ScienceDirect

Case Studies in Chemical and Environmental Engineering

Volume 10, December 2024, 100860

Case Report

Extraction, concentration, and storage of butterfly pea anthocyanin for commercialization

Okta Bani, Taslim, Iriany, Mikael Sinaga, Sherina Violleta

Show more

https://doi.org/10.1016/j.cscee.2024.100860

Get rights and content

Abstract

An attempt has been made to conserve the nutrition of butterfly pea by modifying the production process. In this paper, butterfly pea syrup was prepared through ultrasound assisted extraction (UAE) of butterfly pea flower using water at ultrasound frequency of 40kHz and initial temperature of 50°C for 30 min. Extraction was modified by addition of sugar and citric acid. Before separation of extract, butterfly pea mixture was adjusted to contain 181,25 g sugar and 2.875 g citric acid per L water. The extract was then concentrated by vacuum evaporation at 50°C for 2–6 h. The concentrated extracts were stored at 6°C and 30°C for 4 weeks. Each experiment was run triplicate and the effect of each treatment on the anthocyanin concentration was measured using UV–Vis Spectrophotometer. Results indicated that presence of citric acid and sugar during extraction affect extraction and shelf life of butterfly pea extract. The presence of citric acid and sugar in anthocyanin extracts also prolongs their shelf life.

Keywords

Anthocyanin; Butterfly pea; Ultrasound; Kinetics; Extraction; Evaporation

1. Introduction

Butterfly pea (*Clitoria ternatea*) is a perennial herbaceous plant which is easy to cultivate and capable of increasing soil fertility [1]. Commercially, butterfly peas have been sold dried, powdered, concentrated, and encapsulated powdered. The extract has many health benefits, easy to process, and stable when kept at low temperature without preservatives [[1], [2], [3], [4], [5], [6]]. Among various extraction methods and solvents, a promising one is water extraction at 50–60°C by UAE [7,8]. In one study, UAE was found to be capable of producing 246.46% higher anthocyanin yield compared to conventional extraction. The same study also concluded that the optimal condition for UAE by response surface method was at 50°C for 150 min extraction time and ratio of liquid-to-solid of 15 mL/g [9]. Further study on UAE of butterfly pea under the previous optimal condition revealed that UAE also increased the extracted total phenolics, flavanoids, and antioxidant activities compared to conventional extraction. Furthermore, UAE did not appear to affect the functional group of bioactive compounds structure [10]. Another optimization study found that optimal UAE could be achieved at 40°C for 40 min extraction time and ratio of 10 mL distilled water/mg butterfly pea [11]. While varying optimal conditions were implied under different studies, many suggested that the extraction should be carried out at moderate temperatures.

Aside from the extract, butterfly pea is also consumed as tea and processed into syrup. Butterfly pea syrup (BPS) is produced traditionally by boiling butterfly pea flowers with water and sugar till the syrup forms. This method is believed to be ineffective in preserving the nutritional value of butterfly pea due to many of its heat sensitive components decaying during the boiling. A milder method could produce BPS with better nutritional value. Therefore, this paper aimed to assess the feasibility of BPS creation by UAE and vacuum evaporation at lower temperature and evaluate the stability of its anthocyanin content.

2. Methodology

2.1. Butterfly pea preparation

Butterfly pea flower was washed with water, left to dry in refrigerator for one day, then stored in a closed glass container at 6°C. About 80–200 g (12 g dry base) flowers were mixed with 800 mL 50°C water then blended using a commercial blender (Phillips HR2056/03, 280 Watt) for 30 s to produce butterfly pea slurry. Afterwards, the slurry was added with: nothing, 145 g sugar, 2.3 g citric acid, or 145 g sugar and 2.3 g citric acid.

2.2. Butterfly pea extraction

The butterfly pea slurry was extracted in ultrasonic bath at 40kHz and initial temperature of 50°C for 30 min. After extraction, slurry composition was adjusted to contain 145 g sugar and 2.3 g citric acid (per 800 mL). The extract was separated using a commercial cloth filter.

