UNIVERSITI PUTRA MALAYSIA

STABILIZATION OF ANTHOCYANINS IN MANGOSTEEN (Garcinia mangostana Linn) PERICARP THROUGH INHIBITION OF ENZYMATIC BROWNING AND SPRAY-DRYING

MAHSA ZIABAKHSH DEYLAMI

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By

MAHSA ZIABAKHSH DEYLAMI

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

May 2016

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Specially Dedicated to
My parents who gave me roots to grow and wings to fly

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

STABILIZATION OF ANTHOCYANINS IN MANGOSTEEN (*Garcinia mangostana* Linn) PERICARP THROUGH INHIBITION OF ENZYMATIC BROWNING AND SPRAY-DRYING

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May 2016

Chairman : Professor Russly Abdul Rahman, PhD
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Anthocyanins are natural red colorants with potential health effects. Mangosteen pericarp composes more than half of the fruit weightand is agriculture waste. The pericarp has high anthocyanin content, comparable to some commercial sources of anthocyanin. The main objective of this study was to stabilize anthocyanins of mangosteen pericarp in order to develop an anthocyanin-based food coloring. In this regard the problems to be overcome were the fast enzymatic browning of mangosteen pericarp and intrinsic instability of anthocyanins. Polyphenol oxidase (PPO) is the main enzyme responsible for the pericarp enzymatic browning. Biochemical characteristics of mangosteen pericarp PPO were determined. PPO showed the greatest substrate specificity towards catechol. The optimum temperature and pH for PPO activity were 50°C and 7.5, respectively. Among the inhibitors with different mechanism of action, oxalic acid, cinnamic acid and cysteine were the most potent inhibitors. Blanching significantly (p<0.05) reduced PPO activity but it was not sufficient for complete enzyme inactivation. The activation energies of polyphenol oxidase inactivation, anthocyanin loss and total color changes were 43.11, 26.49 and 18.86 kJ/mol, respectively, which showed PPO inactivation was the most sensitive parameter towards temperature changes. The effect of combinations of oxalic acid (100-180 mM), cinnamic acid (0.5-1.5 mM) and cysteine (0.5-1.5 mM) as an alternative non-thermal enzyme inhibition method was evaluated. The results showed synergistic effect of oxalic acid with cinnamic acid and additive effect of cysteine with the other inhibitors, suggesting the greater potential of combined inhibitors for reducing PPO activity compared to their individual application. Besides, combined chemical treatment significantly (p<0.05) improved anthocyanin and color stability of mangosteen pericarp compared to non-treated pericarp. The application of mild thermal treatment (5 min steaming) with combined chemical treatment (100 mM oxalic acid, 0.5 mM cinnamic acid and 1.528 mM cysteine) resulted in 99.2% inhibition of PPO. In an attempt to stablize anthocyanins of mangosteen pericarp, spray drying with maltodextrin (DE 10) was investigated using varying inlet air temperature (140-

180 ℃), feed pump rate (8-10 rpm) and pericarp to maltodextrin ratio (5:3 to1:1 w/w). The retention of anthocyanins during spray drying was improved at higher pericarp to maltodextrin ratio and lower inlet air temperature. The stability of mangosteen pericarp powder was studied throughout 12 weeks storage at different temperatures (25 and 40 ℃) and relative humidity (50-97%). The results showed both parameters negatively (p<0.05) affected anthocyanin and color stability.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENSTABILAN ANTOSIANIN KULIT MANGGIS (*Garcinia mangostana* Linn) MELALUI PERENCATAN PEMERANGAN ENZIMATIK DAN PENGERINGAN SEMBUR

Oleh

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Antosianin merupakan pewarna merah semula jadi yang bermanfaat terhadap kesihatan. Kulit manggis yang merangkumi lebih separuh daripada berat buah merupakan bahan sisa hasil pertanian. Kulit manggis mempunyai kandungan antosianin yang tinggi, setanding dengan beberapa sumber komersial. Objektif utama kajian ini adalah untuk menstabilkan antosianin daripada kulit manggis dan seterusnya menghasilkan pewarna makanan berasaskannya. Dalam hal ini, masalah yang perlu diatasi adalah pemerangan kulit manggis yang terlalu cepat oleh enzim dan ketidakstabilan unsur intrinsik pigmen antosianin. Polifenol oksidase (PPO) adalah enzim utama yang bertanggungjawab ke atas pemerangan kulit manggis dan ciri-cirinya ditentukan. PPO menunjukkan kekhususan yang paling tinggi terhadap substrat catechol pada suhu optimum 50 ℃ dan pada pH 7.5. Asid oksalik, asid cinnamic dan cysteine adalah antara perencat yang paling berkesan dengan mekanisme yang berbeza. Proses penceluran dapat mengurangkan aktiviti PPO (p<0.05) secara ketara tetapi tidak dapat menyahaktifkan enzim. Tenaga pengaktifan polifenol oksidase inactivation, kehilangan antosianin dan jumlah perubahan warna adalah 52.7, 26.49 dan 18.86 kJ/mol masing-masing. Ini menunjukkan penyahaktifanPPO adalah parameter yang paling sensitif terhadap perubahan suhu.Gabungan asid oksalik (100-180 mM), asid cinnamic (0.5-1.5 mM) dan cysteine (0.5-1.5 mM) sebagai alternatif bukan haba untuk perencatan enzim dikaji. Asid oksalik menunjukkan kesan sinergi dengan asid cinnamic. Sementara cysteine menunjukkan kesan aditif dengan perencat lain. Hasil kajian menunjukkan gabungan perencat memberi potensi yang lebih besar dalam mengurangkan aktiviti PPO berbanding aplikasi individu.Gabungan kaedah kimia meningkatkan kestabilan antosianin dan warna kulit manggis secara ketara (p<0.05) berbanding dengan kulit yang tidak dirawat. Penggunaan rawatan haba sederhana (5 minit pengukusan) dengan kaedah kimia (100 mM asid oksalik, 0.5 asid mM cinnamic dan 1.528 mM cysteine) berjaya merencat 99.2% aktiviti PPO. Dalam usaha untuk menstabilkan antosianin kulit manggis, kaedah pengeringan sembur telah digunakan untuk mengkaji kesan suhu udara masuk (140-180 ℃), kadar pam

