea extract. When comparing conventional and alternative extraction methods, Gamage & Cho (2023) observed that ultrasound and microwave extraction provided greater antioxidant activities against the ABTS radical. Mehmood et al. (2019) also found significantly higher values of antioxidant activity in extracts obtained by ultrasound (931.46 ± 16.91 µg Trolox/g DM) compared to conventional extraction (764.32 ± 23.41 µg Trolox/g MS), indicating the potential of ultrasound as a method of efficient extraction of antioxidant compounds from C. ternatea.

FINAL CONSIDERATIONS

According to the results obtained in the present study, it is possible to verify that the drying of the petals of *Clitoria ternatea* is efficient in the conservation of the product, keeping the humidity within the recommended range and controlling the water activity, which contributes to the microbial stability and facilitates storage and transportation. In addition, the extraction of anthocyanins, a compound of great functional interest and natural coloring, proved to be efficient when performed with the application of ultrasound. To maximize the yield and quality of the final product, it is necessary to seek the optimization of extraction parameters, such as temperature, time, and solvent type.

The results also indicate that the flowers of *Clitoria ternatea* They have potential as a source of antioxidant compounds, with the ability to fight free radicals, reinforcing their value in the production of functional ingredients. Although conventional extraction methods show good results, the use of alternative technologies, such as ultrasonic extraction, is promising both in terms of process efficiency and in representing a more sustainable option in terms of energy consumption and solvent use. This study contributes to the understanding of the potential of *Clitoria ternatea* in industrial processes, emphasizing the need to optimize extraction processes in order to maximize their benefits.

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