2.3. Extract concentration

Butterfly pea extract (BPE) was split into 4 batches, each containing 200 mL filtrate. Each batch was condensed by using rotary vacuum evaporator at 50°C for 0, 2, 4, or 6 h. The condensed BPE was analyzed and stored in dark glass bottle, half in a fridge (6°C) and half at room temperature (30°C). Each experiment was run triplicate, and BPE was analyzed for its anthocyanin content every week for 4 weeks.

2.4. Anthocyanin content analysis

Anthocyanin content analysis follows the procedure by Ref. [12]. First, dilution factor was determined by diluting sample in KCl buffer (pH 1.0) till absorbance at λ_{max} is within linear reading range of spectrophotometer. Sample must be less than 20% of total volume. Spectrophotometer was zeroed by using distilled water at λ_{max} and 700 nm. Two samples were prepared based on the dilution factor, one with KCl buffer (pH 1.0) and the other with natrium acetate buffer (pH 4.5). These dilutions were left to equilibrate for 15 min. Absorbance of each solution was measured at λ_{max} and 700 nm against a blank cell filled with distilled water. The monomeric anthocyanin pigment concentration was calculated by using the following formula:

$$C\left(\frac{mg}{L}\right) = \frac{\left[(\lambda_{max}-\lambda_{700})_{pH\ 1,0}-(\lambda_{max}-\lambda_{700})_{pH\ 4,5}\right]\times MW\times DF\times 1000}{\varepsilon\times 1}$$

MW is molecular weight of predominant anthocyanin in sample, DF is dilution factor, ε is the molar absorptivity of the anthocyanin. The formula is based on a pathlength of 1 cm, which is equal to the cuvette width. To compare between literature, anthocyanin concentration in this paper is expressed as *cyanidin-3-glucoside* (MW=449.2 and ε=26,900).

2.5. Water content analysis

Petri dish was heated at 105 °C for 3 h, then placed in desiccator till cooled down. The dish was weighted (w_1) then ±3 g sample was added, then reweighted (w_2). The sample was dried in oven at 105 °C for 3 h, then placed in desiccator till cooled and weighted (w_3). Water content was calculated by formula:

$$\frac{w_2 - w_3}{w_2 - w_1} \times 100$$

2.6. Anthocyanin yield calculation

Anthocyanin yield of the butterfly pea is calculated as follow:

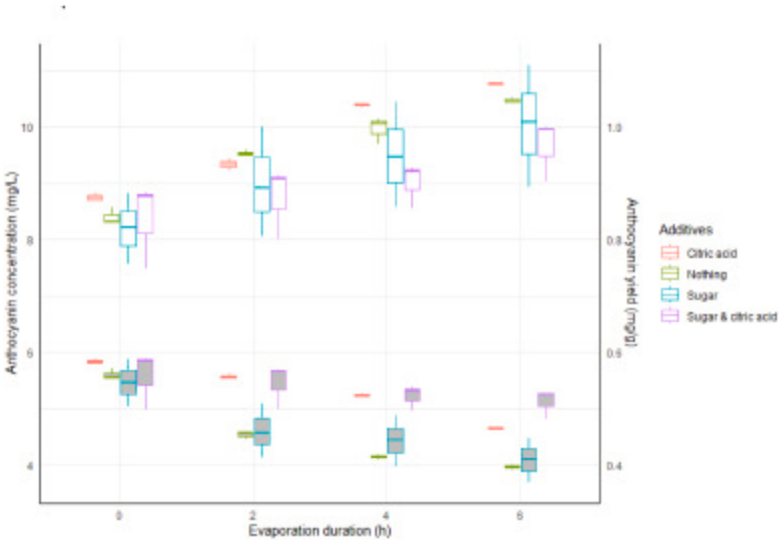
$$\textbf{Yield}\ \left(\frac{\text{mg}}{\text{g}}\right) = \frac{C \times V_c}{w_{bf}}$$

C is anthocyanin concentration in mg/L, V_c is concentrate volume after vacuum evaporation in L, and w_{bf} is the estimated mass of dry butterfly pea flower in 200 mL filtrate (3 g).

