https://doi.org/10.17221/68/2024-CJFS

Phytochemical, antioxidant activity, and thermal stability of *Clitoria ternatea* flower extracts

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Citation: Shiau S.Y., Wang Y., Yu Y., Cai S., Liu Q. (2024): Phytochemical, antioxidant activity, and thermal stability of *Clitoria ternatea* flower extracts. Czech J. Food Sci., 42: 284–294.

Abstract: The demand for beneficial, safe natural pigments is growing. *Clitoria ternatea* (CT) flowers are a promising source of natural blue colours and bioactive compounds. This study extracted intact CT flowers (CTFs) and their powders (CTFPs) with distilled water at various temperatures (30–90 °C) and durations (30–180 min). The total anthocyanin content (TAC) and total phenolic content (TPC) of the CT extracts were analysed by using the pH differential and Folin-Ciocalteu methods, respectively. The antioxidant activity was expressed as the ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. The results showed that different sample types significantly ($P < 0.05$) influenced the TAC, TPC, and antioxidant activity. The optimal TAC and TPC extraction for CTF was at 90 °C for 90 min, and for CTFP at 90 °C for 30 min. Using hot water instead of 60% alcohol organic solvent was feasible for extracting the phytochemicals, particularly with CTF. Ultrasound-assisted extraction was notably effective for anthocyanins from intact flowers. Higher heating temperatures and pH values increased the anthocyanin degradation rate constants, while a higher pH decreased the activation energy. Thus, blue aqueous CT flower extracts, rich in phytochemicals and antioxidants, have the potential for use in foods and nutraceuticals with neutral and acidic pH values.

Keywords: butterfly pea; extraction; anthocyanin; polyphenol; antioxidation; heat degradation

The flowers of *Clitoria ternatea* (CT), commonly known as the butterfly pea have been used in food products for a long time as a natural colourant (Gamage et al. 2021). Due to their rich content of phytochemicals, including anthocyanins, polyphenolics, and flavonoids, phytosterols and tocopherols, CT possesses the ability to inhibit oxidation, inflammation, microbial growth, diabetes, and cancer cell proliferation *in vitro* (Mukherjeea et al. 2008; Shen et al. 2016; Li et al. 2022).

Anthocyanin, a natural pigment that occurs in plants exhibits multiple colours and consists of anthocyanidin (aglycon) and sugars, occasionally linked to organic acids (Jokioja et al. 2021). Ternatins, the main polyacylated anthocyanins found in CT flowers, are the derivatives of delphinidin 3,3',5'-triglucoside and exhibited good stability in both acidic and neutral conditions (Vuong and Hongsprabhas 2021). The stability of anthocyanin was influenced by various factors, such as the chemical structure, temperature, pH, water activity, and others (Enaru et al. 2021; Gamage et al. 2021). Acylation of the glycosyl moieties in anthocyanins, mainly with aromatic acids, enhanced the colour stability through co-pigmentation and self-association reactions (Wallace and Giusti 2019; Jokioja

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Supported by the project (MOST-107-2221-E-127-002) of the Ministry of Science and Technology, Taiwan, and the Foundation Key project (No. 2022ZD04) of Shanghai Sanda University.

https://doi.org/10.17221/68/2024-CJFS

et al. 2021). The extract from CT flowers showed a higher stability within the pH range of 4.0–8.0, and the anthocyanin stability of CT flowers was the highest among five materials (CT, purple sweet potato, red cabbage, grape skin and eggplant peel) tested (Fu et al. 2021).

Anthocyanins are typically extracted using acidic organic alcohols or alcohol-water mixtures (Oancea and Draghici 2013; Ludin et al. 2018). However, due to the low cost, convenience, non-toxicity, and eco-friendliness, water has been employed successfully to extract anthocyanins from CT flowers for food applications (Gamage et al. 2021). Ultrasound-assisted extraction (UAE) is a new, efficient and sustainable non-thermal method for extracting phytochemicals from medical plants (Morata et al. 2021). Ultrasound waves having a frequency of 20–200 kHz can produce air bubbles in extracting solutions. As the bubbles formed by cavitation collapse, large amounts of energy are released. This leads to the depolymerisation of the cell wall polysaccharides in plant materials and the increase in the diffusion rate and extracting yield of phytochemicals (Jeyaraj et al. 2021; Gil-Martín et al. 2022).

Previous studies have primarily extracted CT anthocyanins using powder as the sample, while rarely utilising the flower form as the sample, and conducting comparisons between the two forms. In this study, intact dry CT flowers and their ground powders were used. The 'green' aqueous extraction process involved varying temperatures (30–90 °C) and durations (30–180 min). Then, the phytochemicals and antioxidant activities of the resulting CT flower extracts were analysed. Additionally, the extraction efficiency of the phytochemicals under the assistance of ultrasound was investigated. Lastly, the stability of anthocyanins in the blue extract was assessed under different heating temperatures (60–90 °C), heating durations (0–5 h), and pH levels (2.5–5.5).

MATERIAL AND METHODS

Materials. Dry CT flowers (CTFs) with a moisture content of 8.857 ± 0.081% were procured from a local market. All the chemicals used in the food analysis were of analytical grade.

