



**OPTIMIZATION, KINETICS, THERMODYNAMICS AND STABILITY OF
ULTRASOUND-ASSISTED AND HIGH-PRESSURE CARBON DIOXIDE
EXTRACTION OF ANTHOCYANINS FROM MANGOSTEEN (*Garcinia
mangostana* Linn.) PERICARP**

By

TANG BO

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

June 2023

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

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June 2023

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Faculty : Food Science and Technology

Mangosteen is a genus of *Garcinia* trees widely grown in Southeast Asia, tropical Africa and South American countries. In the food industry, the mangosteen fruit is often processed into juice, jam and frozen mangosteen's pulp. However, the pericarp, which accounts for about two-thirds of the whole fruit weight, is often discarded as agricultural waste. This may not only cause environmental pollution but also seriously affect the added value of the mangosteen. Considering the rich anthocyanin content in mangosteen pericarp and the multiple health benefits of anthocyanins such as antioxidant, anti-inflammatory and vision protection, the extraction of anthocyanins from mangosteen pericarp may be an effective strategy to fully utilized mangosteen pericarp. To date, reports on the extraction of anthocyanins from mangosteen pericarp are still very limited. The main objective of this study is to develop an efficient method for the extraction of anthocyanins from mangosteen pericarp and to conduct a preliminary investigation of its mechanism. First, the differences between conventional solvent extraction (CSE), ultrasound-assisted extraction (UAE) and high-pressure carbon dioxide extraction (HPCDE) in anthocyanin extraction were compared by analyzing the extraction yield, parameters, kinetic and thermodynamic behaviors. Subsequently, response surface methodology (RSM) was applied to optimize the four main parameters in UAE to further increase anthocyanin yield. Finally, the effects of heating and ultrasound treatment on the stability of the mangosteen anthocyanin extract were also investigated. Single-factor experiments showed that amplitude, pressure, temperature and liquid-solid ratio significantly affected the anthocyanin yield and produced the maximum anthocyanin yield at 100%, 25 MPa, 80 °C and 60 mL/g, respectively. In the kinetic analysis, both the second-order kinetic model ($R^2 > 0.99$) and

Peleg's model ($R^2 > 0.95$) were applicable to describe the extraction behavior of anthocyanins. Based on the second-order kinetic model, UAE showed the highest equilibrium concentration with 81.15 mg/100 g, while HPCDE showed the highest rate constant of 0.0149 100 g/mg·min, which was nearly two times higher than that of UAE. Thermodynamic analysis showed that enthalpy changes and entropy changes were positive under all extraction methods, and the Gibbs free energy changes were negative, suggesting that the extraction process was endothermic, irreversible and spontaneous. In addition, the highest anthocyanin yield of 92.52 mg/100 g was obtained under the optimal UAE conditions: amplitude 100%, temperature 80 °C, liquid-solid ratio 60 mL/g for 25 min. There was no significant difference between this result and the predicted value of RSM. In the stability study, heating and ultrasound treatments both accelerated the degradation of anthocyanins, the formation of polymeric colors, the degradation of polyphenols, and the reduction of antioxidant capacity. The concentration of anthocyanin decreased by 6.8%, polymeric color index increased by 24.6%, polyphenol concentration decreased by 9.0%, and DPPH radical scavenging capacity decreased by 17.3% after 30 min of ultrasound treatment at 70 °C. Overall, UAE is a more recommended method for anthocyanin extraction from mangosteen pericarp. Although the high temperature and high amplitude in UAE may lead to degradation of anthocyanins, this amount of degradation is acceptable compared to the increased in yield. The findings of this study will be served as a useful guideline for the application of UAE and HPCDE in mangosteen anthocyanin extraction and help to increase the value of mangosteen.

