



UNIVERSITI PUTRA MALAYSIA

***EXTRACTION, PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT
ACTIVITIES OF XANTHONE FROM MANGOSTEEN (*Garcinia
mangostana* LINN.) PERICARP***

NASRIN ABOUTORAB

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By

NASRIN ABOUTORAB

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of
Doctor of Philosophy**

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DEDICATION

This Thesis is dedicated to my family and many friends.

A special feeling of gratitude to my loving parents, my husband Reza and my sister Mozghan and her lovely children for their endless love, support and encouragement



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Doctor of Philosophy

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ANTIOXIDANT ACTIVITIES OF XANTHONE FROM MANGOSTEEN
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NASRIN ABOUTORAB

August 2017

Chairman : Associate Professor Badlishah Sham Baharin, PhD
Faculty : Food Science and Technology

Garcinia mangostana Linn. (mangosteen) belongs to the *Guttiferae* family; about 2/3 of the fruit weight is pericarp which is thrown out as a waste by the fruit processing industries due to its unpleasant astringency taste, while they are a great source of bio-actives such as polyphenols and xanthenes. Extraction and utilization of these bioactive compounds from the fruit pericarp will be valuable for the fruit industries. Even though the extraction of bioactive compounds was done previously; utilization and further enhancement of those bioactive compounds in the functional product development is still in the budding stage. Owing to the above concern, the first purpose of the present study is to investigate the effect of drying and extraction procedure (solvent used, ultrasound pretreatment, and their combination) on the extraction yield, antioxidant activity, and the levels of total polyphenol compounds (TPC), total xanthenes, and α - mangostin. The second purposes is to encapsulate the xanthone crude extract isolated from mangosteen fruit pericarp powder (MFPP), with maltodextrin at various conditions. The study showed the maximum TPC value (322.86 ± 8.31 mg GAE/g MFPP), α - mangostin (42.233 ± 2.072 mg/g MFPP), high total xanthone (89.360 ± 1.02 mg/ g MFPP), and DPPH radical scavenging activity (120.534 ± 6.526 mM TE /100g MFPP) for ethanol extract of freeze dried MFPP. Furthermore, using 57% ethanol, 5 min direct sonication, 50 % pulser as a pretreatment and 51min solvent extraction resulted in isolating xanthone crude extract containing high amount of α -mangostin (54.633mg/100mg of total xanthone), and total xanthone (115.394 mg/g DW) with optimum TPC (560.518 mg GAE/g MFPP) value and DPPH value (176.785 mM TE/100g MFPP). The MFPP extract can be efficiently encapsulated with maltodextrin at ratio 2.5: 1, 150°C inlet temperature, and flow rate 15 rpm (10 mL/min). The characteristics of the powder encapsulated xanthone crude extract (XCEP) are particle size: 62.95 to 86.98 μ m (D[4,3]- volume weighted), encapsulation yield 62%, tap density:0.201 mg/mL,

hygroscopicity:10.17%, water activity (WA): 0.279, moisture content: 3.44%, water absorption index (WAI): 0.409, water solubility index (WSI): 84.43g/100g, thermal glass transition temperature (T_g °C) : 158.507° C, TPC retention:70%, DPPH radical scavenging activity: 661.83mM TE/g powder, total xanthone: 563.520 mg/g powder and α -mangostin content: 73.910 mg/ g powder. Using encapsulation with spray drying, more than 90% of TPC, total xanthone and α -mangostin was remained unchanged and 85% of antioxidant activity was still hold after 60 days incubation at 4°C and room temperature. Supplemented milk with microencapsulated xanthone crude extract shows good functional properties such as high TPC (319.32mg GAE/ 200mL milk) and DPPH value (298.76 mM TE/200mL milk) comparing to the TPC value of 99.549 mg GAE/ 200mL milk and DPPH value of 35.44 mM TE/200mL milk for control milk during 16 days at 4° C.

As a conclusion, microencapsulated xanthone crude extract showed stable physicochemical and functional properties during long-term storage in form of powder or as supplement into milk. Together these results provide important visions to the potential of encapsulated xanthone crude extract to be used as supplement and application as bioactive compounds in the functional product development.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGEKSTRAKAN, SIFAT FISIKOKIMIA DAN AKTIVITI
ANTIOKSIDA XANTHONE DARIPADA PERIKARPA MANGGIS
(*Garcinia mangostana* LINN.)**

