



UNIVERSITI PUTRA MALAYSIA

**EXTRACTION OF NATURAL PIGMENTS FROM *HYLOCEREUS*
POLYRHIZUS GROWN IN MALAYSIA**

NASSIM NADERI

FSTM 2009 5



**EXTRACTION OF NATURAL PIGMENTS FROM
HYLOCEREUS POLYRHIZUS GROWN IN
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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2008**



**EXTRACTION OF NATURAL PIGMENTS FROM *HYLOCEREUS*
POLYRHIZUS GROWN IN MALAYSIA**

By

NASSIM NADERI

**Thesis submitted to the school of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

May 2009



DEDICATION

This thesis is dedicated to

My parents

Who taught me how to work hard and never give up my dreams

&

Who's loving ways have no bound.



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

EXTRACTION OF NATURAL PIGMENTS FROM *HYLOCEREUS POLYRHIZUS* GROWN IN MALAYSIA

By
NASSIM NADERI
May 2009

Chairman: Professor Mohd Yazid Abd. Manap, PhD

Faculty: Food Science and Technology

The use of natural colors in food is gaining importance because the number of synthetic colorants approved for use in food is legislatively restricted. There is only one type of betacyanin i.e. betanin has been approved as natural food colorant (E-162) and almost entirely obtained from the red beet. Thus, in this study *Hylocereus polyrhizus* fruit, as an alternative source of betacyanin pigments was investigated to meet the ever-increasing commercial demand. Two solvent extraction systems were investigated to determine the efficiency of solvents in recovering color pigments from pulp of *Hylocereus polyrhizus* fruit. Individual betacyanin pigments were identified according to High-performance liquid chromatographic analysis. Quantitative and qualitative differences were determined in betacyanin composition of each concentrated extract. Betanin, phylloactin, hylocerenin and their respective isomers with different ratios were the major betacyanin components of *Hylocereus polyrhizus* fruits. From solvent system selection, the aqueous ethanolic system was found more effective than ethanol in recovering betacyanins. The alteration of betanin:phylloactin peak area ratio in



ethanolic assay revealed the lower stability of phyllocactin that resulted deacylation and betanin formation. Enzymatic treatment method was optimized by means of highest yield of pigment in concentrated extract. Betacyanin retention was monitored in a range of different enzyme dosage (0.1%-2% w/v). The color characteristic of concentrated extracts from solvent extraction assays and enzymatic treatment were compared and monitored along with red beet colorant by using CIELAB system. Chroma (C^*) value was strongly influenced by applied extraction methods. The two extracts obtained from the pulp of fruits by solvent extraction assay provided color tint (h^0) close to the red beet color. Tonality of color extracts from the skin and enzyme-treated shifted into an intense purple color and due to their chroma value displayed darker color. These findings revealed that composition of betacyanin pigments in *Hylocereus polyrhizus* fruit displayed wider color spectrum compare to red beet. Application of betacyanins in *Hylocereus polyrhizus* fruit could have considerable potential for development to be employed in the food industry.



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

**PENGEKSTRAKAN PIGMEN- PIGMEN SEMULAJADI DARIPADA
HYLOCEREUS POLYRHIZUS YANG DITANAM DI MALAYSIA**

Oleh

NASSIM NADERI

May 2009

Pengerusi : Professor Mohd Yazid Abd. Manap, PHD

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Penggunaan pewarna semulajadi semakin mendapat perhatian kerana bilangan pewarna sintetik yang diluluskan oleh pihak berkuasa adalah terhad. Hanya sejenis betacianin iaitu betanin telah diluluskan sebagai pewarna makanan semulajadi (E-162) dan ianya didapati daripada ubi merah. Maka, kajian dengan menggunakan buah-buahan *Hylocereus polyrhizus* sebagai sumber gantian bagi pigmen betacyanin telah dijalankan untuk menemui keperluan komersial yang semakin meningkat. Dua ujian pengekstrakan pelarut terpilih telah dijalankan bagi menentukan kecekapan pelarut dalam pemulihan pigmen warna daripada pulpa *Hylocereus polyrhizus*. Individu pigmen betacianin dicam berasaskan kepada Analisis kromatografi cecair berprestasi tinggi. Perbezaan di antara kuantitatif dan kualitatif telah dipertahatkan bagi komposisi betacianin dalam setiap kepekatan ekstrak. Betanin, phylloactin, hilocerenin dan isomernya dengan nisbah yang berbeza merupakan komponen betacianin yang utama bagi buah-buahan *Hylocereus polyrhizus*. Daripada pilihan sistem pelarut, larutan etanol yang dikaji didapati lebih