suapan (8-10 rpm) dan nisbah kulit manggis kepada maltodekstrin DE 10 (5:3-1:1 w/w). Retensi pigmen antosianin meningkat semasa proses pengeringan sembur dengan penggunaan maltodekstrin pada nisbah yang lebih tinggi dan suhu udara masuk yang lebih rendah. Kajian penyimpanan serbuk sepanjang 12 minggu menunjukkan kedua-dua faktor suhu (25 dan 40 ℃) dan faktor kelembapan relatif (50-97%) memberi kesan negatif (p<0.05) terhadap kestabilan antosianin dan warna.

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I certify that a Thesis Examination Committee has met on 31 May 2016 to conduct the final examination of Mahsa Ziabakhsh Deylami on her thesis entitled "Stabilization of Anthocyanins in Mangosteen (*Garcinia mangostana* Linn) Pericarp Through Inhibition of Enzymatic Browning and Spray-Drying" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF SYMBOLS AND ABBREVIATIONS

L	Liter	
v/v	Volume per volume	
w/w	Weight per weight	
min	Minute	
rpm	Rotation per Minute	
g	Gram	
M	Molarity	
mL	Milliliter	
μL	Microliter	
U	Unite of Enzyme Activity	$\Delta A\ min^{-1}\ 0.001$
C_t	Enzyme Activity in Treated Sample	
C_c	Enzyme Activity in Control Sample	
mM	Millimolar	
IC_{50}	50% Inhibitory Concentration	
ANOVA	Analysis of Variance	
p	Significance Difference	
mg	Milligram	
SD	Standard deviation	
MW	Molecular Weight	g/mol
DF	Dilution Factor	
ε	Molar Absorptivity Coefficient	L/cm/mol
l	Path Length	cm
t	Time	min or h
$t_{1/2}$	Half-life Time	min or h
E_a	Activation Energy	$kJmol^{-1}$

R	Universal Gas Constant	8.314J/mol K
T	Temperature	°K or °C
k	Rate constant	min^{-1}
k	Boltzman constant	1.381×10^{-23} JK^{-1}
h	Lanck's constant	6.626×10^{-34} Js
L^*	Lightness	
a^*	Redness or Greenness	
b^*	Yellowness or Blueness	
ΔE	Total Color Difference	
CRD	Complete Randomized Design	
R^2	Correlation Coefficient	
χ^2	Chi-square	
SEM	Standard Error of Mean	
RSM	Response Surface Methodology	
h	Hour	
η	Apparent Viscosity	s^{-1}
μm	Micrometer	
T_g	Glass Transition Temperature	°C

CHAPTER 1

GENERAL INTRODUCTION

Color is the first characteristics of a food product that is noticed and it predetermines the expectation of consumer for flavor and quality. It may even affects consumer perception of flavor and taste (Hoegg and Alba, 2006). Color may be added to food products for enhancing the color that already presents in food, minimizing color variation between processing batches, restoring color lost during food processing and adding color to uncolored food. Food colorants can be classified based on their origin as natural colors, natural-identical colors, synthetic colors and inorganic colors (Mortensen, 2006). Natural pigments are obtained from natural edible sources, usually plant material. Insects, algae, cyanobacteria and fungi are also the other sources of natural colorants(Mortensen, 2006). Natural-identical colors are produced by chemical synthesis and have an identical chemical structure to colorants found in nature. Synthetic colorants are chemically synthesized and are not found in nature.

In recent years, food containing synthetic colors has been fallen into disfavor with consumers, partly due to an increasing trend to avoid foods containing artificial additives and also some drawbacks of synthetic colors have been widely discussed for example a link between the consumption of certain azo-dyes and their effect on childhood behavior. This has led to a preference for using natural sources of colorant in the food industry and removal of artificial colors from food products. Colors from natural sources are generally permitted. Natural colors can be used in a form of coloring foodstuff. A plant product with coloring property is called a "coloring foodstuff", which is manufactured by physical processes that results in a concentrate or powder.

