A COMPARATIVE STUDY ON THE EXTRACTION OF BETACYANIN IN THE PEEL AND FLESH OF DRAGON FRUIT

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ABSTRACT

Dragon fruit (*Hylecereus polyrhizus*) is a well known for the rich nutrient contents and used to improve many health problems. Several studies had shown the effect of various parameters on the betacyanin concentration in peel and flesh of dragon fruit. However, the comparison on the effect of different parameters to the betacyanin concentration in peel and flesh of dragon fruit has not been extensively studied. There is also lack of information on the antimicrobial potential and mineral profile in dragon fruit. This study was carried out to compare the effect of several parameters on the betacyanin concentration of peel and flesh of dragon fruit by subjecting the samples to different type of solvent, varied composition of weight of sample to volume of solvent, different length of heating time and a series of temperature. Thus, identify the optimum parameters to extract the highest yield of betacyanin in each sample. The antimicrobial potential was investigated by applying *Escherichia coli* and *Bacillus subtilis* in the samples. The mineral profile was analyzed by Atomic Absorption Spectrophotometer. The result showed the highest yield of betacyanin in peel when extracted using acetone with the weight of sample to the volume of solvent of 1:1 (w/v) within 4 minutes at temperature of 90°C. For the flesh, the optimum parameters is by using acetone with the weight of sample to the volume of solvent at 1:1 (w/v) within 5 minutes at temperature of 130°C. Betacyanin showed no indication of antimicrobial properties and calcium was detected in flesh of dragon fruit. This preliminary research had found that dragon fruit flesh have a higher stability compared to the peel. However, both have a great potential to be utilize as an antioxidant and natural colorant. These initial finding should be further studied in more controlled condition for a better understand on the betacyanin properties to maximize the total potential in dragon fruit.

ABSTRAK

Buah naga (*Hylecereus polyrhizus*) amat terkenal dengan kekayaan kandungan nutriennya dan telah banyak digunakan untuk mengatasi pelbagai masalah kesihatan. Beberapa kajian yang lepas telah menunjukkan kesan pelbagai parameter terhadap kepekatan betasianin pada kulit dan isi buah naga. Walaubagaimanapun, tidak banyak kajian dalam membezakan kesan parameter yang berbeza terhadap kepekatan betasianin di dalam kulit dan isi buah naga. Terdapat juga kekurangan maklumat mengenai potensi anti mikroorganism dan profil mineral pada buah naga. Kajian ini dijalankan untuk membezakan kesan beberapa parameter terhadap kepekatan betasianin pada kulit dan isi buah naga apabila didedahkan dengan jenis pelarut berlainan, variasi nisbah berat sampel kepada isipadu pelarut, tempoh pemanasan yang berbeza dan suhu bersiri. Dengan itu, dapat mengesan parameter optimum dalam mengekstak hasil betasianin yang tertinggi daripada kulit dan isi buah naga. Potensi mikroorganism telah dikaji dengan memasukkan *Escherichia coli* dan *Bacillus subtilis* ke dalam sampel. Profil mineral juga telah dianalisis menggunakan Spektofotometer Penyerapan Atom. Keputusan menunjukkan kulit menghasikan kandungan betasianin tertinggi apabila diekstrak dengan menggunakan aseton dengan nisbah berat sampel kepada isipadu pelarut 1:1 selama 4 minit pada suhu 90°C. Untuk isi, parameter optimum adalah dengan menggunakan aseton dengan nisbah berat sampel kepada isipadu pelarut 1:1 selama 5 minit pada suhu 130°C. Betasianin tidak menunjukkan tanda-tanda ciri anti microorganism dan Kalsium telah dikesan pada isi buah naga. Kajian awal ini telah menemukan bahawa isi buah naga mempunyai kestabilan yang lebih tinggi berbanding kulit. Walaubagaimanapun, kedua-duanya mempunyai potensi yang baik untuk digunakan sebagai antioksidan dan pewarna semulajadi. Penemuan ini seharusnya dikaji dengan lebih lanjut dalam keadaan yang lebih terkawal untuk pemahaman yang lebih kukuh tentang ciri-ciri betasianin untuk memaksimumkan keseluruhan potensi pada buah naga.