Preparation of the *Clitoria ternatea* flower extracts. In this study, parts of the CTFs (500 g) were ground and sifted using a 60-mesh screen. The resulting powder of the CT flowers (CTFP), characterised by a particle size of less than 0.25 mm, was made and stored in a desiccator cabinet until further use. Distilled water was employed as the solvent for extracting the CTF and CTFP. The 2% (*w*/*v*) extracts (CTFE and CTFPE) were prepared in a water bath shaker by mixing 4 g of the dry CT flowers and powders with 200 mL of distilled water at 60 rpm (revolutions per minute) and temperatures ranging from 30 to 90 °C. The extraction process lasted for different periods (30–180 min) at 60 rpm. After extraction, the solutions were immediately filtered, cooled and diluted with distilled water (about 5–7 mL) to a final volume of 200 mL. Moreover, the traditional 60% ethanol extraction method operated at 40 °C for 30 min (Escher et al. 2020) was used as a control for comparison.

UAE was employed for the CT flowers and powders using an ultrasonicator (T650CT; Shanghai Zollo, China). According to the method (Salacheep et al. 2020) with some modifications, the UAE conditions operated at 162.5 W and 20 kHz were set at 60 °C for 30 min of extraction time.

Total anthocyanin content. The total anthocyanin contents (TACs) of the CT extracts were analysed using the pH differential method (Lee et al. 2005). In this method, a 1 mL sample of the extract was mixed with 4 mL of a pH 1.0 buffer containing 0.025 mol·L^{-1} KCl adjusted with HCl, as well as 4 mL of a pH 4.5 buffer consisting of 0.4 M sodium acetate. The absorbance of the samples at 520 nm and 700 nm wavelengths was recorded. The TACs of the CTFE and CTPE were calculated using the following equation:

$$TAC\ (\text{mg·kg}^{-1}\ \text{DB}) = \frac{A \times F \times MW \times 1\,000 \times V}{\varepsilon \times X \times W} \qquad (1)$$

where: A – absorbance $[(A_{520\ \text{nm}} - A_{700\ \text{nm}})_{\text{pH}\ 1.0} - (A_{520\ \text{nm}} - A_{700\ \text{nm}})_{\text{pH}\ 4.5}]$; F – dilution factor; MW – molecular weight of cyanidin-3-glucoside (MW = 449.2 g·mol^{-1}); ε – molar extinction coefficient of cyanidin-3-glucoside (ε = 26 900 L·mol^{-1}·cm^{-1}); X – path length (cm); V – volume of the extract (mL); W – CTF or CTFP sample weight (g); DB – dry weight basis.

Total phenolic content. The total phenolic contents (TPCs) of the CT extracts were determined using the Folin-Ciocalteu reagent, following the method (Shiau et al. 2023), with gallic acid as a standard. TPC was expressed by mg gallic acid equivalent (GAE) per kg sample (dry weight, DB).

Antioxidant activity. The radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity of the extracts was performed by using the methods (Liyana-Pathirana and Shahidi 2007) and using Trolox as a standard. The result was expressed as mg Trolox equivalent (TE) per kg sample (DB).

Thermal stability of anthocyanin. The thermal degradation of the anthocyanins in the CT extracts was performed by different heating temperatures (60, 70, 80, and 90 °C), heating times (0, 1, 2, 3, 4, and 5 h), and pH values (2.5, 3.5, 4.5, and 5.5). The procedure included transferring 4 mL of the CT extract into a glass test tube with a screw cap, followed by adding 4 mL of a 0.1 $\text{mol}\cdot\text{L}^{-1}$ citric acid – a 0.2 $\text{mol}\cdot\text{L}^{-1}$ Na_2HPO_4 buffer to adjust the sample solution's pH to 2.5–5.5. After thorough mixing, the sample solution was heated for various durations at a specified temperature. The thermal degradation kinetics of the anthocyanins was evaluated using a first-order reaction model, as seen in Equation 2.

$$\ln\left(\frac{C_t}{C_0}\right) = -k \times t \tag{2}$$

where: C_0 – initial monomeric anthocyanin content; C_t – monomeric anthocyanin content after t (h) heating at a given temperature and pH; k – reaction rate constant.

The half-life ($t_{1/2}$), the time needed for the 50% degradation of the anthocyanins, was calculated by the Equation 3:

$$t_{1/2} = \frac{\ln 2}{k} \tag{3}$$

Statistical analysis. IBM SPSS Statistics (version 20) was used to analyse the data in two replicates for the different treatments, unless stated otherwise. A one-way analysis of variance (ANOVA), Duncan's new multiple range tests, and Pearson correlation coefficients were conducted to judge the statistical significance ($P < 0.05$) of the differences among the values.

RESULTS AND DISCUSSION

Total anthocyanin content (TAC) of the *Clitoria ternatea* extracts. The pH values of the CTFE and CTFPE prepared was 5.50–5.53 and 5.43–5.46, respectively, and they were not obviously changed by the different extraction temperatures and times. Table 1 lists the effect of the extraction temperature on the TAC of the CTFE and CTFPE. With a constant extraction time of 30 min, the TAC of both CTFE and CTFPE significantly increased as the extraction temperature rose from 30 to 90 °C. Within this temperature range, the TAC of the CTFE was obviously increased by 531.60%, while that of the CTFPE was increased by 9.03%. The increase in the TAC by increasing the extraction temperature was partly attributed to the elevation of the internal energy in the pigment molecules, leading to an increase in the molecular diffusivity and solubility. Another reason might be related to the changes in the plant cell membrane structure and permeability caused by the extraction at high temperatures, because the altered membrane permeability facilitated the extraction of cellular solutes, including the anthocyanins, by the solvents.

