INCLUSION COMPLEX FORMATION BETWEEN NATURAL DYE EXTRACTED FROM PITAYA FRUIT SKIN AND β-CYCLODEXTRIN: KINETIC AND THERMODYNAMIC STUDY

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ABSTRACT

The purpose of this study is to investigate the kinetic and thermodynamic parameters of inclusion complex formation between the natural dye and β-cyclodextrin (β-CD). The natural dye was extracted from skin pitaya fruit by using water extraction. The kinetic and thermodynamic studies were carried out by using UV-Vis spectroscopy in liquid phase. Effects of pH value, concentration of β-CD, temperature and ionic strength on the inclusion interactions were studied to obtain the information about the mechanism of inclusion complex. From the experiment, the best conditions for the natural dye to encapsulate into the β-CD can be determined. The pH, concentration of β-CD and temperature were determined to be pH 5, 0.004M of concentration and temperature of 25^{o}C, respectively. Formation constant with 1:1 molar ratio was calculated using the modified Benesi Hildebrand equation. The formation constant was determined in the varied temperature condition. The formation constant was decreased as temperature increased. During the time, the thermodynamic parameters which is changes of enthalpy (ΔH^{o}), changes of entropy (ΔS^{o}) and free Gibss energy (ΔG^{o}) were determined. In order to identify the thermodynamic parameters, the Van Hoff equation and the Free Gibss energy were used. From the equation, the change of enthalpy (ΔH^{o}) and the change of entropy (ΔS^{o}) are about -3.22 kJ/mol and 50.40 kJ/mol, respectively. Based on the thermodynamic parameters results, the inclusion process was deduced to be an exothermic. Since the reaction was reversible, so the reaction of the inclusion complex formation can be concluded to be spontaneously in the forward direction. The solid inclusion complex of natural dye and β-CD prepared by co-precipitation method were characterized. The characterization consist four samples which are natural dye itself, pure substance of β-CD, physical mixture and also the inclusion complex in solid form. The Fourier Transform Infrared (FTIR) was applied to examine the functional group of the guest and host molecule in the inclusion. The morphology surface of the samples was observed under Scanning Electron Microscope (SEM). Powder X-Ray Diffractometer (PXRD) was used to investigate the crystalline structure in the samples. In order to identify the thermal analysis such as percentage weight loss due to the water molecules contents, decomposition temperature, exothermic or endothermic peak assigned, the Thermogravimetry Analysis (TGA) and Differential Scanning Calorimetry (DSC) were used. From TGA result, the percentage weight loss due to the water molecule content for natural dye, β-CD, physical mixture and solid inclusion are 8.56%, 9.23%, 14.23% and 29.89%, respectively. The decomposition temperature showed that the solid inclusion complex decompose at 300^{o}C which is higher than natural dye and β-CD. It could be proved that the natural dye is included in the cavity since the solid inclusion complex decomposes after natural dye.

ABSTRAK

Tujuan kajian ini ialah untuk mengkaji parameter termodinamik dan kinetik formasi kompleks antara pencelup semulajadi dan β-siklodekstrin (β-CD). pencelup semulajadi telah diekstrak daripada kulit buah pitaya dengan menggunakan pengekstrakan air. Kajian kinetik dan termodinamik ini telah dijalankan dengan menggunakan spetroskopi ultra lembayung dalam fasa cecair. Kesan pH, kepekatan β-CD, suhu dan kekuatan ionik terhadap interaksi inklusi telah dikaji bagi mendapatkan maklumat mengenai mekanisma kompleks inklusi. Daripada eksperimen yang telah dijalankan, kondisi terbaik untuk pengkapsulan pencelup semula jadi dengan β-CD dapat ditentukan. Kondisi terbaik bagi pH, kepekatan β-CD dan suhu masing-masing adalah pada pH 5, 0.004M kepekatan β-CD dan suhu 25^{o}C. Pembentukan malar dengan nisbah molar 1:1 telah dihitung dengan menggunakan persamaan *Benesi Hildebrand* yang telah diubah suai. Pembentukan malar telah ditentukan berdasarkan kepelbagaian keadaan suhu. Pembentukan malar berkurangan apabila suhu dinaikkan. Dalam masa yang sama, parameter termodinamik seperti perubahan entalpi (ΔH^{o}), perubahan entropi (ΔS^{o}) dan tenaga bebas Gibbs (ΔG^{o}) dapat ditentukan. Untuk mengenalpasti parameter termodinamik, persamaan *Van Hoff* dan *Free Gibss* telah digunakan. Daripada persamaan tersebut, perubahan entalpi (ΔH^{o}) dan perubahan entropi (ΔS^{o}) masing-masing adalah -3.22kJ/mol dan 50.40kJ/mol. Berdasarkan hasil keputusan parameter termodinamik, proses inklusi dalam kajian adalah proses eksotermik. Memandangkan tindakbalas pencantuman formasi kompleks adalah tindakbalas berbalik, maka dapat disimpulkan bahawa tindakbalas yang berlaku adalah tindakbalas ke arah depan secara spontan. Pencantuman kompleks antara pencelup semula jadi dan β-CD dalam bentuk pepejal disediakan dengan menggunakan kaedah ko-pemendakan dan seterusnya pepejal ini telah diperincikan. Perincian terdiri daripada empat sampel iaitu pencelup semula jadi, bahan tulen β-CD, campuran fizikal dan juga kompleks inklusi dalam bentuk pepejal. Spektroskopi infra merah (FTIR) digunakan untuk meneliti kumpulan berfungsi bagi setiap sampel. Permukaan morfologi setiap sampel dikaji dengan menggunakan mikroskop elektron imbasan (SEM). Sinar-X difraktometer serbuk (XRD) digunakan bagi menentukan struktur hablur dalam setiap sampel. Bagi mengenalpasti analisis terma yang melibatkan aspek seperti kandungan molekul-molekul air, suhu penguraian, puncak eksotermik dan endotermik, analysis termogravimetri (TGA) dan kalorimetri imbasan pembezaan (DSC) telah digunakan. Dari hasil TGA, kehilangan peratusan berat disebabkan kandungan molekul air untuk pencelup semula jadi, β-CD, campuran fizikal dan pepejal pencantuman kompleks masing-masing ialah 8.56%, 9.23%, 14.23% dan 28.89%. Suhu penguraian menunjukkan pepejal kompleks inklusi mengurai pada suhu 300^{o}C di mana suhu tersebut adalah lebih tinggi berbanding dengan suhu pencelup semulajadi dan β-CD. Ia menunjukkan bahawa pencelup semulajadi telah masuk ke dalam kaviti β-CD kerana penguraian pepejal kompleks inklusi berlaku selepas pencelup semulajadi mengurai.