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LIST OF SYMBOLS

W	Weight
V	Volume
L	Liter
Ml	Mililiter
min	Minute
°C	Degree Celcius
ppm	Part per Million
DAA	Day After Flower Anthesis
ROS	Reactive Oxygen Species
mg	Miligram
Nm	Nanometer
Km	Kilometer
Hr	Hour
Min	Minutes
HPLC	High Performance Liquid Chromatography
AAS	Atomic Absorption Spectrophotometer
Rpm	Rotation per Minute
UV-vis	Ultraviolet- visible
A	Absorbance
DF	Dilution Factor
MW	Molecular Weight
E	Molar Extinction Coefficient
I	Path length of cuvette
SC	Betacyanin Concentration
cm	Centimeter

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CHAPTER 1

INTRODUCTION

1.1 Research Background

1.1.1 Dragon Fruit

Dragon Fruit (*Hylocereus polyzhirus*) is also known as 'buah naga' in Malaysia is belong to the family of Cactacae and order of Caryophyllales. It is becoming popular in Malaysia due to its attractive colors, sweet, juicy pleasant taste and has been considered the most beautiful in cactus family. It contains numerous edible soft black seed covered by mucilage distributed in the pulp of the fruit. The fruit have a unique appearance with green tipped overlapping scales covering the fruit. Oblong shaped fruits of genus *Hylocereus* origination from Latin America and known as red pitaya derive from climbing epiphytes belonging to the Cactacae family. Due to crassulacean Acid Metabolic (CAM), members of this family exhibit extraordinary high water use efficiency with low water requirement. (Raveh, 1998 & Winter, 1996). There are three cultivars of dragon fruit: *Hylocereus undatus*, red skin with white flesh; *Hylocereus polyrhizus*, red skin with red flesh and *Selinecereus megalanthus*, yellow skin with white flesh. In the research, the main target is to investigate *Hylocereus polyrhizus* due to its nutirition content, attractiveness and pleasant taste that are potentially marketable.

1.1.2 Potential

Comparing with other local fruits, although dragon fruit are sold at slightly higher price, there is still strong demand for dragon fruit. This is due to strong consumer demand for more natural product which is more safety and health benefit because of the public concern about possible or proven harmful effects of artificial food colorants in food processing industries, thus the trend towards replacement of synthetic colorant by natural product has been increasing although it have higher cost.(Boyd, 1998; Jackman and Smith, 1996), especially since several synthetic azo-dyes have been recently associated with hyperactivity in preschool children (McCann *et al.*, 2007). Natural colorants have a vast economic significant because the dye trade has a world market worth RM12.3 billion/ year (Harivaindaran *et al.*,2008). Showing a stable appearance in the pH range from 3 to 7, betaleins have a great potential in colouring a broad array of food. In this view, betacyanins from *H.polyrhizus* are most promising, not only as colouring agents buat also in possessing antiradical potential (Escribano and Pedre).

1.1.2.1 Antioxidant in Dragon Fruit

In the research, the ascorbic acid and betacyanin was identified as some of the natural antioxidant compound detected from previous study. Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidised to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. (Lee & Kader, 2000). The red-violet betacyanins and the yellow betaxanthins belong to the betalein pigments. (Cai et al., 2005). The uniqueness of betalains is their N-heterocyclic nature with betalamic acid being their common biosynthetic precursor. (Anderson& Francis, 2004). Natural antioxidants are primarily plant phenolics that may occur in all parts of plants, such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt & Hudson, 1990). In the research, our main focus is based on the betacyanin

antioxidant from the peel and flesh of dragon fruit. Plant phenolics are multifunctional and can act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers (Mathew & Abraham, 2006). All the phenolic classes have the structural requirements of free radical-scavengers and have potential as food antioxidants (Bandoniene & Murkovic, 2002).

1.1.2.2 Mineral Profile

Calcium accounts for one to two percent of adult human body weight. Over 99 percent of total body calcium is found in teeth and bones. Calcium is a versatile carrier of signals regulating many aspects of cellular activity such as fertilization to create a new life and programmed cell death to end it. Calcium homeostasis is strictly controlled by channels, pumps and exchangers functioning as gates for calcium entry and release. Calcium controls virtually all cellular functions including energy metabolism, protein phosphorylation and de-phosphorylation, muscle contraction and relaxation, embryogenesis and subsequent development, cell differentiation and proliferation, gene expression, secretion, learning and memory, membrane excitability, cell-cycle progression and apoptosis. (Krebs and Michalak, 2007)

Ferum is vital part of chemical component that make up hemoglobin and plays an important role in oxygen transport around our bodies. About 70 percent of iron in the human body is found in red blood cell. Insufficient iron level in the body can suffer anemia causes tiredness, muscle weakness, and loss of concentration. (The Element Iron,1999)

1.1.2.3 Antimicrobial Analysis

It has been proposed that betacyanins play a role in fungal resistance. Betanin containing beet seedlings have a higher degree of resistance to the plant pathogenic fungus *Phthium debaryum* than non pigmented seedlings. At 50 ppm, betanin reduces the growth of the causal agent of damping off by 50%. (T.J Mabry, 1980). Red pitaya was discovered contain betalein component from previous study. However, there is no research yet to be done to discover the antimicrobial characteristic in dragon fruit flesh.