At all the extraction used temperatures, CTFPE had a higher TAC than CTFE, especially at lower extraction temperatures (Table 1). When intact CT flowers were ground into powder (CTFP), the structure of the tissue and cell were disrupted. The fine particles, conducive

Table 1. Effect of the extraction temperature on the phytochemicals and antioxidant activity of the *Clitoria ternatea* flower extracts under a fixed 30-min extraction time

Extraction temperature (°C)	TAC ($\text{mg}\cdot\text{kg}^{-1}$ DW)		TPC ($\text{g GAE}\cdot\text{kg}^{-1}$ DW)		Radical DPPH scavenging activity ($\text{g TE}\cdot\text{kg}^{-1}$ DW)	
	CTFE	CTFPE	CTFE	CTFPE	CTFE	CTFPE
30	147 ± 16^{e}	1 051 ± 28^{c}	6.7 ± 0.1^{e}	23.3 ± 0.3^{c}	7.6 ± 0.4^{e}	24.5 ± 1.0^{a}
45	225 ± 18^{d}	1 075 ± 25^{bc}	9.9 ± 0.3^{d}	23.7 ± 0.4^{c}	10.9 ± 0.9^{d}	25.1 ± 1.1^{a}
60	339 ± 19^{c}	1 075 ± 29^{bc}	11.7 ± 0.3^{c}	25.6 ± 0.5^{b}	14.2 ± 0.8^{c}	25.5 ± 1.3^{a}
75	669 ± 44^{b}	1 119 ± 25^{ab}	21.3 ± 0.3^{b}	26.0 ± 0.4^{b}	17.8 ± 0.9^{b}	25.7 ± 1.1^{a}
90	931 ± 55^{a}	1 146 ± 28^{a}	25.4 ± 0.8^{a}	27.9 ± 0.6^{a}	21.0 ± 1.0^{a}	26.7 ± 1.2^{a}

$^{a-e}$ Means in the same column marked with different letters are significantly different ($P < 0.05$); values are means ± standard deviations ($n = 3$); TAC – total anthocyanin content; TPC – total phenolic content; DPPH – 2,2-diphenyl-1-picrylhydrazyl; DW – dry weight; GAE – gallic acid equivalent; TE – Trolox equivalent; CTFE – *Clitoria ternatea* flower extract; CTFPE – *Clitoria ternatea* flower powder extract to mass and heat transfers, contributed to the higher TAC of the CTFPE compared to the CTFE. Cisse et al. (2012) reported that a decrease in particle size (2 cm – 150 μm) of roselle calyces significantly reduced the extraction time of anthocyanins.

Both the temperature and time of extraction can affect the extractability of bioactive compounds and their bioactivity. Generally, a longer extraction time can lead to the increased extractability of a heat-stable soluble substance. CTF extracted at a high temperature is similar to steeping CT flower petals directly in hot water (90–100 °C) to make tea. In Figure 1A, the TACs of the CTFE obviously increased with the increase of extraction time from 30 to 180 min. At temperatures of 30, 60, and 75 °C, the TAC value of the CTFE increased by 171.37, 111.91, and 26.48%, respectively. However, the CTFE extracted for 90 min at 90 °C had the highest TAC (1 094 mg·kg^{-1}) among all the extraction con-