Key words: anthocyanin, mangosteen, ultrasound-assisted extraction, high-pressure carbon dioxide extraction

SDG: GOAL 3: Good Health and Well-being, GOAL 9: Industry, Innovation and Infrastructure, GOAL 12: Responsible Consumption and Production

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**OPTIMASI, KINETIK, TERMODINAMIK DAN KESTABILAN
PENGEKSTRAKAN ANTOSIANIN DARI KULIT MANGGIS (*Garcinia
mangostana* Linn.) DENGAN BANTUAN ULTRASOUND DAN TEKANAN
TINGGI KARBON DIOKSIDA**

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Manggis ialah genus pokok *Garcinia* yang ditanam secara meluas di Asia Tenggara, Afrika dan negara-negara Amerika Selatan. Di dalam industri makanan, buah manggis sering diproses menjadi jus, jem dan isi manggis beku. Walau bagaimanapun, kulit manggis yang menyumbang kira-kira dua pertiga daripada berat keseluruhan buah, sering dibuang sebagai sisa pertanian. Ini bukan sahaja boleh menyebabkan pencemaran alam sekitar tetapi juga menjejaskan nilai keseluruhan dan status manggis secara serius. Memandangkan kandungan antosianin yang kaya di dalam kulit manggis yang memberi pelbagai manfaat seperti antioksidan, anti-radang dan perlindungan penglihatan, pengekstrakan antosianin daripada kulit manggis adalah merupakan strategi yang baik untuk memanfaatkan sepenuhnya penggunaan kulit manggis. Sehingga kini, laporan mengenai pengekstrakan antosianin daripada manggis adalah sangat terhad. Objektif utama kajian ini adalah untuk membangunkan kaedah yang cekap untuk pengekstrakan antosianin daripada manggis dan untuk menjalankan penyiasatan awal mekanismenya. Pertama, perbezaan antara pengekstrakan pelarut konvensional (CSE), pengekstrakan yang dibantu ultrabunyi (UAE) dan pengekstrakan menggunakan karbon dioksida pada tekanan tinggi (HPCDE) dalam pengekstrakan antosianin akan dibandingkan dengan menganalisis hasil pengekstrakan, parameter, kinetik dan termodinamik. Selepas itu, metodologi permukaan tindak balas (RSM) digunakan untuk mengoptimumkan empat parameter utama di UAE untuk meningkatkan lagi hasil antosianin. Akhir sekali, kesan pemanasan dan rawatan ultrabunyi terhadap kestabilan ekstrak antosianin daripada manggis turut disiasat. Eksperimen faktor tunggal menunjukkan bahawa amplitud, tekanan, suhu dan nisbah cecair-pepejal memberi kesan ketara kepada hasil antosianin dan menghasilkan hasil antosianin maksimum masing-masing pada 100%, 25 MPa, 80 °C dan 60 mL/g. Dalam analisis kinetik, kedua-dua model kinetik tertib