berkesan dalam memulihkan betacianin berbanding dengan etanol. Pengubahsuaian kawasan nisbah tertinggi betanin:pilocactin dalam etanol yang dikaji menunjukkan kestabilan pilocactin yang rendah telah menyebabkan pembentukan deacylation and betanin. Kaedah rawatan dengan enzim adalah dioptimumkan dengan cara hasil tertinggi pigmen dalam ekstrak pekat. Pengekalan Betasianin diawasi dalam satu lingkungan dos enzim yang berbeza (0.1%-2% w/v). Ciri warna kepekatan yang diekstrakkan dari ujian pengestrakan pelarut dan rawatan enzim telah dibandingkan dan diperhatikan bersama dengan pewarna merah ubi dengan menggunakan Sistem CIELAB. Nilai Kroma (C^*) amat dipengaruhi oleh kaedah-kaedah pengestrakan yang digunakan. Dua hasil ekstrak yang didapati daripada pulpa buah-buahan oleh ujian pengestrakan pelarut membekalkan warna (h^o) yang hampir kepada warna ubi merah. Kualiti nada warna yang diekstrakkan dari kulit dan enzim yang dikaji menunjukkan satu warna ungu yang tumpu dan disebabkan oleh nilai kromanya menunjukkan warna yang lebih gelap. Penemuan ini menunjukkan komposisi pigmen betasianin dalam *Hylocereus polyrhizus* mempamerkan spektrum warna lebih luas berbanding dengan ubi merah. Aplikasi betacianin dalam *Hylocereus polyrhizus* akan mempunyai potensi yang tinggi untuk digunakan dalam industri makanan.



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I certify that an Examination Committee has met on to conduct the final examination of Nassim Naderi on her Master of Science thesis entitled “Extraction of Natural Pigments from *Hylocereus polyrhizus* grown in Malaysia” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree. Members of the Examination Committee were as follows:

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DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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TABLE OF CONTENTS

	Page
DEDICATION	iii
ABSTRACT	iv
ABSTRAK	vi
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATION	xx
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	
2.1 Physical basis of color	4
2.2 Physiology and attributes of color	6
2.3 Measurement of color	7
2.4 CIE system of colorimetry	8
2.4.1 Uniform color space and color differences formula	9
2.5 Natural food color market	13
2.6 Color additives	15
2.6.1 Synthetic food colorants	15
2.6.2 Natural food colors	16
2.6.3 Natural identical food colors	19
2.6.4 Coloring food stuffs	19
2.7 Betalain pigments	21
2.7.1 Red beet (<i>Beta vulgaris</i> L. ssp. <i>vulgaris</i> ‘Garden Beet Group’)	29
2.7.2 Amaranthaceae plants (<i>Amaranthus</i> & <i>Celosia</i>)	30



2.7.3	Cactus fruits	31
2.8	<i>Hylocereus polyrhizus</i> fruits	32
2.8.1	Habitant	34
2.8.2	Potential uses	34
2.9	Extraction of betacyanin pigments from plant tissues	37
2.10	Detection of pigments	39
2.11	Tracking color and pigment changes	40
III	PHYSICAL ATTRIBUTES AND CHEMICAL COMPOSITION OF SKIN AND PULP OF <i>HYLOCEREUS POLYRHIZUS</i>	
3.1	Introduction	43
3.2	General Materials	45
3.2.1	Plant material	45
3.2.2	Chemicals	46
3.3	Methods	46
3.3.1	Sample preparation	47
3.3.2	Chemical and physical analysis	48
3.3.3	Pigment extraction	50
3.3.4	Betacyanin content determination	50
3.3.5	HPLC characterization of betacyanins	51
3.3.6	Color analysis	52
3.3.7	Statistical analysis	52
3.4	Results and discussions	53
3.4.1	Characterization and chemical composition	53
3.4.2	HPLC analysis to determine the betacyanin pattern of Skin and pulp, total pigment content and color characteristic	56
3.5	Summary	64