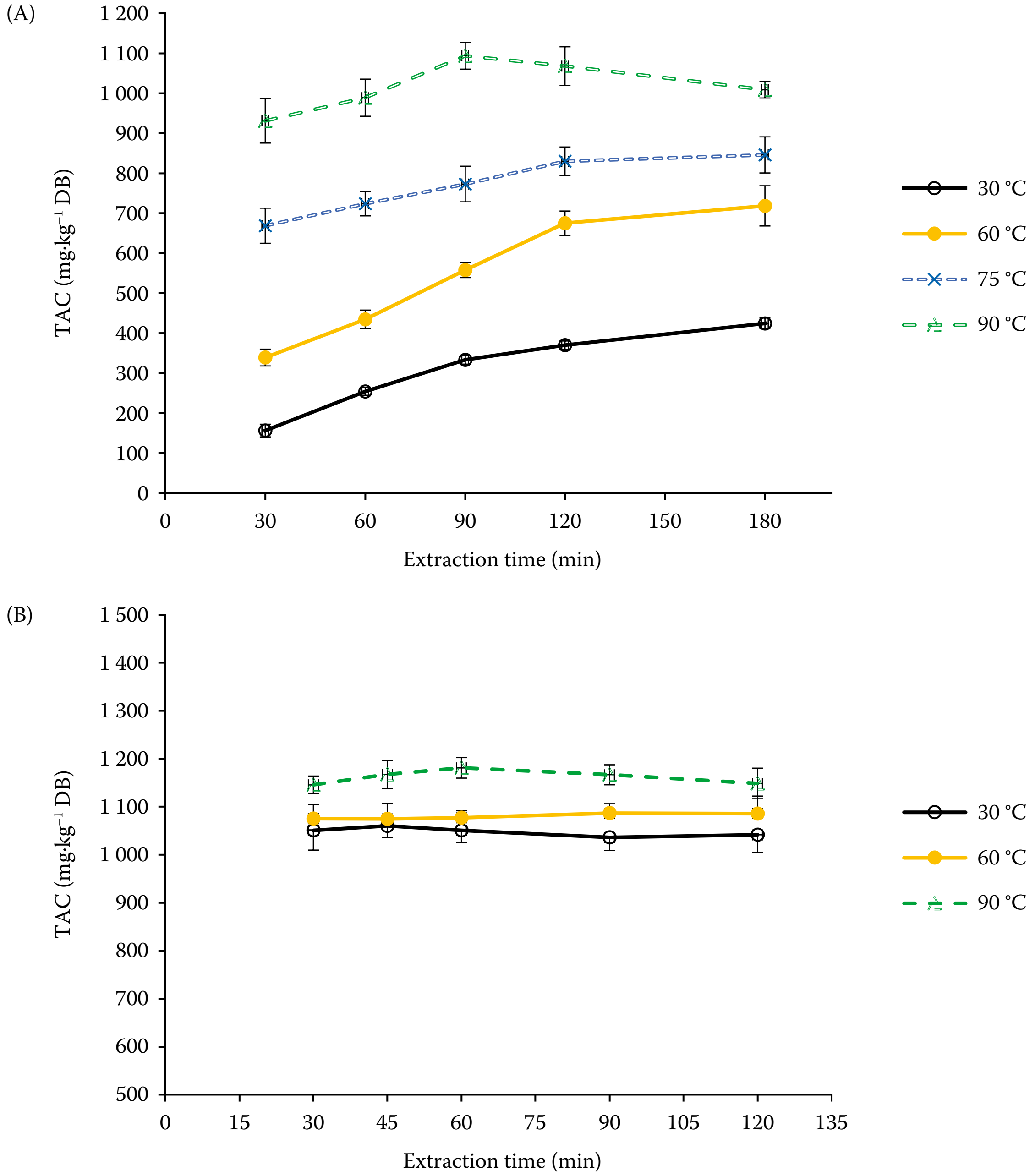

Figure 1. Effect of the extraction time on the total anthocyanin contents of the (A) *Clitoria ternatea* flower extract and (B) *Clitoria ternatea* flower powder extract

TAC – total anthocyanin content; DB – dry weight basis ditions tested, and it increased by 17.48% compared to the extraction for 30 min at the same temperature. Then the TAC value was significantly reduced by a longer extraction time (180 min).

Figure 1B displays the TACs of the CTFPE extracted over various durations (30–120 min) at 30, 60, and 90 °C. The change in the TAC of the CTFPE was less than 3.08%; hence, the extraction time had an insignificant effect ($P > 0.05$) on the TAC of the CTFPE at the same extraction temperatures. The result may be due to the high solubility of ternatins and the fine CTFP particles. Based on the results from Table 1 and Figure 1, the optimal extraction conditions for anthocyanins from the CTF and CTFP were 90 °C for 90 min and 75–90 °C for 30 min, respectively.

Several articles on anthocyanins using aqueous extraction methods have reported the optimal or adequate conditions for dried ground CT flowers. These conditions included 60 °C for 60 min (Ahmad et al. 2020), 80 °C for 30 min (Vuong and Hongsprabhas 2021), 90 °C for 5 min (Voss et al. 2020), 100 °C for 5 min (Cisse et al. 2009), and 100 °C for 30 min (Zakaria et al. 2018). These results are not consistent, which are possibly related to the tested extraction temperature and time ranges, particle size of the samples, solid-liquid ratio, stirring rate during extraction, and analytic method of the anthocyanins. To our knowledge, there is very limited research on the extraction of anthocyanins using intact CT flower petals.

By the 60% ethanol extraction method operated at 40 °C for 30 min, the TACs of the obtained CTFP and CTFPE were 442 $mg\cdot kg^{-1}$ and 1 302 $mg\cdot kg^{-1}$, respectively. The former, which corresponded roughly to the value extracted at 63.1 °C for 30 min, had a much lower TAC than that (931 $mg\cdot kg^{-1}$) of the CTFE extracted at 90 °C for 30 min in Table 1. The latter, on the other hand, exhibited a 13.69% higher TAC than the optimal extraction conditions for the CTFP at 90 °C for 30 min. The comparative experimental results indicate that using hot water for extracting anthocyanins from CT flowers is highly suitable. Ludin et al. (2018) indicated that the powder of CT flowers could be used to extract more anthocyanins when using absolute ethanol as a solvent, compared to water and non-polar solvents. At 45 °C for 45 min of extraction, an aqueous extract of CT petal powder had a higher TAC than a 50% ethanolic extract (Netravati et al. 2022).

Total phenolic content (TPC) and antioxidant activity of the *Clitoria ternatea* extracts. Similar to the TAC, the TPC of both the CTFE and CTFPE significantly increased when the extraction temperature increased from 30 °C to 90 °C (Table 1). The ratio of the TPC increase in the CTFE and CTFPE was 276.87% and 19.86%, respectively. Moreover, CTFPE obviously had a higher TPC than CTFE, especially at extraction temperatures below 75 °C. This should be due to the disruption of the cell and tissue structures and the increase in the molecular mobility and solubility by the high temperature. The DPPH radical scavenging activity of CTFE was significantly increased by increasing the extraction temperature from 30 to 90 °C (Table 1). However, the antioxidant activity of CTFPE was insignificantly ($P > 0.05$) affected by the extraction temperature.

In Figure 2A, the TPC of the CTFE obviously increased with the increase in the extraction time. The TPC values of the CTFE extracted for 30, 60, 75, and 90 °C increased by 94.47, 84.64, 24.57 and 11.45%, respectively. As the extraction temperature increased, the time required to reach its maximum point of the TPC was shortened. At 30 °C, it took 180 min; at 60 °C, it took 120 min; at temperatures between 75–90 °C, it took 90 min. However, the TPCs of the CTFPE extracted at 30, 60, and 90 °C were not significantly affected by the extraction time, as shown in Figure 2B, because the change in the TPC values ranged from 1.61% to 4.29%. To obtain the highest total polyphenolic contents, the CTF and CTFP should be extracted for 90 min and 30 min at 90 °C, respectively.