Anthocyanins, carotenoids, betalains and chlorophyll are natural colors that are widely used in every day foodstuff. Anthocyanins are water-soluble pigments, which are responsible for red to blue color of many fruits and vegetables. They are mainly used as natural red colors. To date, there is no report of anthocyanin toxicity. Anthocyanins have also health beneficial effects such as high antioxidant activity, reduction of risks of cardiovascular diseases, stroke, cancer and anti-inflammatory activity(He and Giusti, 2010). Anthocyanins can be found in wide range of fruits and vegetables such as grapes, blackcurrants, cherries, raspberries, strawberries, red cabbage, red onion, purple corn, red potato, hibiscus flowers, black rice, and eggplants. In order to make anthocyanin application economic, the source should be abundant and has high anthocyanin concentration. That is the reason why grape pomace, a byproduct of wine industry, is the most common source for anthocyanin extraction(Mortensen, 2006).

Anthocyanins are susceptible to degradation through number of factors such as the high temperatures, pH (mainly pH higher than 7), presence of light, presence of ascorbic acid, sulfite, enzymes among other factors (Santos and Albarelli, 2013). Anthocyanins are more stable at lower moisture content. Spray drying is a common method evaluated for production of anthocyanin powder.

Mangosteen (*Garcinina mangostana* Linn.) is well known as "the queen of fruits". It is cultivated in Central America, tropical Africa and mainly Southeast Asia (Dembitsky et al., 2011). Fruits consist of two parts, the purplish pericarp and the white inner pulp. The pulp is sweet and juicy and is served fresh or it is processed into juice, jam and syrup. The pericarp composes more than half of the fruit weightand is considered as agriculture waste (Chisté et al., 2010). The purple color of the mangosteen pericarp is mainly due to anthocyanins. Palapol et al.(2009) has reported anthocyanin concentration of 182.4 to 423.5 mg/100gin mangosteen pericarp. High anthocyanin content of mangosteen pericarp is comparable to that of some commercial sources of anthocyanin such as grapes, 6-600 mg/100g and red cabbage, 25 mg/100 (Giusti and Wrolstad, 2001). Based on availability and anthocyanin concentration, mangosteen pericarp is a promising source of natural color. Nevertheless, the rapid browning of pericarp after it is cut or crushed limits its use as a red colorant. Enzymatic browning, mainly polyphenol oxidase activity is the reason for this discoloration.Among the fruits susceptible to enzymatic browning such as lulo, mangosteen, mango, banana passionfruit, papaya and guava, mangosteen pulp showed one of the highest activity, while polyphenol oxidase activity in mango and banana passionfruit was lower (Falguera et al., 2012). Enzymatic browning negatively affects the color, taste, flavor, and nutritional value of food products.

Mangosteen pericarp is a rich source of fiber (Winuprasith and Suphantharika, 2013) and different phenolic compounds. Several studies have been conducted on processing of the pericarp. They focused specially on xanthons (Chaovanalikit et al., 2012; Cheok et al., 2013; Suvarnakuta et al., 2011) and some on anthocyanins (Cheok et al., 2013; Chisté et al., 2010).In spite of high polyphenol oxidase activity in mangosteen pericarp and the obvious fast browning, in none of the studies any pretreatment step was applied to control the enzyme activity in the pericarp. Suvarnakuta et al. (2011) suggested enzymatic degradation may contribute to xanthon loss during drying of mangosteen pericarp.

Many strategies have been proposed to prevent enzymatic browning among which thermal treatment is the most conventional process. Using enzyme inhibitors is an alternative non-thermal method, among which the use of acidulants is very common. Enzymatic browning of fruits is pH dependent. Lowering the pH is important for the control of discoloration even in acidic fruits. Although PPO activity may be negligible at the natural pH value of the fruit, it may be sufficient to cause browning (Vámos‐Vigyázó and Haard, 1981).

It is necessary to apply an efficient enzyme inactivation method while the other quality parameters of product are well preserved. To reach this goal, choice of inactivation method should be made based on characteristics of polyphenol oxidase. Variation in the characteristics of polyphenol oxidase from different plant sources is a well known phenomenon. In case of mangosteen pericarp no information is available in literatures on characterisitics of its polyhenol oxidase.
The main objective of this study is to stabilize anthocyanins of mangosteen pericarp in order to develop an anthocyanin-based coloring foodstuff. This goal offers unique challenges, among which are to inhibit enzymatic browning and to protect anthocyanins against environmental influences.

Therefore, the specific objectives of this thesis are:

1- To determine the biochemical characteristics of polyphenol oxidase of mangosteen pericarp;
2- To determine and quantify the effects of thermal treatment on polyphenol oxidase activity, anthocyanin stability and color changes of mangosteen pericarp;
3- To evaluate the effect of combined polyphenol oxidase inhibitors on polyphenol oxidase activity and to evaluate their effects on anthocyanin and color stability of mangosteen pericarp;
4- To assess the retention of mangosteen pericarp anthocyanins during spray drying and to determine the effect of process variables on powders properties;
5- Tomonitor the changes in anthocyanin and color of mangosteen pericarp powder during storage.

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