IV EFFECT OF DIFFERENT SOLVENTS IN EXTRACTION AND BETACYANIN CONSTITUENTS OF *HYLOCEREUS POLYRHIZUS*

4.1	Introduction	66
4.2	Material and methods	68
4.2.1	Plant material and chemicals	68
4.2.2	Solvent and reference substances	68
4.2.3	Preparation of the concentrated betacyanin extract	68
4.2.4	Photometric quantification of total betacyanins	69
4.2.5	High-Performance Liquid Chromatography (HPLC)-UV Visible Detector Analysis	70
4.2.6	Other analytical measurements	71
4.2.7	Statistical analysis	71
4.3	Results and discussions	72
4.3.1	Isolation of the concentrated betacyanin extracts by solvent system selection	72
4.3.2	Pigment characterization and betacyanin retention in concentrated extracts	76
4.4	Summary	81

V EFFECT OF ENZYME TREATMENT ON THE YIELD OF BETACYANIN PIGMENTS AND CONSTITUENTS

5.1	Introduction	82
5.2	Materials and methods	85
5.2.1	Plant material	85
5.2.2	Chemical and standard	85
5.2.3	Enzymatic treatment	86
5.2.4	Preparation of the concentrated betacyanin:solvent system	86
5.2.5	Determination of sugars by HPLC analysis	87
5.2.6	HPLC conditions for organic acids	87
5.2.7	Betacyanin quantification	89
5.2.8	Determination of betacyanin retention by HPLC analysis	89



5.2.9	Statistical analysis	90
5.3	Results and discussion	91
5.3.1	Enzymatic treatment	91
5.3.2	Monitoring matrix constituent in concentrated betacyanin extracts by applying different dosage of enzyme & solvent extraction	92
5.3.3	Individual betacyanin patterns in concentrated betacyanin extract for enzyme screening	95
5.4	Summary	104
VI	COLOR EVALUATION OF CONCENTRATED BETACYANIN EXTRACTS FROM <i>HYLOCEREUS POLYRHIZUS</i> IN COMPARISON WITH RED BEET COLOR	
6.1	Introduction	106
6.2	Material and methods	108
6.2.1	Plant material and chemicals	108
6.2.2	Isolation of concentrated extracts	108
6.2.3	Spectral measurement	110
6.2.4	Color measurement by CIEL* a* b* system	110
6.2.5	Statistical analysis	112
6.3	Results and discussions	113
6.3.1	Spectra characteristic of each colorant	113
6.3.2	Color characteristic of concentrated natural extracts	115
6.3.3	Visual impact of hue and chroma in color differences of concentrated extracts	118
6.4	Summary	124
VII	SUMMARY, CONCLUSION AND RECOMMENDATION	
7.1	Summary	126
7.2	Conclusion and recommendation	132
	REFERENCES	135
	APPENDICES	144
	PUBLICATION AND CONFERENCES FROM THE THESIS	152
	BIODATA OF STUDENT	153



LIST OF TABLES

Table		Page
2.1	Natural red food color market share by product	14
2.2	Natural food color market	18
2.3	Caryophyllales and distribution of betalains	23
2.4	Main betacyanins and betaxanthins in edible plant source of betalains	25
2.5	Summary of external factors and pigment-specific that affect betacyanin stability	27
3.1	Grading system of red-purple <i>Hylocereus polyrhizus</i> in Malaysia	45
3.2	Chemical composition of skin and pulp of <i>Hylocereus polyrhizus</i> fruits in Malaysia	55
3.3	Total contents and relative concentrations (expressed as percentage of the total HPLC peak area) of betacyanins found in analyzed skin and pulp of <i>Hylocereus polyrhizus</i>	58
3.4	Relative concentration (Expressed as the percentage of the total HPLC peak area) of the six betacyanins found in analyzed <i>H. polyrhizus</i> from Malaysia and Israel	60



3.5	Chromatic parameters of the extracted fruit pulp and skin obtained from <i>Hylocereus polyrhizus</i> fruits	62
4.1	Characteristic of liquid concentrated extracts using solvent assays	74
4.2	Pigment characteristics of concentrated extract using organic and aqueous organic extraction assay and betanin/isobetanin, betanin/phyllocactin peak area ratios at 540 nm in <i>H. polyrhizus</i> pigment preparation	79
5.1	Concentration ranges and calibration equations of reference compounds used for calibration of the HPLC analysis	90
5.2	HPLC qualitative and quantitative data of sugars & organic acids in concentrated betacyanin extracts from <i>H. polyrhizus</i> fruit pulp, applying different extraction methods	94
5.3	Relative chromatogram areas and betacyanin ratios of major betacyanins in concentrated color extracts from <i>H. polyrhizus</i> fruits	96
5.4	Betacyanin retention in extracted colors obtained through enzymatic treatments monitored at λ_{\max} by spectrophotometric & by HPLC analysis	100
6.1	Chromatic parameters in concentrated betacyanin from <i>H. polyrhizus</i> using three different solvent systems	116