The TPC values of the CT flowers and powders using 60% ethanol extraction method were 19.1 $g\ GAE\cdot kg^{-1}$ and 31.5 $g\ GAE\cdot kg^{-1}$, respectively. The former, which corresponded roughly to the value of the CTFE extracted at 71.5 °C for 30 min in Table 1, was a much lower TPC than that (28.1 $g\ GAE\cdot kg^{-1}$) of the CTFE extracted at 90 °C for 90 min in Figure 2. However, the alcohol extraction of the CT powders exhibited a 12.95% higher TPC than the optimal extraction condition for the CTFPE at 90 °C for 30 min. Netravati et al. (2022) reported that both aqueous and 50% ethanolic extracts of a CT petal powder had a similar TPC at 45 °C for 45 min of extraction.

In Figure 3, the CTFE extracted at 90 °C had the highest DPPH scavenging activity and was insignificantly affected by the extraction time. The DPPH scavenging activity of the CTFE obviously increased with the increase in the extraction time at 30–75 °C. The scavenging activity of the CTFE extracted for 30, 60, and 75 °C increased by 14.61, 48.42, and 16.42%, respectively. The strong DPPH scavenging activities of the CTFE obtained by extraction at 60 °C for 120–180 min and at 75 °C for 90–180 min showed no significant difference compared to those extracted at 90 °C. Similar to the TAC and TPC,

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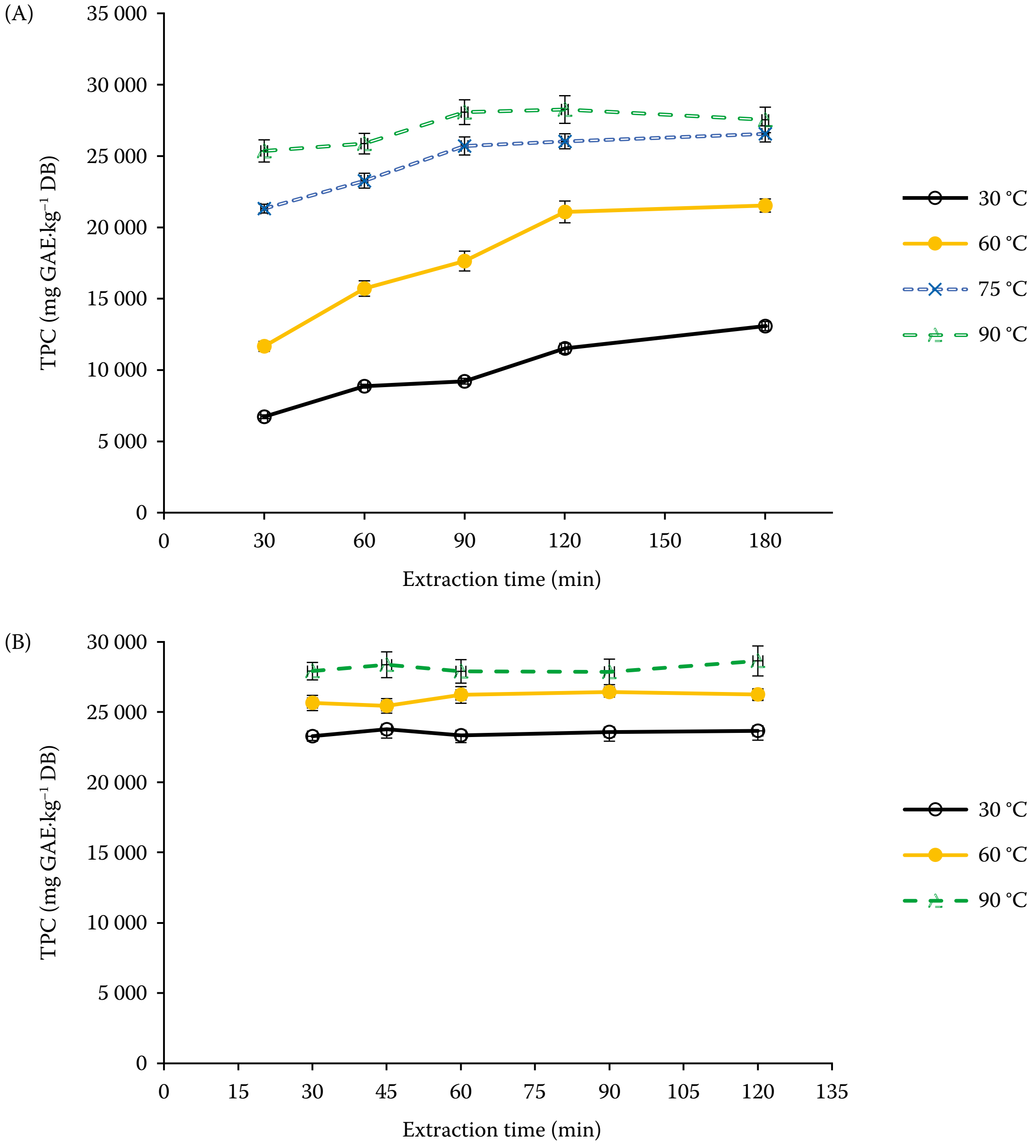

Figure 2. Effect of the extraction time on the total polyphenolic contents of the (A) *Clitoria ternatea* flower extract and (B) *Clitoria ternatea* flower powder extract

TPC – total polyphenolic content; GAE – gallic acid equivalent; DB – dry basis

the DPPH scavenging activities of the CTFPE extracted at 30, 60, and 90 °C were not significantly affected by the extraction time (data not shown). To obtain the optimal DPPH scavenging activity, CTF and CTFP could be extracted for 30 min at 90 °C.