1.2 Problem Statement

The motivation of the research is to compare the effect of different parameters to on the betacyanin concentration in peel and flesh of dragon fruit. A few conditions were discovered through different parameters that will give the highest yield of betacyanin from peel and flesh of dragon fruit. The parameter varied in the research is type of solvent, sample to solvent ratio, heat exposure time and temperature toward the betacyanin concentration in peel and flesh of dragon fruit extract.

It has been proposed that betacyanins plays a role in fungal resistance. The research is to verify the antimicrobial properties of the active compound in dragon fruit extract. There are also lacks of comparative study of mineral profile in the peel and flesh of dragon fruit with other local fruit.

This preliminary research is done to compare the effect of different parameters to the betacyanin content in peel and flesh of dragon fruit, to compare the mineral profile in peel and flesh of dragon fruit. Also to identify the antimicrobial properties in dragon fruit extract. Thus, identify the potential of the dragon fruit and solution for peel disposal problem by converting it from waste to wealth.

1.3 Statement of Objective

The aim of the study is to compare the effect of different parameters on the betacyanin concentration in peel and flesh of dragon fruit using spectrophometric method. Beside that, work also done to identify the antimicrobial properties of betacynin and to compare the mineral profile in peel and flesh of dragon fruit.

1.4 Research Scope

The scope of research is included as below:

I. To compare the effect of water, acetone and ethanol as extraction solvent to the betacyanin concentration in peel and flesh of dragon fruit.

II. To compare the effect of sample to solvent ratio (w/v) at 2:1, 1:1, 1:2, 1:3, and 1:4 to betacyanin extract in peel and flesh of dragon fruit.

III. To compare the effect of heat exposure time at 2, 4, 6, 8, 10 minutes to betacyanin extract in peel and flesh of dragon fruit.

IV. To compare the effect of temperature at 90°C, 100°C, 110°C, 120°C, 130°C to betacyanin extract in peel and flesh of dragon fruit.

V. To investigate antimicrobial properties of dragon fruit extract on *Escherichia coli* and *Bacillus subtilis.*

VI. To compare the mineral content of ferum and calcium in dragon fruit's peel and flesh extract.

1.5 Rational and Significance of Study

The dragon fruit peel and flesh need to be extract in order to obtain the concentrated betacyanin from dragon fruit that believes have a good potential as antioxidant, natural dye and other benefits content to human life. The previous research had revealed that betacyanin as one of the active compound in the dragon fruit. The interest of the food industry in betalains has grown since they were identified as natural antioxidants which may have positive health effects in humans. (Tesoriera et al, 2004). Previously, beetroot has been the most important natural betacyanin source for red colour, However, there is a demand for alternative compounds due to the unfavorable earthly flavor caused by geosmin and pyrizine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines (Esquel *et al.,* 2007). Less work has been done on the comparative study on the effect of different parameters to the betacyanin concentration between the flesh and peel of dragon fruit. This information is important in food processing procedure as it can reduce the loss of dragon fruit antioxidant potential in the dragon fruit product and to discover the optimum parameter needed to extract dragon fruit peel and flesh to produce the highest quality of its potential.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

2.1.1 Dragon Fruit Definition

Figure 2.1: Dragon Fruit (*Hylocereus polyrhizus*)

Dragon fruit or red pitaya belongs to the Cactaceae family from the subfamily Cactoidea of the tribe Cactea. Among the red pitaya species *Hylocereus polyrhizus* (Red flesh) fruits are edible and it has a great source of vitamin C and water soluble fiber (Mizrahi *et al,* 1999). The flesh of the fruit is deep red-purple in colour when ripened. From the description by Dr Teddy F. Tepora, the fruit is round and unique in appearance and has been considered the most beautiful cactus family. It is called 'dragon fruit' because of the bright pink to red, green tipped overlapping scales of peel covering the

fruit. The fruit is sweet, juicy, crispy and has a taste of pear, kiwi and watermelon. It has no distinct flavor. Dragon fruit is cultivated in Vietnam, Malaysia, Taiwan, China, Okinawa, Israel and Southern China (Rebecca *et al.,* 2008). Comparing with the previous source of natural colorant of red beet and amaranth, *Hylocereus polyrhizus* are highly appealing in the European and United States market.

Dragon fruit is a fast growing and developing fruit. Under Malaysia condition, the flesh and fruit will change from creamy white to full red-violet colour within 26-28 days after flower anthesis (DAA) while peel took 1-2 days longer for the green colour changed to red. The fruit is ready for harvest once the peel has turned full red but not later than 35 DAA as fruit start to crack and split and thus the quality deteriorate. (Phebe *et al.,* 2009)