kedua ($R^2 > 0.99$) dan model Peleg ($R^2 > 0.95$) boleh digunakan untuk menerangkan proses pengekstrakan antosianin. Berdasarkan model kinetik tertib kedua, UAE menunjukkan kandungan keseimbangan yang tertinggi dengan 81.15 mg/100 g, manakala HPCDE menunjukkan pemalar kadar yang tertinggi iaitu pada 0.0149 100 g/mg·min, iaitu hampir dua kali lebih tinggi daripada UAE. Analisis termodinamik menunjukkan bahawa perubahan entalpi dan perubahan entropi adalah positif di bawah semua kaedah pengekstrakan, dan perubahan tenaga bebas Gibbs adalah negatif, menunjukkan bahawa proses pengekstrakan adalah endotermik, tidak boleh dimundurkan dan spontan. Di samping itu, hasil antosianin tertinggi adalah sebanyak 92.52mg/100 g yang diperolehi di bawah keadaan optimum UAE: amplitud 100%, suhu 80 °C, nisbah cecair-pepejal 60 mL/g selama 25 minit. Tiada perbezaan yang signifikan antara bacaan ini dan nilai ramalan RSM. Dalam kajian kestabilan, pemanasan dan rawatan ultrabunyi kedua-duanya mempercepatkan degradasi antosianin, pembentukan warna polimer, degradasi polifenol, dan pengurangan kapasiti antioksidan. Kepekatan antosianin berkurangan sebanyak 6.8%, indeks warna polimer meningkat sebanyak 24.6%, kepekatan polifenol menurun sebanyak 9.0%, dan kapasiti penghapusan radikal DPPH menurun sebanyak 17.3% selepas 30 minit rawatan ultrabunyi pada 70 °C. Secara keseluruhan, UAE adalah kaedah yang lebih disyorkan untuk pengekstrakan antosianin daripada kulit manggis. Walaupun suhu dan amplitud tinggi di UAE boleh menyebabkan degradasi antosianin, penurunan kecil ini boleh diterima berbanding dengan peningkatan hasil antosianin. Dapatan kajian ini boleh dijadikan garis panduan yang berguna untuk aplikasi UAE dan HPCDE dalam pengekstrakan antosianin manggis dan membantu meningkatkan nilai manfaat manggis.

Kata kunci: Antosianin, Manggis, Pengekstrakan yang dibantu ultrabunyi, Pengekstrakan menggunakan karbon dioksida pada tekanan tinggi

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TABLE OF CONTENTS

ABSTRACT	Page
ABSTRAK	i
ACKNOWLEDGEMENTS	iii
APPROVAL	v
DECLARATION	vi
LIST OF TABLES	viii
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiii
	xv
 CHAPTER	
 1 INTRODUCTION	 1
1.1 Background	1
1.2 Problem statement	2
1.3 Objectives	4
 2 LITERATURE REVIEW	 5
2.1 Mangosteen	5
2.1.1 Overview	5
2.1.2 Utilization of fruit waste	6
2.1.3 Functional organic compounds in mangosteen pericarp	6
2.2 Anthocyanin	8
2.2.1 Structure	8
2.2.2 Biological activities	9
2.3 Extraction methods	13
2.3.1 Conventional solvent extraction	13
2.3.2 Ultrasound-assisted extraction	15
2.3.3 High-pressure carbon dioxide extraction	16
2.3.4 Other extraction methods	17
2.4 Factors Affecting Anthocyanin Stability	19
2.4.1 Temperature	19
2.4.2 Ultrasound	21
2.4.3 Other factors	23
 3 MATERIAL AND METHODS	 27
3.1 Sampling and reagents	27
3.1.1 Plant sample preparations	27
3.1.2 Chemicals	27
3.2 Conventional solvent extraction	28
3.3 Ultrasound-assisted extraction	28
3.4 High-pressure carbon dioxide extraction	29
3.5 Single factor experiments	30
3.6 Kinetics of extraction	31
3.6.1 Second-order kinetic model	32
3.6.2 Peleg's model	32
3.6.3 Activation energy	33
3.7 Effective diffusion coefficient during extraction	33
3.8 Thermodynamic analysis of extraction	34