LIST OF FIGURES

Figure		Page
2.1	The electromagnetic spectrum. (A)Spectral power distribution of light (B)Visible spectra	5
2.2	Three-dimensional color system (A) How hue, saturation (chroma), and lightness are related to each other in a three dimensional color system. (B) A color space based on hue, chroma and lightness uses cylindrical coordinates.	10
2.3	CIE L*a*b* color space- When a color is expressed in CIE L*a*b*, L* defines lightness; a* denotes the red/green value; and b* the yellow/blue value. This three-dimensional space provided a logical framework within which the relationship between two or more colors can be calculated.	12
2.4	Chemical structure of betalamic acid (A) the main chromophore of betalains. Predominant betaxanthins (e.g. indicaxanthin from <i>Opuntia ficus-indica</i>)(B), betacyanin (e.g. betanin from <i>Beta vulgaris</i>)(C)	22
2.5	Structures of betanin and isobetanin	28
2.6	Two year old <i>Hylocereus polyrhizus</i> plant growing on trellis, modern agricultural farm, Kluang, Johor, Malaysia (Oct, 2007)	33
2.7	<i>Hylocereus polyrhizus</i> fruits (Grade B:356-450 gr)-Variety grown in Kluang, Johor, Malaysia	33
2.8	Chemical structures of nonacylated betacyanins in fruits from Cactecea: (A) Betanin, Isobetanin and acylated betacyanins: (B) Phyllocactin, Isophyllocactin (C) Hylocerenin, Isohylocerenin	35



3.1	Chromatogram registered at 540 nm corresponding to an extract obtained from skin and pulp of fresh <i>Hylocereus polyrhizus</i> fruits	57
4.1	HPLC pattern of extracted betacyanins from fruit pulp of <i>Hylocereus polyrhizus</i> (a) purified juice (B) Aqueous ethanolic assay (C) ethanolic assay (D) red beet (a) betanin, (a')isobetanin, (b)phyllocactin, (b')Isophyllocactin, (c) hylocerenin, (c') isohylocerenin	77
5.1	HPLC profile of concentrated betacyanin extract obtained through enzymatic treatment with different dosage of enzyme (0.1%-2%); from(A-F)	97
5.2	Correlation between enzyme concentration and predominant betacyanin pigments	98
5.3	Relative peak area changes (%) of betanin, isobetanin, phyllocactin and total peak areas in extracted colorants	102
6.1	Uv/vis spectrum of color extracts made from the <i>Hylocereus polyrhizus</i>	114
6.2	Hue angle of different concentrated betacyanin extract (<i>Hylocereus polyrhizus</i>) compare to commercial red beet at chroma level	119
6.3	Mean values of lightness(L [*]), chroma (C [*]), hue(h ^o) and betacyanin concentration(mg/L) of concentrated betacyanin colorants obtained from different assays	121
6.4	Two-dimensional color space of the betacyanin-based food colorants studied	123



LIST OF ABBREVIATION

ANOVA	:	Analysis of Variance
AOAC	:	Association of Official Analytical Chemists
CFR	:	Code of Federal Regulations
CIE	:	International Commission on Illumination, Vienna
DOPA	:	Dihydroxyphenylalanine
RGB	:	Color's Additive Primaries; Red, Green, Blue
E-162	:	Beetroot Red; Betanin
EU	:	European Union
FDA	:	Food and Drug Administration
HPLC	:	High-Performance Liquid Chromatography
mg	:	milligram
min	:	minute
mL	:	milliliter
M ± SD	:	Mean ± standard deviation
nm	:	Nanometer
TS	:	Tinctorial strength
USD	:	U.S. Dollars
UV	:	Ultra Violet
ε	:	Molar absorbance



CHAPTER I

INTRODUCTION

The development of foods with an attractive appearance is an important goal in the food industry. Increasingly, food producers are turning to natural food colors, since certain artificial color additives have demonstrated negative health issues following their consumption (Griffiths, 2005). Because of the deficiencies of existing natural food colorants, the demand for natural pigments is repeatedly raised by the food industry. This demand can be fuelled by research to offer a more natural-healthy way of coloring foods and provide a clean label declaration. Therefore, part of plant pigment research is seeking new sources of pigments. This will not only directed in finding natural alternatives for synthetic dyes, but also discover new procedures for the pigment production. The diversity of tropical and subtropical vegetation offers a promising range of unknown plant compounds that might prove applicable for the needs of humans.