Generally, a substance rich in anthocyanins and total polyphenolics has good antioxidant activities. In the CTFE, there was a significantly ($P < 0.01$) positive correlation between the TAC, TPC, and radical DPPH scavenging activity, with correlation coefficients (r-values) ranging from 0.876 to 0.976. However, for the CTFPE, only a positive correlation between the TAC and TPC was observed, with an r-value of 0.916**.

Ultrasound-assisted extraction (UAE) effect (TAC, TPC, and DPPH). The extraction of bioactive compounds from foods using UAE is influenced by various factors, such as the ultrasound power and frequency, temperature, time, solvent polarity, and solid-liquid ratio (Jeyaraj et al. 2021). Salacheep et al. (2020) reported that the highest extraction efficiency of anthocyanins

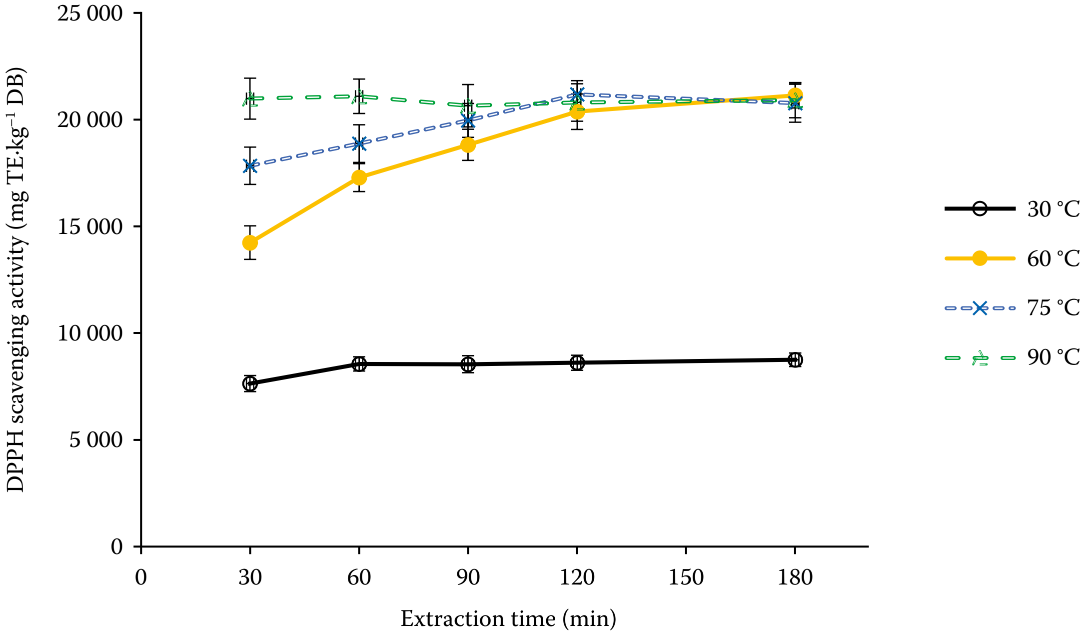

Figure 3. Effect of the extraction time on the radical DPPH scavenging activity of the *Clitoria ternatea* flower extract

DPPH – 2,2-diphenyl-1-picrylhydrazyl; TE – Trolox equivalent; DB – dry weight basis

and polyphenols from CTF powders was achieved by using 60 °C for 60 min and 40 °C for 45 min at 160 W, respectively. Chong and Gwee (2015) reported that the optimal conditions of TAC in CT flowers by UAE were the extraction of 150 min at 50 °C and 240 W.

In Table 2, the UAE significantly affects the phytochemicals and antioxidant activity of the CT extracts. The ultrasound notably enhanced the extractability of the anthocyanins (+66.70%) and polyphenolics (+45.30%) from the unground CT Flowers. Although the CTFP extracted by UAE exhibited a mildly higher TPC than the CTFPE without the ultrasonic treatment, the extract with the UAE had a lower TAC and DPPH scavenging activity than that without the UAE.

Ternatins in CT flowers contain both sugar and organic acid moieties, making them highly soluble in water and relatively stable to heat. The extraction yield of the anthocyanins from the intact CT flowers at 60 °C was less than that at high temperature, and hence using UAE could yield significant effects. For the CT powders, their anthocyanins were easily released from the fine particles into the aqueous solvent. Once released, they might be susceptible to degradation under ultrasound due to the oxidation by air bubbles and free radicals, leading to a decrease in the extracted anthocyanin content. Santos and Martins (2023) reported that the increase in both the extraction temperature and electric power variables resulted in a system with

Table 2. Effect of the ultrasound-assisted extraction on the phytochemicals and antioxidant activity of two *Clitoria ternatea* flowers extracts for 30 min at 60 °C

Extraction	TAC (mg·kg^{-1} DW)	TPC (g GAE·kg^{-1} DW)	Radical DPPH scavenging activity (g TE·kg^{-1} DW)
CTFE	339 ± 19^{d}	11.7 ± 0.4^{d}	14.2 ± 0.8^{c}
CTF-UAE	565 ± 29^{c}	16.9 ± 0.6^{c}	14.4 ± 0.8^{c}
CTFPE	1 075 ± 29^{a}	25.6 ± 0.5^{b}	25.4 ± 1.2^{a}
CTFP-UAE	1 001 ± 25^{b}	26.9 ± 0.6^{a}	22.6 ± 1.0^{b}