Oleh

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Pengerusi : Profesor Madya Badlishah Sham Baharin, PhD
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Garcinia mangostana Linn. (manggis) berasal dari keluarga *Guttiferae* ; kira-kira 2/3 daripada berat buah adalah perikarpa yang dibuang sebagai bahan buangan oleh industri pemprosesan buah-buahan disebabkan rasa astringen yang kurang menyenangkan, sedangkan ianya adalah sumber bahan bio-aktif seperti polifenol dan xanthone. Pengekstrakan dan penggunaan bahan bioaktif dari perikarpa buah-buahan adalah sangat bernilai untuk industri buah-buahan. Walaupun pengekstrakan sebatian bioaktif telah dilakukan sebelum ini, penggunaan dan peningkatan lanjutan sebatian bioaktif dalam pembangunan produk berfungsi masih pada tahap awal. Oleh kerana kebimbangan yang dinyatakan, tujuan pertama kajian yang dijalankan adalah untuk menyiasat kesan prosedur pengeringan dan pengekstrakan (pelarut yang digunakan, pra-rawatan ultrasonik, dan gabungannya) ke atas hasil pengekstrakan, aktiviti antioksidan dan tahap jumlah sebatian polifenol (TPC), jumlah xanthone dan α -mangostin. Tujuan kedua adalah bagi melaksanakan pengkapsulan ekstrak xanthone mentah yang diasingkan dari serbuk perikarpa buah manggis (MFPP), dengan maltodekstrin pada pelbagai keadaan. Kajian menunjukkan bahawa nilai maksima TPC (322.86 ± 8.31 mg GAE/g MFPP), α - mangostin (42.233 ± 2.072 mg/g MFPP), jumlah xanthone tinggi (89.360 ± 1.02 mg/ g MFPP) dan aktiviti memerangkap radikal DPPH (120.534 ± 6.526 mM TE /100g MFPP) untuk ekstrak etanol bagi MFPP yang melalui pengeringan pembekuan. Tambahan pula, menggunakan 57% etanol, sonifikasi langsung selama 5 minit, pendenyut 50% sebagai pra-rawatan dan pengekstrakan menggunakan pelarut selama 51minit, menghasilkan pengasingan ekstrak xanthone mentah mengandungi jumlah α -mangostin yang tinggi (54.633mg/100mg daripada jumlah xanthone keseluruhan) dan jumlah xanthone keseluruhan (115.394 mg/g DW) dengan nilai TPC optima (560.518 mg GAE/g MFPP) dan nilai DPPH (176.785 mM TE/ 100g MFPP). Ekstrak MFPP boleh dikapsulkan dengan maltodekstrin pada nisbah 2.5: 1, suhu salur masuk 150°C, dan

kadar aliran 15rpm (10 mL/ min). Ciri-ciri serbuk ekstrak xanthone mentah (XCEP) yang dikapsulkan adalah saiz partikel: 62.95 hingga 86.98 μ m (D[4,3]- ditimbang isipadu), hasil pengkapsulan 62%, ketumpatan ketik: 0.201 mg/mL, sifat higroskopik: 10.17%, aktiviti air (WA): 0.279, kandungan kelembapan: 3.44%, indeks penyerapan air (WAI): 0.409, indeks kebolehlarutan air (WSI): 84.43g/100g, suhu peralihan kaca terma (Tg° C) : 158.507° C, pengekal TPC: 70%, aktiviti memerangkap radikal DPPH: 661.83mM TE/g serbuk, xanthone keseluruhan: 563.520 mg/g serbuk dan kandungan α -mangostin : 73.910 mg/ g serbuk. Melalui pengkapsulan menggunakan kaedah pengeringan sembur, lebih daripada 90% TPC, xanthone keseluruhan dan α -mangostin kekal tanpa perubahan dan 85% daripada aktiviti antioksidasi masih dikekalkan selepas pengeringan 60 hari pada 4°C dan suhu bilik. Susu yang ditambah dengan ekstrak mentah xanthone melalui mikropengkapsulan menunjukkan sifat berfungsi yang baik seperti TPC yang tinggi (319.32mg GAE/ 200mL susu) dan nilai DPPH (298.76 mM TE/200mL susu) berbanding dengan nilai TPC (99.549 mg GAE/ 200mL susu) dan nilai DPPH (35.44 mM TE/200mL susu) bagi susu kawalan selama 16 hari pada 4° C.

