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 MALAYSIA

NASSIM NADERI

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, phyllocactin, 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:phyllocactin 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^o) 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**

Fakulti : **Sains Makanan dan Teknologi**

Pengunaan pewarna semulajadi semakin mendapat perhatian kerana bilangan pewarna sintetik yang diluluskan oleh pihak berkuasa adalah terhad. Hanya sejenis betacianin iaitu betanin telah dilusluskan 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 dipertahatikan bagi komposisi betacianin dalam setiap kepekatan ekstrak. Betanin, phyllocactin, 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 pembentukkan 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 pengekstrakan 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 pengekstrakan yangdigunakan. Dua hasil ekstrak yang didapati daripada pulpa buah-buahan oleh ujian pengekstrakan 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.

ACKNOWLEDGEMENTS

A word of thanks is given to GOD, the source of all knowledge, by whose abundant grace this work came to fruition.

I would like to begin by thanking Professor Dr. Mohd Yazid Abd Manap, my thesis supervisor. Your guidance encouragement and faith in my success meant more to me than you can imagine. It will always be appreciated.

My sincere thanks go to Professor Dr. Hasanah Mohd Ghazali for accepting to be expert on the thesis committee and her important suggestions and her fruitful discussions.

I strongly appreciated the support, advice, freedom and the confidence they granted me during the last two years.

I would like to thank all members of the Biochemical lab for their technical help, suggestions and the pleasant atmosphere.

Finally, my thoughts go to my parents for their essential logistical help, supports, encouragement and their love.

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:

Name of Chairperson, PhD
Title: Assoc. Prof. Badlishah Sham Baharin
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Name of Examiner 1, PhD
Title: Assoc. Prof. Azizah Abdul Hamid
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Name of Examiner 2, PhD
Title: Prof. Dr. Nazamid Saari
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Name of External Examiner, PhD
Title:
Name of Faculty:
Name of University:
(External Examiner)

BUJANG KIM HUAT, PHD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

Mohd Yazid Abd. Manap, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Hasanah Mohd. Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

HASANAH MOHD. GHAZALI , PHD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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.

NASSIM NADERI

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 (Purve and Lotto, 2002). Color is associated with light waves and their wavelength distributions. Light, the visible part of the electromagnetic spectrum, consist 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 selection absorption of different amounts of the wavelengths within these limits determines the colors of object (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 consist of three dominant colors; red, green and blue that they called additive primaries. Combination of two pure additive primaries produces a subtractive primary (cyan, magenta, and yellow) whereas the combination of all three cause white color (Purves et al., 2002).