3. Results and discussion

3.1. Effect of sugar and citric acid on anthocyanin yield

Initial water content analysis revealed that fresh butterfly pea contains 84–90% water. Due to the possibility of interference of sugar and/or citric acid in anthocyanin analysis using spectrophotometer UV–Vis, samples of BPE was first prepared by UAE with water at 40kHz and initial temperature of 50 °C for 30 min. BPE was split into three samples: one as pure extract, one added with sugar to concentration of 181.25 g/L, and one added with sugar to concentration of 181.25 g/L and citric acid to concentration of 2.875 g/L. All samples were analyzed for its λ_{max}. It was found that addition of sugar and/or citric acid did not change λ_{max} value (all λ_{max}=549 nm) although the absorbance reading decreased. The value of λ_{max} agree well with literature [3,13]. The decrease is within acceptable range and indicates that all samples must be adjusted to contain similar sugar and citric acid concentration to avoid bias during measurement of anthocyanin concentration. The effect of additives during extraction and treatment of evaporation on anthocyanin concentration and yield is in Fig. 1, while the ANOVA on anthocyanin yield is in Table 1.



Download: Download high-res image (219KB)

Download: Download full-size image

Fig. 1. The effect of sugar and citric acid on anthocyanin yield.

Table 1. ANOVA for the effect of additives during extraction, duration of evaporation, and the interaction on anthocyanin yield.

Source	Sum of Squares	Degree of freedom	Mean of Squares	*F*	p-value
All	0.1967	47			
Additives	0.0638	3	0.0213	25.5841	1.2400E-08
$t_{evaporation}$	0.0881	3	0.0294	35.3423	2.8445E-10
Interaction	0.0182	9	0.0020	2.4343	3.0922E-02
Error	0.0266	32	0.0008		

In Fig. 1, anthocyanin concentration (white fill) increases with longer evaporation. This increase is expected as water evaporates, and anthocyanin accumulates. However, the treatment of evaporation consistently reduced anthocyanin yield as in Fig. 1 (gray fill) and further confirmed in the ANOVA result in Table 1. This phenomenon is because thermal treatment during evaporation promotes anthocyanin degradation [3,5,14,15].

In Table 1, the significance of interaction implies that there are distinct trends in yield reductions among each extraction treatment. Without evaporation (0 h evaporation duration), anthocyanin concentrations and yields of BPE extracted using various additives