$^{a–d}$Means in the same column marked with different letters are significantly different ($P < 0.05$); values are means ± standard deviations ($n = 3$); TAC – total anthocyanin content; TPC – total phenolic content; DPPH – 2,2-diphenyl-1-picrylhydrazyl; DW – dry weight; GAE – gallic acid equivalent; TE – Trolox equivalent; CTFE – *Clitoria ternatea* flower extract; CTF-UAE – *Clitoria ternatea* flower by ultrasound-assisted extraction; CTFPE – *Clitoria ternatea* flower powder extract; CTFP-UAE – *Clitoria ternatea* flower powder by ultrasound-assisted extraction

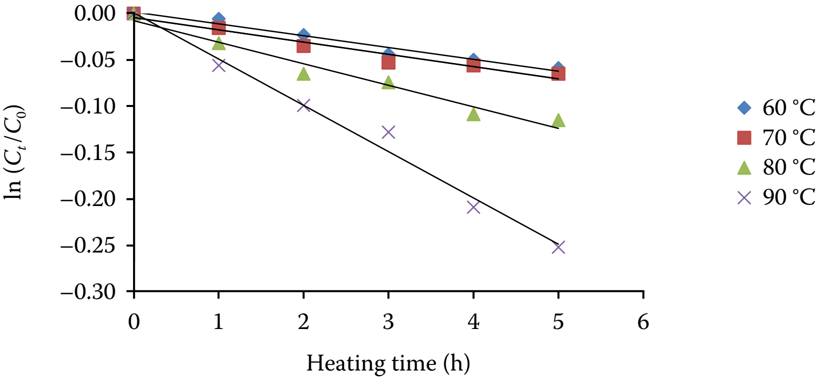

Figure 4. Degradation curves of the *Clitoria ternatea* anthocyanins during various heating temperatures at pH 2.5

C_t – monomeric anthocyanin content after t (h) heating at a given temperature and pH; C_0 – initial monomeric anthocyanin content

higher levels of free radicals and an elevated medium temperature, leading to a synergistic action in the degradation of bioactive compounds.

Thermal stability of the anthocyanins in the *Clitoria ternatea* extracts. The thermal stability of the blue anthocyanin extracts from CT flowers was analysed at various heating temperatures (60–90 °C), heating times (0–5 h), and pH values (2.5–5.5). The results showed that all the thermal degradation data of CTFPE anthocyanin can be fitted well by a first-order reaction kinetic model (Equation 2), with a 0.83–0.99 of R^2 range. The thermal degradation of anthocyanins from the CT extract at pH 2.5 is depicted in Figure 4.

In this study, Figure 5 shows the kinetic parameters for the thermal degradation of anthocyanins in the CTFE at different heating temperatures and pH values. Generally, the degradation rate constant (k-value) of the CT anthocyanins increased with the increase in the heating temperature and pH value, and ranged from 0.0131 h^{-1} to 0.0541 h^{-1} as shown in Figure 5A. However, when the anthocyanins were heated at 90 °C, the k-values at pH 2.5 and 3.5 were higher than those at pH 4.5 and 5.5. This indicated an interaction effect between the pH and the heating temperature. Under the conditions of heating at 90 °C and pH 5.5, the k-value of the CT extract was 0.0461 h^{-1} (Figure 5A). According to Equation 2, after 2 h of heating, its anthocyanin content should be 91.19% of the original. Hence, a mild decrease in the TAC might be reasonable, considering the lengthy extraction time of CT flowers at 90 °C, as depicted in Figure 1.

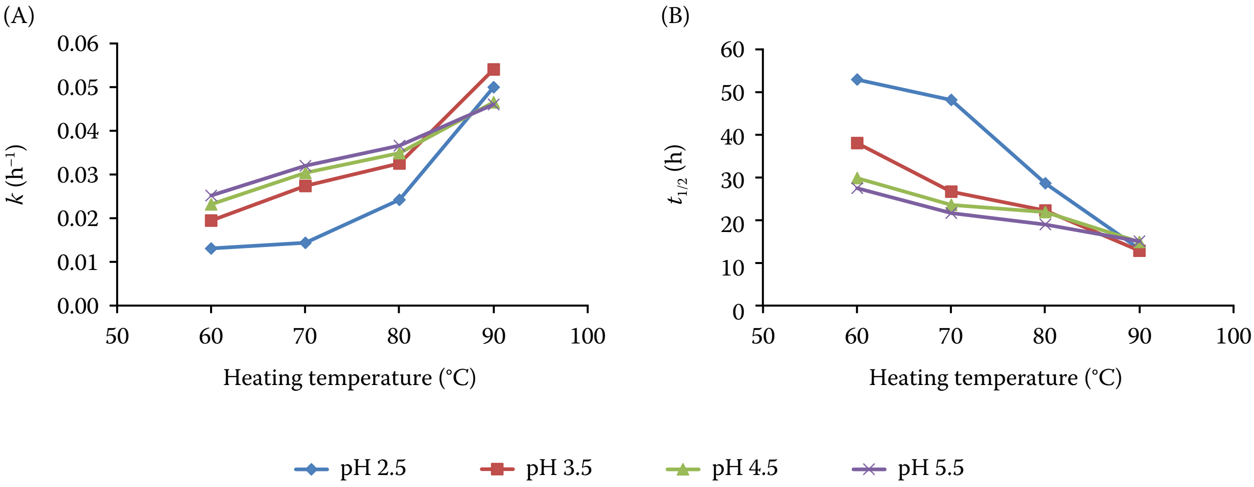

Figure 5. Kinetic parameters for the thermal degradation of the anthocyanins from the *Clitoria ternatea* flowers at different heating temperatures and pH values: (A) rate constant, (B) half-life