3.9	Response surface methodology	35
3.10	Validation of model	36
3.11	Stability of anthocyanins under heating and ultrasound treatment	35
3.11.1	Preparation of anthocyanin extract	36
3.11.2	Heating and ultrasound treatment	36
3.11.3	Kinetics of degradation	37
3.11.4	Thermodynamic analysis of degradation	38
3.12	Determination of total anthocyanins content	39
3.13	Total total phenolic content	40
3.14	Polymeric color index	40
3.15	DPPH radical scavenging activity	41
3.16	Data analysis	41
4	RESULTS AND DISCUSSION	42
4.1	Effects of UAE parameters on TAC	42
4.2	Effects of HPCDE parameters on TAC	44
4.3	Analysis of extraction kinetics	46
4.4	Estimation of the effective diffusion coefficient	51
4.5	Thermodynamics properties of CSE, UAE and HPCDE	52
4.6	RSM optimization of UAE	53
4.6.1	Experimental data obtained with the CCD design	53
4.6.2	Validation of fitting the RSM models	57
4.6.3	Analysis of RSM model	61
4.6.4	Optimal extraction conditions and experimental validation	62
4.7	The effects of heating and thermo-ultrasonic treatments on the stability of mangosteen anthocyanins	64
4.7.1	The effects of heating and thermo-ultrasonic treatment on the degradation of anthocyanins	64
4.7.2	The effects of heating and thermo-ultrasonic treatment on the formation of polymeric color	69
4.7.3	The effects of heating and thermo-ultrasonic treatment on the degradation of phenolic compounds	70
4.7.4	The effects of heating and thermo-ultrasonic treatment on antioxidant activity	72
4.7.5	The competitive behaviors between anthocyanins extraction and degradation	73
5	SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	75
5.1	Summary	75
5.2	Conclusion	76
5.3	Recommendations for future research	77
	REFERENCES	78
	APPENDICES	97
	BIODATA OF STUDENT	104
	LIST OF PUBLICATION	105

LIST OF TABLES

Table		Page
2.1	Application of different extraction methods in the extraction of anthocyanins	18
3.1	CSE extraction parameters	28
3.2	The experimental design of single factor experiments	31
3.3	Five levels of the independent variables in the extraction	35
4.1	Parameters of kinetic models applied for the ultrasound-assisted extraction	48
4.2	Effective diffusion coefficient under conventional extraction (CSE), ultrasound-assisted extraction (UAE) and high-pressure carbon dioxide extraction (HPCDE)	50
4.3	Thermodynamic parameters under CSE, UAE and HPCDE	51
4.4	Response surface design (CCD) and experimental results	53
4.5	Analysis of variance (ANOVA) for the response surface quadratic model of total anthocyanins content (TAC)	56
4.6	Analysis of variance (ANOVA) for the response surface quadratic model of total phenolic content (TPC)	57
4.7	First-order kinetic data on the degradation of anthocyanin during heating and thermo-ultrasonic treatment	65
4.8	Thermodynamic data of anthocyanin degradation during heating and thermo-ultrasonic treatment	66
4.9	Zero-order kinetic data for the formation of polymeric color in heating and thermo-ultrasonic treatment	68
4.10	First-order kinetic data of phenolic degradation in heating and thermo-ultrasonic treatment	70

LIST OF FIGURES

Figure		Page
2.1	The structure of mangosteen fruit	5
2.2	The monomeric anthocyanins isolated from mangosteen pericarp	8
2.3	The structure of anthocyanin molecular	31
2.4	Structural reversible chemical transformations of anthocyanins	20
2.5	Mechanism of degradation of anthocyanins by scission and polymerization	20
2.6	The chemical structures of anthocyanin at different pH	25
2.7	Mechanism of enzymatic degradation and browning of anthocyanins	26
3.1	Flow chart of experimental design	27
3.2	Schematic diagram of ultrasound-assisted extraction system	29
3.3	Schematic diagram of high-pressure carbon dioxide extraction system	30
4.1	The effects of various combination between amplitude (A), temperature (B), and liquid-solid ratio (C) on TAC in UAE	42
4.2	The effects of various combination between pressure (A), temperature (B), and liquid-solid ratio (C) on TAC in HPCDE	44
4.3	Kinetic curves of second order and Peleg's model of anthocyanins extraction using CSE (A), UAE (B), and HPCDE (C)	45
4.4	Correlation between the actual and predicted values for TAC (A) and TPC (B)	58
4.5	Response surface and contour plots for the UAE of TAC from mangosteen pericarp	60