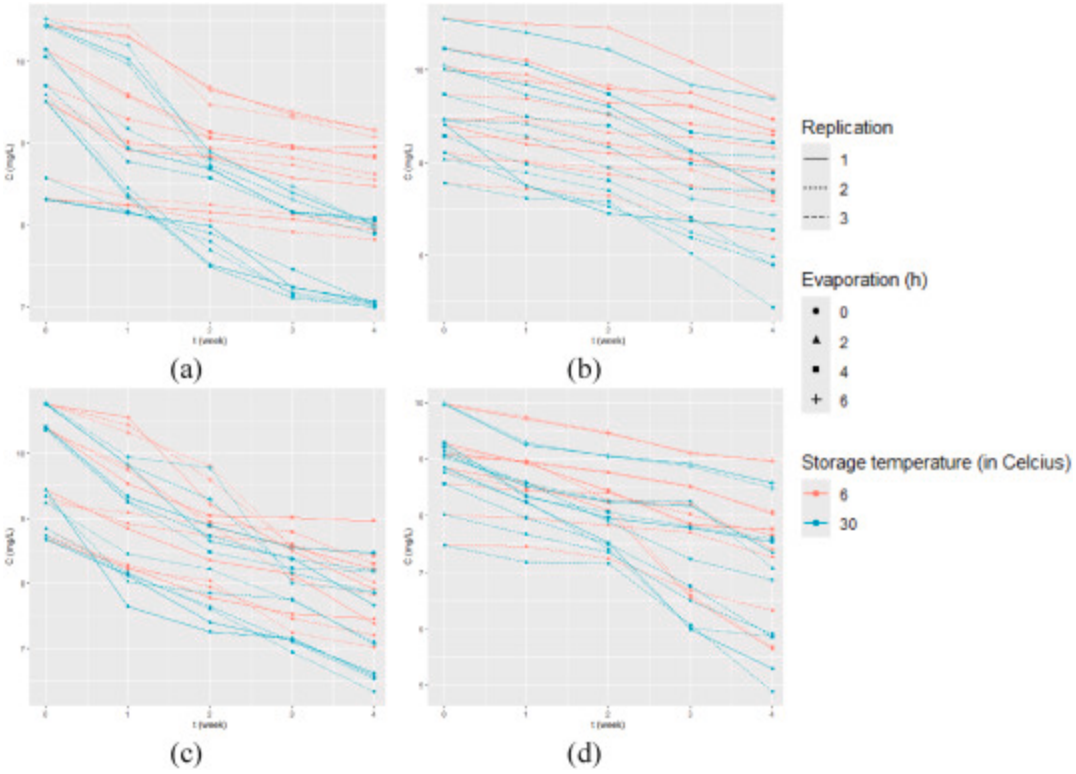
have only deviated slightly from each other with presence of citric acid producing slightly higher anthocyanin yield. This effect is likely due to the pH alteration, in which anthocyanins in butterfly pea shift to flavylium cation form which is very soluble in water [16]. Interestingly, BPEs with addition of citric acid during extraction seems to impede anthocyanin reduction during evaporation despite all sample being equalized at the end of extraction as observed in Fig. 1. A possible reason for this phenomenon is that presence of citric acid may assist or hinder the extraction of certain extractives in butterfly pea flower which may stabilize or destabilize anthocyanin. However, a more thorough study is required to verify this verdict. In this study, the anthocyanin extraction yield is 0.396–0.583 mg/g. The yield is lower than most literature but in similar order of magnitude [9,11,17,18]. The difference is likely due to differences in extraction conditions such as solvent to dry mass ratio.

3.2. Anthocyanin degradation of BPE

Anthocyanin degradation of BPE was studied across various factors: presence of additives during extraction, duration of evaporation, and storage temperature. The changes in anthocyanin concentration of BPE are in Fig. 2. The degradation was modelled using zero, and first order kinetics expressed in Equations (1), (2)) as they are most common models for anthocyanin degradation.

$$C_0 - C = kt \tag{1}$$

$$\ln C_{A0} - \ln C_A = kt \tag{2}$$



Download: Download high-res image (926KB)

Download: Download full-size image

Fig. 2. Changes in anthocyanin concentration of BPE extracted using: (a) water, (b) sugar solution, (c) citric acid solution, and (d) mix of sugar and citric acid solution.

The degradation constants (k) for each kinetic model for each factor were estimated by linear regression using R software. The summary of R^2 values for each kinetic model is in Table 2. Based on the R^2 values, zero order model provides the best fit for the data. The model fits better for more data sets (having higher R^2) and has higher consistency (lower variance). This finding agrees with [14], although many literatures found first order kinetics more appropriate [3,19,20]. The different fit in kinetic models is likely due to presence of sugar and citric acid during the storage of BPE, whence both substances were found to affect stability of anthocyanins [5,15].

Table 2. Summary of R^2 values for each kinetic model.

Model order	**Count of best fits**	***R* [2]**			
		Average	**Variance**	**Min**	**Max**
0	54	0.96727	0.0008	0.8393	0.9998

Model order	Count of best fits	R^2			
		Average	Variance	Min	Max
1	42	0.96586	0.0009	0.8307	0.9990

The estimated k values of both models were compared between each factor. The values were analyzed using ANOVA and the results are in Table 3. ANOVA results suggest that only presence of additives and storage temperature have significant effect on the degradation kinetics for both models, while evaporation duration has negligible effect on degradation. The effect of storage temperature on anthocyanin degradation has been widely confirmed with higher temperatures generally correspond to faster degradation [3,5,14,16,20], although an anomalous behavior was also observed [20]. For evaporation, while the process itself reduces anthocyanin yield, there is no reason for the treatment to have lasting effect which could alter anthocyanin degradation.