2.2 Betalains

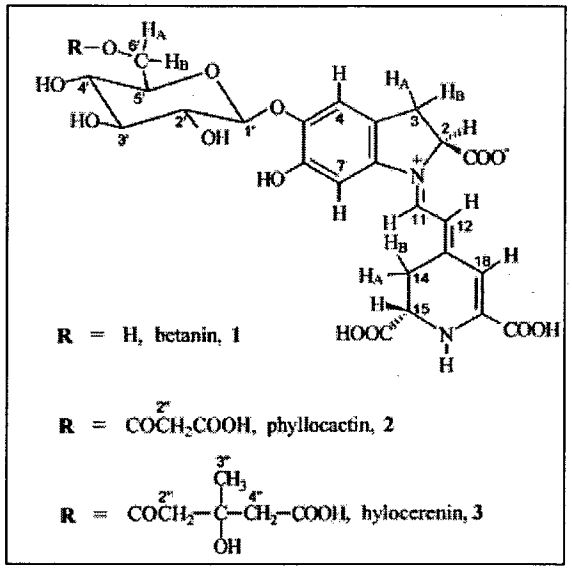

Figure 2.2: Chemical Structures of Analyzed Betacyanin in *Hylocereus polyrhizus*

Betalains are nitrogenous vacuolar pigments and important chemotaxanomical markers found in 13 families within the plant kingdom and in some members of the Basidiomycetes. It has never been found co-existing with the widely distributed plant pigment antocyanon (Stinzing and Carle, 2004).

Betalains is divided into the red-purple betacyanins and yellow-orange betaxanthins which comprice about 55 different structures and promise great variation of colour array to the food industry. There are at least seven known betalain was identified from the previous research namely betanin, isobetanin, phyllocactin, isophyllocactin, betanidi and bougainvillien-r-I (Stintzing *et al.,* 2002).

2.3 Benefits

Hylocereus polyrhizus is rich in fibers, vitamin C, minerals and phytoalbumins which are highly valued for their antioxidant properties. The dragon fruit helps the digestive process, prevent colon cancer and diabetes, neutralize toxic substances such as heavy metal, reduce cholesterol levels and high blood pressure and consumed regularly the dragon fruit can help against asthma and cough. It is also rich with potassium, protein, fiber, sodium and calcium which goods for health than other fruits.(Khalili *et.al*,2006). Previous study had found that both flesh and peel of red dragon fruit are rich in polyphenols and were good sources of antioxidant. The antiproliferative study revealed that the peel component was a stronger inhibitor of the growth of B16F10 melanoma cancer cells than the flesh (L.C. Wu *et al.,* 2005).

2.3.1 Betacyanin as Antioxidant

Betalains (betacyanins and betaxanthins), showed an antiradical effect when measured by the destruction of the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radical generated by the horse-radish peroxidase/hydrogen peroxide-mediated oxidation of ABTS. The antiradical activity of betacyanins was greater than that of the betaxanthins and increased with the pH of the reaction medium (Escribano, *et al.,* 1997). As shown for anthocyanins, betacyanins may also function as protective UV-filters (L. Chalker-Scott, 1999) and (H.A Stafford, 1994).

2.3.1.1 Definition

According to United State Institute of Health, antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Antioxidants are nature's way of fighting off potentially dangerous molecules in the body. Such molecules come in the form of synthetic chemicals such as pesticides, plastics, and chlorine byproducts and are called free radicals. Free radicals are unstable molecules that essentially feed off of otherwise healthy molecules in order to survive. Every day tens of thousands of free radicals are generated within the body, causing cell damage that can lead to chronic and degenerative diseases if left unchecked. (Leigh Erin Connealy, M.D, 2008)

2.3.1.2 Oxidation

Oxidative rancidity is initiated by oxygen free-radicals or by the reaction of molecular oxygen with pre-formed organic free-radicals from polyunsaturated fatty acids composing fats and oils. Oxidation may be prevented or delayed by antioxidants, these substances being organic molecules of either synthetic or natural origin which can scavenge the oxygen free-radicals involved in fatty acid oxidation.

Synthetic antioxidants are the most popular and widely used antioxidants, however concerns about it safe to both human and animal health is encouraging research on substances from natural origin showing antioxidant properties. Few natural antioxidants have been proved to be effective when compared to synthetic products in the same experimental conditions (B.A.Valenzuela, K.S.Nieto, 1996).

2.3.1.3 Mechanism

Antioxidants help to prevent the occurrence of oxidative damage to biological macromolecules caused by reactive oxygen species (Lindley, 1998). Reactive oxygen species (ROS) are constantly generated in vivo, both by "accidents of chemistry" and for specific purposes (Wang, *et al.,* 1996). Active oxygen forms superoxide, hydrogen peroxide (H_2O_2) and hydroxy radicals (OH) is a by- product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. Active oxygen and free radicals are produced by certain chemical carcinogens and play a role in carcinogenic process (Cerutti, 1985).

The higher antioxidant properties of certain compounds are related to their increased ability to donate a hydrogen atom to free radicals. Antioxidants reduce the primary radicals to non-radical chemical species and are thus converted to oxidize