4.6	Response surface and contour plots for the UAE of TPC (Total phenolic content) from mangosteen pericarp	61
4.7	The effects of ultrasound and heating treatment on anthocyanins concentration	63
4.8	The effects of ultrasound and heating treatment on polymeric color index	67
4.9	The effects of ultrasound and heating treatment on phenolics concentration	69
4.10	The effects of ultrasound and heating treatment on DPPH radical scavenging activity	71
4.11	Schematic diagram of extraction and degradation mechanisms of anthocyanin and polyphenol during ultrasonic treatment	72

LIST OF ABBREVIATIONS

CSE	Conventional solvent extraction
UAE	Ultrasound-assisted extraction
HPCDE	High-pressure carbon dioxide
TAC	Total anthocyanins content
TPC	Total phenolics content
RSM	Response surface methodology
ROS	Reactive oxygen species
IC50	Half-maximal inhibitory concentration
PLE	Pressurized liquid extraction
SFE	Supercritical fluid extraction
MAE	Microwave-assisted extraction
EE	Enzymatic extraction
PPO	Polyphenol oxidase
C3G	Cyanidin-3-glucoside
GAE	Garlic acid equivalent
DW	Dry weight
ANOVA	Analysis of variance
R ²	Regression coefficient
CCD	Central composite design
RMSE	Root mean square error
HPLC	High pressure liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance



CHAPTER 1

INTRODUCTION

1.1 Background

Mangosteen is a genus of *Garcinia* trees widely grown in Southeast Asia, tropical Africa and South American countries. It is widely loved for its sweet taste and aromatic smell and has the reputation of being the queen of fruits. The flesh of ripe mangosteen is white flap-like, accounting for about 30% of the whole fruit weight, and the pericarp is purple-red, accounting for 70% of the whole fruit weight. Phytochemical analysis has shown that the mangosteen pericarp contains a variety of active substances such as xanthenes, anthocyanins, and phenolic compounds. Among them, the extraction and biological activity of xanthone is the most widely studied, due to its abundant content in the mangosteen pericarp. However, limited researches have been conducted on anthocyanin, which is also abundant in mangosteen pericarp and has bioactive functions.

Anthocyanins are a group of flavonoids with a diphenylchroman skeleton, which are widely found in the fruits, flowers and roots of plants. Anthocyanin is categorized as a pigment which gives them varying degrees of red or purple color. There have been numerous reports on the extraction of anthocyanins from fruits such as grapes, blackberries and blueberries and on the exploration of their bioactivity (Pereira et al., 2019; Gowd et al., 2018; X. Li et al., 2017). The researchers pointed out that that anthocyanin extracts have multiple biological activities such as antioxidant, anti-tumor, anti-obesity, antibacterial, and anti-inflammatory effects (Dzah et al., 2020; Y. Huang & Zhou, 2019; Yan et al., 2018). Nowadays, anthocyanin has been widely used in the production of food and cosmetics.

Due to the rich anthocyanin content in mangosteen pericarp (23.54 - 292 mg/100 g) and the low-cost characteristics of mangosteen pericarp, this makes the use of mangosteen pericarp as a low-cost source of anthocyanins a potentially viable alternative (Cheok et al., 2013a; Hiranrangsee et al., 2016). At the same time, the full utilization of mangosteen pericarp can also greatly increase the added value of mangosteen and reduce the potential environmental pollution caused by the random disposal of mangosteen pericarp. The extraction of anthocyanin from mangosteen pericarp is an essential operation before it can be used in food or cosmetics. In the studies of Cheok et al. (2013) and Ziabakhsh Deylami et al. (2016), anthocyanins were successfully extracted from mangosteen pericarp using UAE and CSE methods respectively. Cheok et al. (2013) investigated the effect of ultrasound amplitude, extraction time on the yield of anthocyanin but did not control the temperature which also plays an important role in the extraction (diffusion) process. This may affect the accurate assessment of ultrasonic extraction of anthocyanin from mangosteen. Therefore, it is necessary to explore

and optimize the mangosteen anthocyanin extraction more comprehensively. In addition, kinetic and thermodynamic analyses of the extraction process as well as stability analyses of the extracted products are also important means of evaluating the extraction method. Ziabakhsh Deylami et al. (2016) analyzed the kinetic process of anthocyanin extraction from mangosteen by CSE method by peleg's model. However, the analysis of kinetic and thermodynamic analysis related to the extraction of anthocyanin from mangosteen under UAE and HPCDE has been rarely reported. In order to have a more comprehensive understanding of the extraction of anthocyanin from mangosteen by UAE and HPCDE, it is necessary to carry out related studies.