Table 3. ANOVA for k values across various factors.

Zero order model					
Source	**Sum of Squares**	**Degree of freedom**	**Mean of Squares**	***F***	**p-value**
All	3.3749	95			
Additives	0.4655	3	0.1552	9.0928	2.63E-05
$t_{evaporation}$	0.0834	3	0.0278	1.6295	0.188291
$T_{storage}$	1.3245	1	1.3245	77.6208	1.01E-13
Error	1.5016	88	0.0171		
First order model					
Source	**Sum of Squares**	**Degree of freedom**	**Mean of Squares**	***F***	**p-value**
All	0.0540	95			
Additives	0.0049	3	0.0016	5.6728	0.001341
$t_{evaporation}$	0.0012	3	0.0004	1.3782	0.254713
$T_{storage}$	0.0224	1	0.0224	77.3187	1.09E-13
Error	0.0255	88	0.0003		

Values of *k* for each model are averaged across insignificant factors and tabulated in Table 4. In Table 4, degradation constants for BPE extracted using water and sugar solution share a close value for each model and storage temperature. This suggests that addition of sugar during extraction has minimal effect on the process itself, and the resulting BPE closely resembles each other. However, addition of citric acid during extraction leads to faster degradation of BPE for each model and storage temperature, with addition of sugar to the citric acid solution seems to slightly decrease the degradation rate. Since samples were equalized at the end of extraction, this suggests that citric acid and sugar are not the main source of this accelerated degradation. This result contradicts the positive effect of citric acid during initial treatments, in which samples extracted with addition of citric acid preserved greater yield than those without. It is also notable that anthocyanin degradation for BPE aided with citric acid had a higher increase in degradation rate at 6°C than at 30°C as evidenced by the leap in the *k* values. Another point worth mentioning is the combined effect of sugar and citric acid on extraction, which decreases anthocyanin yield and anthocyanin degradation. Based on these two observations, it is probable that additional extractives from extraction aided by citric acid produce fast decaying intermediates which may act as catalyst for anthocyanin degradation. However, there is still no report on this remark and the investigation of this phenomena is beyond the scope of this study. The *k* values obtained in this study are also lower than other literatures. For comparison, the *k* values for first order model from various literatures were 0.0136/d (0.095/w) at 4°C [3], 0.0247/d (0.173/w) at 25°C [3] 0.0248/d (0.174/w) at 27°C [19], 0.0012/min (12/w) at 28°C [14], and 0.06/d (0.420/w) at 30°C [20]. For zero order model, the *k* value was 0.1219 mg/L·min (1228 mg/L·w) at 28°C [14].

Table 4. Anthocyanin degradation constants.

Additives	$T_{storage}$ **(°C)**			
	6		**30**	
	0^{th} order (mg/L·w)	**1st order (w^{-1})**	**0^{th} order (mg/L·w)**	**1st order (w^{-1})**
W	0.2681±0.0103	0.0287±0.0001	0.5758±0.0272	0.0663±0.0003
S	0.2563±0.0056	0.0294±0.0001	0.5331±0.0075	0.0666±0.0003
C	0.4929±0.0177	0.0547±0.0002	0.6573±0.0117	0.0756±0.0001
A	0.3435±0.0332	0.0426±0.0007	0.5343±0.0233	0.0690±0.0006

4. Conclusions

This work confirms the high importance of environmental sciences in different fields as shown in a lot of papers published before [[21], [22], [23]]. Anthocyanin from butterfly peas were successfully extracted using ultrasound assisted extraction. Among the extraction additives tested in this study, citric acid was found to increase extraction yield and preserve anthocyanin during evaporation. Sugar alone did not affect extraction and evaporation, however when mixed with citric acid, it decreased the efficacy of citric acid. Kinetics study on anthocyanin degradation revealed that modification on extraction of anthocyanin could affect its degradation, however the mechanism by which this process affected degradation is still unclear. Addition of sugar and citric acid to BPE could also prolong the shelf life of anthocyanin. It is suggested that preparation of BPS using UAE should be administered with water alone, followed by addition of sugar and citric acid afterwards. Further study should also be proceeded to affirm the effect of sugar and citric acid on BPE yield during vacuum evaporation.