Sebagai kesimpulan, ekstrak mentah xanthone yang dimikrokapsulkan menunjukkan sifat fisikokimia dan sifat berfungsi yang stabil semasa tempoh penyimpanan jangka panjang dalam bentuk serbuk atau sebagai bahan tambahan dalam susu. Oleh itu, keputusan yang diperolehi memberikan wawasan yang amat penting kepada potensi ekstrak mentah xanthone yang dikapsulkan untuk digunakan sebagai bahan tambahan dan diaplikasi sebagai sebatian bioaktif dalam pembangunan produk berfungsi.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
≤	Equal or less than
°	Degree
°C	Degree centigrade
μm	Micrometre
ANOVA	Analysis of Variance
CO ₂	Carbon dioxide
e.g.	Exempli gratia
(EXCEP)	Encapsulated Xanthone Crude Extract Powder
FAO	Food and Agriculture Organization
FDMP	Freeze Dried Mangosteen Pericarp
FDMFP	Freeze Dried Mangosteen Fruit Pericarp
FRAP	Ferric Ion Reducing Antioxidant Power
g	Gram
GLM	General Linear Model
GRAS	Generally Recognized As Safe
h	Time (hour)
i.e.	That is
MD	Maltodextrin
MFP	Mangosteen Fruit Pericarp
mg/mL	milligram per milliliter
min	Minute
mL	millilitre
mL/min	millilitre per minutes
mm	millimetre
nm	nanometre
p	probability
pH	Power of hydrogen ion

s	Second
α	alpha
β	Beta
(SC-Co ₂)	Supercritical Fluid Co ₂
UHT	Ultra-High Temperature
v/v	volume per volume
w/v	Weight per volume
w/w	Weight per weight
WPI	Whey protein isolate
$\mu\text{g/ mL}$	microgram per milliliter
μL	microliters
$\mu\text{l/ mL}$	microliters per milliliter
RSM	Response Surface Methodology
SEM	Standard Error of Mean
CRD	Complete Randomized Design
R^2	Correlation Coefficient
T_g	Glass Transition Temperature
L^*	Lightness
a^*	Redness or greeness
b^*	Yellowness or Blueness

CHAPTER 1

GENERAL INTRODUCTION

Scientific research shows that consumption of fruits and vegetables can lessen the occurrence of degenerative diseases such as inflammation, arthritis, heart disease, cancer, immune system decline and brain dysfunction. These protective effects partly come from the existence of different antioxidants in fruits and vegetables that can inhibit or interrupt the formation of oxidizable substrate chain reactions (Zhou, Lin, Wei, & Tam, 2011; Jung, Su, Keller, Mehta, & Kinghorn, 2006). In regard to these effects, there is crucial demand for incorporating some of these useful phytochemicals into our food regime by producing what are known as functional foods or nutraceuticals.

As the manufacturing of processed fruit and foodstuffs increase, the rate of fruit wastes produced also increases rapidly. In general, processed organic raw materials generated more than 50% w/w wastes of their original source. These huge amount of organic wastes could be a solemn environment problem but almost all of these wastes contain ingredients with high nutritional or functional properties (fragrances, proteins, pigments, vitamins, antimicrobial, antioxidants, etc.) and can be taken into account as by-products for reuse (Romo-Hualde, Yetano-Cunchillos, González-Ferrero, Sáiz-Abajo, & González-Navarro, 2012).

Mangosteen is one of those fruits with high amount of phytochemical components in its pericarp (peel, rind, and hull). Mangosteen, mango's teen (English name), mangotanier, mangos tan, and manggis (local Malay name) are the common names of this fruit. It is known as "the queen of tropical fruits" because of its delicious taste (Obolskiy, Pischel, Siriwatanametanon, & Heinrich, 2009). *Garcinia mangostana*. Linn. (GML) belongs to the *Guttiferaceae* family and is a perennial tropical plant. It is native in the Southeast Asia and has been cultured in the tropical rainforests of Malaysia, Thailand, Hawaii, Brazil, Northern Australia, Central America, Southern India, and Indonesia (Ji, Avula, & Khan, 2007).

The edible fruit arils are made up of 4 to 8 triangular pieces of white, juicy, and soft flesh with slightly or noticeably acidic flavor with very pleasant taste. The fruit has a range of 3.4 cm – 7.5 cm (diameter) and 6 mm – 10 mm (thickness). It has dark to red purple pericarp and red cross-section with purplish white on the inside. The fruit also contains yellow latex and purple staining juice (Obolskiy et al., 2009) as it constitutes around 2/3 of the whole fruit weight (Zarena, Manohar, & Udaya Sankar, 2012). The peel of mangosteen fruit contains variety of secondary polyphenol metabolites such as prenylated and oxygenated xanthenes (Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, & Pérez-Rojas, 2008; Peres, Nagem, & De Oliveira, 2000), and has been used in curing diarrhea, infected wounds, dysentery, abdominal pain, chronic ulcer, and suppuration (Zhou et al., 2011). This part of the fruit has been used in Thai medicine for curing wounds, skin infections and diarrhea