1.2 Problem statement

In the food industry, the mangosteen fruit is often processed into juice, jam or frozen mangosteen pulp, while the pericarp, which makes up most of the fruit weight, is often discarded as a production waste. In Malaysia alone, approximately 12,600 tons of mangosteen pericarp are produced in 2021 (Nasrullah et al., 2021). This is obviously not conducive to the high-value utilization of mangosteen. At the same time, the environmentally friendly disposal of waste mangosteen pericarp may further increase the production costs of enterprises. Therefore, it is necessary to conduct relevant studies to more fully exploit the value of mangosteen pericarp.

Extraction is often an essential unit operation in the utilization of mangosteen anthocyanins. Conventional solvent extraction (CSE) is often used in anthocyanin extraction due to its simple operation and inexpensive equipment. However, the disadvantages of CSE, such as high solvent consumption, time-consuming, and low yield, have been bothering researchers and companies related to anthocyanin extraction. Therefore, the development of new extraction techniques with higher yields and efficiency has been a key research area to expand the industrial applications of anthocyanins. Currently, Ultrasound-assisted extraction (UAE), high-pressure carbon dioxide extraction (HPCDE), supercritical fluid extraction (SFE), and other emerging technology have been developed and applied to the laboratory-scale or industry-scale extraction. In the extraction of anthocyanins, the UAE method often shows the advantages of high yield, high extraction rate and low equipment cost, which makes it widely used in the extraction of biologically active substances. In the case of HPCDE, the study of Xu et al. (2010) pointed out that HPCDE was able to significantly reduce the extraction time by about 50%. Compared with CSE, this is undoubtedly a huge advantage. Therefore, in this study, based on the pursuit of higher extraction efficiency and greater extraction yield, the UAE and HPCDE methods were chosen for the extraction of anthocyanin from mangosteen.

UAE has been favored by a large number of researchers and applied in the extraction of anthocyanins due to its easy operation and low maintenance of the equipment. Various sono-physical effects induced by ultrasonic cavitation in UAE cause mechanical damage to the tissue structure of plant materials and

therefore increase the extraction efficiency of targeted compounds (Fu et al., 2020). Compared to CSE, UAE showed significant yield increases and efficiency enhancement in the extraction of anthocyanins from black carrot, blue butterfly pea flower and grapes (Sucheta et al., 2020; Mazza et al., 2019; Mehmood et al., 2019). Meanwhile, the significant role of UAE in enhancing the yield of mangosteen anthocyanin was reported in previous studies (Cheok et al., 2013a; Hiranrangsee et al., 2016). In a previous study by Ziabakhsh Deylami et al. (2016), they investigated the effect of bleaching pretreatment at different temperatures on the extraction of anthocyanins from mangosteen pericarp. The authors investigated the kinetic characteristics of PPO enzyme inactivation and anthocyanin degradation at different temperatures by First-order kinetic model and Weibull model. Also, the authors applied Peleg's model to describe the kinetic parameters under conventional solvent extraction (CSE). However, to date, we have not seen any studies on the kinetics of the UAE extraction of anthocyanin from mangosteen pericarp. Kinetic parameters such as rate constants, equilibrium concentrations, can be used to quantitatively characterize the extraction behavior of anthocyanins, which is useful for a comprehensive understanding of the characteristics of different extraction techniques. Also, the kinetic model can help engineers to predict the anthocyanin yield, which is beneficial to guide the industrial production of anthocyanin. Therefore, in addition to comparing the anthocyanin yields among different techniques, it is also necessary to perform kinetic analysis.