k – reaction rate constant; $t_{1/2}$ – half life

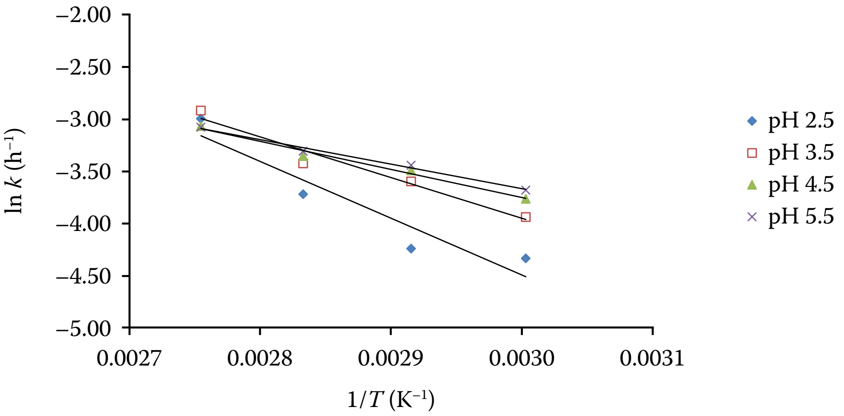

Figure 6. Arrhenius equation of the *Clitoria ternatea* anthocyanins at various pH values

k – reaction rate constant; *T* – absolute temperature

In Figure 5B, the $t_{1/2}$ values were generally reduced by increasing the heating temperature and pH value. Among the tested treatments, the CT anthocyanins at 60 °C and pH 2.5 had the highest $t_{1/2}$ value (52.94 h). Moreover, the $t_{1/2}$ values at 60–80 °C were obviously higher than those at 90 °C (12.87–15.07 h). Although the anthocyanins of the CT flower at pH 3.6–5.4 exhibited stable properties at 60 °C and 70 °C, while the rate constant of thermal degradation significantly increased above 70 °C (Marpaung et al. 2017). In strong acidic conditions, anthocyanin does exist as a stable flavylium cation (Enaru et al. 2021). Oancea and Draghici (2013) reported that the degradation rate constant of anthocyanins from red onions increased almost 17 times as the pH value was increased from 1.0 to 9.0 during storage at room temperature.

The Arrhenius model, Equation 4, is a classic empirical equation used for chemical reactions and can be used to describe the temperature dependence of the degradation reaction rate of anthocyanins.

$$k = k_0 e^{-\frac{Ea}{RT}} \tag{4}$$

where: k – reaction rate constant; k_0 – pre-exponential factor; Ea – activation energy (J·mol^{-1}); R – gas constant (R = 8.314 J·mol^{-1}·K^{-1}); T – absolute temperature (K).

Similar to previous studies (Danisman et al. 2015), Figure 6 demonstrates that temperature-dependent rate constants obey the Arrhenius relationship (R^2 = 0.90–0.99). The activation energies of the degradation reaction for anthocyanins at pH 2.5, 3.5, 4.5, and 5.5 were 45.23, 32.43, 22.36, and 19.52 kJ·mol^{-1}, respectively. Notably, at pH 2.5, the highest activation energy was observed, indicating that the degradation reaction of anthocyanins at this pH was more sensitive to temperature changes compared to higher pH values. Marpaung et al. (2017) reported that the activation energy for the thermal degradation of anthocyanins in a CT extract at pH 7 ranged from 83.21 kJ·mol^{-1} to 101.15 kJ·mol^{-1}, which was dependent on the headspace volume in bottles. The activation energies of roselle extracts (pH 2.23–2.55), blackberry (pH 2.68), blood orange (pH 3.53), and grape juice (pH 3.34) were 47–61, 37.23, 66.04, and 64.89 kJ·mol^{-1}, respectively (Cisse et al. 2009; Danisman et al. 2015).

CONCLUSION

CT flowers are a potential source of blue natural colours and rich bioactive compounds. Different sample types significantly affect the extraction of phytochemicals and antioxidant properties from CT flowers. Under different extraction temperatures and durations, the TAC, TPC, and DPPH radical scavenging activity of CTFE were all lower than those of CTFPE. Due to the intact tissues and slow mass transfer of the CTF, the extraction temperature is an important influencing factor, often requiring longer extraction times. The optimal extraction conditions for both anthocyanins and polyphenols from CTF and CTFP were at 90 °C in hot water for 90 min and 30 min, respectively. Additionally, the optimal conditions for the radical DPPH scavenging activities from both forms of CT flowers were 90 °C for 30 min. Both the TAC and TPC of CTFE extracted in optimal conditions were obviously higher than those of the control extract (60% ethanol). However, for the powder type samples, the TAC and TPC values of the CTFPE were approximately 87% of the control.

Hence, substituting water for alcohol organic solvents should be feasible, especially when using the CTF sample. Using ultrasound for anthocyanin extraction from CTF compared to CTFP was obviously effective.

The thermal degradation of the anthocyanins from the CT extract fitted a first-order reaction model. Generally, the rate constants (*k*) of the thermal degradation for anthocyanins increased with the increase in heating temperature and pH value. The Arrhenius equation could be used to describe the relationship between the rate constant of the thermal degradation and the temperature. The activation energy of the CT anthocyanins ranged from 19–45 $\text{kJ}\cdot\text{mol}^{-1}$ and decreased by increasing the pH value of the extract. Hence, the blue aqueous extract of CT flowers, which contains high levels of phytochemicals and exhibits good antioxidant activity, has the potential to be utilised in food products and nutraceuticals having neutral and acidic pH values.

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Received: April 10, 2024
Accepted: July 25, 2024