(Jung et al., 2006). Numerous commercial merchandise such as herbal cosmetics, nutritional supplements, and pharmaceutical products added mangosteen as the constituent into their products (Aisha, Abu-Salah, Ismail, & Majid, 2012). In the United States, consumption of many botanical dietary supplements made from *Garcinia mangostana* have increased due to their potent antioxidant properties (Jung et al., 2006). In order to reach the highest extraction yield of xanthone from mangosteen, many extraction methods have been used such as solvent extraction method and Soxhlet extraction. Soxhlet method is one of the common methods which use high concentration of organic solvent at high extraction temperature for better extraction of relatively polar xanthenes (Zarena et al., 2012). Supercritical carbon dioxide (SC-CO₂) extraction method is an environmental friendly extraction method for obtaining higher extraction yield of xanthone from *Garcinia mangostana* (Zarena, Udaya Sankar, 2011). Using the optimized ethanol modified Supercritical fluid extraction (SFE) resulted in more pure xanthone extract with higher antioxidant activity obtained from the mangosteen pericarp during 8 hours extraction process (Zarena, Sachindra, & Udaya Sankar, 2012). Ultrasound accelerated extraction method also has been used for the extraction xanthenes from the mangosteen fruit pericarp (MFP) (Yoswathana, 2013).

By using different drying methods with various extraction methods and solvents, different kinds of xanthenes at various concentrations could be extracted from different parts of *Garcinia mangostana*. Although some researchers have been carried out on the individual effect of drying methods, type of solvents or extraction method on extraction yield, TPC value, and amount of total xanthone isolated from different parts of mangosteen fruit extract; there is still few empirical investigation on the synergistic effects of different drying methods and various extractant solvents on extraction yield, TPC value, antioxidant properties, amount of total xanthone, and α -mangostin of xanthone crude extract isolated from Malaysian mangosteen fruit pericarp (MMFP). Also, no previous study has investigated the effect of indirect (under different levels of sonication power and time) and direct ultrasound pre-treatment (under various sonication pulser, and sonication time) on the antioxidant activity and amount of extracted total xanthone, and α -mangostin isolated through solvent extraction method from ultrasound pre-treated MMFP.

Based on the MFP is a great source of xanthenes with variety of functional properties and due to increasing interest to incorporate nutraceutical into foodstuffs, the MFP agricultural waste can be considered as an incredible source to isolate those natural antioxidant from the by-product from mangosteen.

The astringency of polyphenols is one of the unpleasant attributes of these compounds which limit their applications in food products (Teixeira, Afonso, Pinto, & Barbosa, 2003). In regard to low aqueous solubility of xanthenes and their unpleasant astringent taste, micro-particle encapsulation systems could stabilize their bioactivities as well as mask the unwanted astringency. To date, no research has been done on the encapsulation of xanthenes crude extract isolated from MMFP.

In this work, first of all, we determined the individual and synergistic effects on type of solvent and drying method; selected the appropriate solvent and drying method; indicated the optimum sonication pre-treatment and solvent extraction conditions to isolate xanthone crude extract from MMFP powder with high extraction yield, antioxidant activities, xanthone, and α -mangostin amount. In the next step, we produced the microencapsulated xanthone crude extract from ultrasound pre-treated MMFP powder; added the encapsulated xanthone crude extract as a functional additive into milk and monitored the changes in TPC value and antioxidant activity of produced powder during 60 days at room temperature, 4°C, and after adding into the milk during 16 days of storage at 4°C.

Even though the extraction of bioactive compounds and antioxidants in mangosteen pericarp was done previously, utilization and further enhancement of those bioactive compounds in the functional product development is still in the budding stage. Owing to the above concern the objectives of this study are:

1. To investigate the effect of drying method (oven drying/freeze drying) and extraction solvent (water/ethanol/methanol) on the extraction yield, antioxidant activity of the extracts, and the levels of total phenol compounds (TPC), total xanthone, and α -mangostin.
2. To investigate the effect of extraction procedure (solvent extraction, ultrasound pretreatment, and their combination) on the extraction yield, antioxidant activity of the extracts, and the levels of total phenol compounds (TPC), total xanthone, and α -mangostin.
3. To encapsulate the xanthone crude extract isolated from mangosteen fruit pericarp powder (MFPP) with maltodextrin at various conditions for production of encapsulated xanthone crude extract powder with good functional properties, and acceptable physicochemical powder properties.
4. To monitor the changes in functional property (TPC value and antioxidant activity) stability of produced encapsulated xanthone crude extract powder (incubated for 60 days at room temperature and 4°C), and milk supplemented with different concentrations of encapsulated xanthone crude extract powder (incubated for 16 days of storage at 4°C).

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