CRediT authorship contribution statement

Okta Bani: Writing – review & editing, Resources, Funding acquisition, Formal analysis, Conceptualization. **Taslim:** Supervision, Investigation, Conceptualization. **Iriany:** Software, Formal analysis, Conceptualization. **Mikael Sinaga:** Visualization, Methodology, Data curation. **Sherina Violleta:** Visualization, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors would like to thank Lembaga Penelitian Universitas Sumatera Utara (LP USU) for the research funding under TALENTA 2022 grant No 240/UN5.2.3.1/PPM/KP-TALENTA/2022.

Data availability

Data will be made available on request.

References

[1] G.K. Oguis, E.K. Gilding, M.A. Jackson, D.J. Craik
Butterfly pea (clitoria ternatea), a cyclotide-bearing plant with applications in agriculture and medicine
Front. Plant Sci., 10 (2019)
Google Scholar ↗

[2] S.A.T. Lakshan, N.Y. Jayanath, W.P.K. Mendis Abeysekera, W.K.S.M. Abeysekera
A commercial potential blue pea (*Clitoria ternatea* L.) flower extract incorporated beverage having functional properties
Evidence-Based Complementary and Alternative Medicine 2019, 1–13 (2019)
Google Scholar ↗

[3] X. Fu, *et al.*
Spectral characteristic, storage stability and antioxidant properties of anthocyanin extracts from flowers of butterfly pea (clitoria ternatea L.)
Molecules, 26 (2021), p. 7000
Crossref ↗ View in Scopus ↗ Google Scholar ↗

[4] T. Thilavech, *et al.*
Clitoria ternatea flower extract attenuates postprandial lipemia and increases plasma antioxidant status responses to a high-fat meal challenge in overweight and obese participants
Biology, 10 (2021), p. 975
Crossref ↗ View in Scopus ↗ Google Scholar ↗

[5] G.C. Vidana Gamage, Y.Y. Lim, W.S. Choo
Anthocyanins from clitoria ternatea flower: biosynthesis, extraction, stability, antioxidant activity, and applications
Front. Plant Sci., 12 (2021)
Google Scholar ↗

[6] R. Mattioli, A. Francioso, L. Mosca, P. Silva
Anthocyanins: a comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases
Molecules, 25 (2020), 10.3390/molecules25173809 ↗
Preprint at
Google Scholar ↗

[7] A.Q. Syafa'atullah, A. Amira, S. Hidayati, M. Mahfud
Anthocyanin from butterfly pea flowers (Clitoria ternatea) by ultrasonic-assisted extraction
AIP Conference Proceedings Vol. 2237 020069, American Institute of Physics Inc. (2020)
Google Scholar ↗

[8] E.J. Jeyaraj, Y.Y. Lim, W.S. Choo
Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals
J. Food Sci. Technol., 58 (2021), pp. 2054-2067
Crossref ↗ View in Scopus ↗ Google Scholar ↗

[9] F.C. Chong, X.F. Gwee
Ultrasonic extraction of anthocyanin from *Clitoria ternatea* flowers using response surface methodology
Nat. Prod. Res., 29 (2015), pp. 1485-1487
View article Crossref ↗ View in Scopus ↗ Google Scholar ↗

[10] A. Mehmood, *et al.*
Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (Clitoria ternatea L.)
Ultrason. Sonochem., 51 (2019), pp. 12-19
View PDF View article Google Scholar ↗