Meanwhile, HPCDE is also an emerging technique that can be used for anthocyanin extraction. In HPCDE, the high-pressure carbon dioxide in the extraction vessel is rapidly released after the treatment is completed, which can cause a rapid expansion in the volume of carbon dioxide dissolved in the extraction solvent or diffused into the plant material. This micro-explosion effect will play a destructive role in the plant material, which in turn can accelerate the extraction. At the same time, in HPCDE, there is a large amount of polar extraction solvent (water) in the extraction vessel, which gives it an advantage over the supercritical fluid technique in the extraction of polar compounds such as anthocyanins. The method was first proposed and applied to the extraction of anthocyanin from red cabbage by Xu et al. (2010). Subsequently, it attracted the attention of researchers. HPCDE takes advantage of the low viscosity and high diffusion rate of supercritical/ subcritical carbon dioxide, as well as the explosive effect of the treatment to achieve the destruction of plant tissue structure and ultimately accelerate the diffusion of anthocyanins. At present, the technology has been applied to the extraction of bioactive substances in various plant materials such as purple cabbage, purple potato, jaboticaba, and strawberry-tree (Alexandre et al., 2018; Lao et al., 2020; Santos & Meireles, 2011; Z. Xu et al., 2010). In the extraction of anthocyanins from purple potatoes and red cabbage, HPCDE showed 10-45% yield improvement and about half of the extraction time reduction in comparison to CSE (Lao et al., 2020; Z. Xu et al., 2010). To the best of our knowledge, there are limited studies that apply HPCDE technology in mangosteen pericarp for anthocyanin extraction.

In addition to the focus on the yield of anthocyanins, the investigation of possible extraction mechanisms is equally important. In UAE, although ultrasound

treatment has a significant effect in increasing the yield of anthocyanins, the sono-chemical effects in cavitation may negatively affect the stability of anthocyanins, phenolic acids, flavonoids and other bioactive substances (X. Wu et al., 2022). The collapse of the cavitation bubble releases a large amount of energy, resulting in extremely high temperatures and pressures (up to 5000 K, 500MPa) in a localized area. The extreme conditions lead to the hydrogen-oxygen bonds in some water molecules being broken, subsequently producing hydrogen and hydroxyl radicals that can react with antioxidant components in a redox manner (Fu et al., 2020). In addition, transient high temperatures can also cause thermal degradation of temperature-sensitive substances. Several previous studies have reported that ultrasound and heating treatments had different degrees of negative effects on the stability and antioxidant capacity of anthocyanins (X. Wu et al., 2022; W. Li et al., 2020; Cao et al., 2019). A large number of previous studies have focused more on the acquisition of higher yields during extraction, but the negative effects of ultrasound during extraction have not been adequately evaluated. Therefore, it is necessary to investigate the effect of ultrasound on the stability and antioxidant capacity of mangosteen extracts in order to better understand the mechanism of ultrasound in mangosteen extraction.

1.3 Objectives

Therefore, this study includes several research objectives as follows:

1. To compare the differences between conventional solvent extraction (CSE), ultrasound-assisted extraction (UAE) and high-pressure carbon dioxide extraction (HPCDE) to enhance the extraction yield of anthocyanin from mangosteen pericarp by single-factor experiments, kinetic and thermodynamic analyses.
2. To optimize four main parameters of ultrasound-assisted extraction (UAE) in extraction of total anthocyanins content (TAC) and total phenolic content (TPC) from mangosteen pericarp by response surface methodology (RSM).
3. To Investigate the effects of ultrasound and heating treatment on anthocyanin concentration, phenolic concentration, polymeric color index and antioxidant capacity of mangosteen anthocyanin extract.

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