The most common plant pigments are carotenoids, chlorophylls, anthocyanins and betalains. Most research has been focused on carotenoids and anthocyanins but betalains have recently gained interest in food science. Commonly broad arrays of anthocyanin-containing extracts are used for food coloring purposes; so far, there is only one single betalainic source that has been extensively used in the food industry worldwide. Compared to anthocyanins, betalains are ideal for coloring low-acid foodstuff as they maintain their color over a wide array of pH, from 3 to 7. The most important betalainic sources for natural red coloring are selected varieties of red beet, commercial



preparations of which are mainly composed of the red-purple betanin and its C15-isomer isobetanin.

Betacyanins are important constituents of betalain pigments. They are the main compounds associated with the red color exhibited by flowers, fruits, and other plant tissues. Red beet has been established in the market as the oldest and most abundant red food colorant, called betanin, that is known as E-162 in the European Union (Castellar et al., 2003) and as 73.40 in the chapter 21 of the Code of Federal Regulations (CFR) section of the Food and Drug Administration (FDA) in the USA (Griffiths, 2005). Practically it has been used to color foods such as yoghurt, confectionery, ice creams, syrups, sausages and processed meats. However, the typical earthy flavor caused by geosmin and high nitrate concentrations associated with the formation of carcinogenic nitrosamines (Stintzing and Carle, 2004a; Strack et al., 2003) may affect its commercial use. Furthermore, the risk to carry-over earth-bound microorganisms from the raw material in red beet is a crucial point (Herbach et al., 2006a; Stintzing and Carle, 2004). Thereby, there is a high demand for other alternative compounds that can be substitute for red beet. In recent years fruits from the *Cactaceae* have been introduced as a promising betalain source. The fruits of this family plant displayed a broader color spectrum and were devoid of the mention drawbacks in red beet (Stintzing and Carle, 2007).

Selected species of cactus fruits recognized as purple-fleshed *Hylocereus polyrhizus* have very recently been suggested as promising betacyanin source. The primary findings on the pigment potential and the scarce knowledge about color characteristics



encouraged more investigations. Preliminary data from different *Hylocereus* genotype were promising and further investigations are needed for process optimisation with respect to color shade and pigment yield. The main obstacle in these fruits is the pectic substances. Method of degradation of pectic material is necessary to facilitate pigment release, increasing yield and reducing processing wastes.

In Malaysia fruits of *Hylocereus polyrhizus* (genus *Hylocereus*, *Cactaceae*) have been cultivated as fruit crop locally. Red color pigments from fruits of *H. Polyrhizus* could be an interesting possibility as alternative to betanin from red beet. In view of the fact that most natural food colorants are supplied as concentrated juices or extracts and to get a better understanding of how pigment profile and hue are related in making decision for commercial applicability, the present work aimed to:

1. To extract betacyanin pigments from pulp and skin of *Hylocereus polyrhizus*.
2. To identify the constituents of the betacyanin pigments from pulp of *Hylocereus polyrhizus*.
3. To evaluate the effect of enzyme treatment on yield of betacyanin constituent.
4. To compare the color of extract from *Hylocereus polyrhizus* to that of red beet.

CHAPTER II

LITERATURE REVIEW

2.1. Physical Basis of Color

Color is a mental response to the stimulus that a visible radiation produces on the retina, which is transmitted to the brain by the optical nerve (Wandell, 1985). It is introduced as a psychophysical concept which is related at the same time to the psychology of the observer and physiology of vision and the spectral radiant energy of a source of light (Purves and Lotto, 2002). Color is associated with light waves and their wavelength distributions. Light, the visible part of the electromagnetic spectrum, consists of waves (Fig. 2.1 A). Each wave is introduced by its wavelength and measured in nanometers. Visible wavelengths are those between the violet and red ends of the spectrum, near 400 and 700 nm, respectively (Fig. 2.1 B). The selective absorption of different amounts of the wavelengths within these limits determines the colors of objects (Wandell, 1996). Physically, the color of an object is measured and represented by spectrophotometric curves, which are plots of fractions of incident light (that is reflected or transmitted) as a function of wavelength throughout the visible spectrum relative to a reference (Harold, 2001). Visible spectrum region consists of three dominant colors; red, green and blue that they are called additive primaries. Combination of two pure additive primaries produces a subtractive primary (cyan, magenta, and yellow) whereas the combination of all three causes white color (Purves et al., 2002).