[11] S. Salacheep, *et al.*
Optimization of ultrasound-assisted extraction of anthocyanins and bioactive compounds from butterfly pea petals using Taguchi method and Grey relational analysis
J. Food Sci. Technol., 57 (2020), pp. 3720-3730
Crossref ↗ View in Scopus ↗ Google Scholar ↗

[12] Anthocyanins
Handbook of Food Analytical Chemistry, John Wiley & Sons, Inc., Hoboken, NJ, USA (2005), pp. 5-69, 10.1002/0471709085.ch18 ↗
Google Scholar ↗

[13] S. Saha, *et al.*

Anthocyanin profiling using UV-vis spectroscopy and liquid chromatography mass spectrometry

J. AOAC Int., 103 (2021), pp. 23-39, 10.5740/jaoacint.19-0201 ↗

Preprint at

View in Scopus ↗ Google Scholar ↗

[14] P. Charurungsipong, C. Tangduangdee, S. Amornraksa, S. Asavasanti, J. Lin

Improvement of anthocyanin stability in butterfly pea flower extract by Co-pigmentation with catechin

E3S Web of Conferences, vol. 141, EDP Sciences (2020)

Google Scholar ↗

[15] B.-S. Chu, J.D. Wilkin, M. House, M. Roleska, M.A. Lemos

Effect of sucrose on thermal and PH stability of clitoria ternatea extract

International Journal of Food Processing Technology, 3 (2016)

Google Scholar ↗

[16] B. Enaru, G. Drețcanu, T.D. Pop, A. Stănilă, Z. Diaconeasa

Anthocyanins: factors affecting their stability and degradation

Antioxidants, 10 (2021), 10.3390/antiox10121967 ↗

Preprint at

Google Scholar ↗

[17] H.W. Chen, Y.W. Chang

Encapsulation of Clitoria ternatea extract in liposomes by synergistic combination of probe-type ultrasonication and high-pressure processing

J. Food Saf., 40 (2020)

Google Scholar ↗

[18] E.J. Jeyaraj, Y.Y. Lim, W.S. Choo

Effect of organic solvents and water extraction on the phytochemical profile and antioxidant activity of clitoria ternatea flowers

ACS Food Science and Technology, 1 (2021), pp. 1567-1577

Crossref ↗ View in Scopus ↗ Google Scholar ↗

[19] A.M. Marpaung, M. Lee, I.S. Kartawiria

The development of butterfly pea (clitoria ternatea) flower powder drink by Co-crystallization

Indonesian Food Science & Technology Journal, 3 (2020), pp. 34-37

Crossref ↗ View in Scopus ↗ Google Scholar ↗

[20] A.M. Marpaung, N. Andarwulan, P. Hariyadi, D.N. Faridah
Thermal degradation of anthocyanins in butterfly pea (clitoria ternatea L.) flower extract at pH 7
Adv. J. Food Sci. Technol., 5 (2017), pp. 199-203
Google Scholar ↗

[21] A.S.N. Al-Kamali, A.A. Al-Hazmi, M.H.M. Alhousami, M.A. Al-Masany
Synthesis and antibacterial activity of some novel thieno[2,3-c]pyridazines using 3-amino-5-phenyl-2-ethoxycarbonylthieno[2,3-c]pyridazine as a starting material
Arab. J. Chem., 7 (2014), pp. 775-780
View PDF View article View in Scopus ↗ Google Scholar ↗

[22] Y. El Bakri, *et al.*
1,4,9,9-tetramethyloctahydro-4,7-(epoxymethano)azulen-5(1H)-one, a natural product as a potential inhibitor of COVID-19: extraction, crystal structure, and virtual screening approach
J. King Saud Univ. Sci., 35 (2023)
Google Scholar ↗

[23] M.M. Sebaiy, *et al.*
Review: instrumental analytical techniques for evaluating some anti-infective drugs in pharmaceutical products and biological fluids
Current Chemistry Letters, 13 (2024), pp. 491-502
Crossref ↗ View in Scopus ↗ Google Scholar ↗

Cited by (0)