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R	ideal gas constant
T	temperature
ΔG	Gibbs free energy change
ΔH	enthalpy change
ΔS	entropy change
n	the order of diffracted beam
λ	wavelength of the incident X-ray beam
d	distance between adjacent planes of atoms (the *d*-spacings)
θ	single of incidence of the X-ray beam
K	formation constant
ΔF	change in the fluorescence of natural dye by addition of CD
Q	quantum yield for the complex
k	instrumental constant
I	Ionic Strength
C_i	concentration of *i*th ion
Zi	charge of *i*th ion
k’	individual proportionality constant
F	final fluorescence
F	initial fluorescence
ΔA	Absorbance

LIST OF ABBREVIATIONS

CB[6]	Cucurbit[6]bit
CD	Cyclodextrin
CGTase	glycosyl transferase
CNMR	Carbon Nuclear Magnetic Resonance
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared
GAS	Gas Anti-Solvent
HCl	hydrochloric acid
HNMR	Hidrogen Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
KBr	Potassium Bromide
L	Ligand
LD_{50}	Lethal Dose 50%
ND	Natural dye
NMR	Nuclear Magnetic Resonance
OFAT	One Factor At Time
PCA	Precipitation with Compressed Fluid Anti-solvent
PGSS	Particle from Gas-Saturated Solutions
PXRD	Powder X-Ray Diffractometer
RESS	Rapid Expansion of Supercritical Solvents
S	Substrate
SEM	Scanning Electron Microscope
TGA	Thermogravimetry Analysis
VOC	Volatile Organic Compound
α-CD	α-cyclodextrin
β-CD	β-cyclodextrin
γ-CD	γ-cyclodextrin

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Nowadays, natural dye has been progressively applied in textile application. Natural dye is one of the products that has commercial value as it is safe to the environment and consumers. Natural dyes are colorants obtained from biological matter through mechanical retention, covalent chemical bonds formation or complexes with salts or metals formation, physical absorption or by solutions (Harivaindaran et al., 2008). Plant is a major source that can produce natural dye. Natural colorants from plant sources are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes (Stintzing et al., 2002). There are plenty of plants that can be sources of natural dyes such as henna leaves (Shaukat et al., 2009), *Hibiscus mutabilis* (Padma et al., 2007), *Curcuma longa* (turmeric), *Gardenia jasmoides ellis* (gardenia) and *Carthamus tinctorius L* (safflower) (Shin et al., 2008). Natural dyes have a big potential to be commercialized due to it having a vast economic significance. The trade contributes to a world market worth of £ 2.5 billion per year. This research proposes a natural dye from dragon fruit peel that has fluorescent properties and is suitable to be used for colorant either in textile or food industries.

From the previous studies, dragon fruit or as known as pitaya from the Cactacaea family has been a promising betalains source (Stintzing et al., 2003) instead of the varieties red beet species. It can be used in the food colorant or as a replacement of the synthetic dyes. At the same time, this research is focuses on these natural pigments from dragon fruit in textile application especially in the batik industries. This is because the synthetic dyes that are mostly used in the textile industries may cause water pollution and give harmful effects to sensitive skin users. However, the major problem when dealing with natural dyes is the colour is not fasting on the fabric. The colour is easily removed from the textile. This research also proposed a new idea or technology that can overcome this problem. The property of dyes can be improved with cyclodextrins encapsulation.

Cyclodextrins (CDs) are from a family of natural or synthetically modified cyclic molecules, consisting of typically six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units. These cyclic oligomers connecting seven glucose units via α (1,4) linkages, having a toroidal shape with an apolar inside and two hydrophilic rims, thus allowing their solubilization in water (Cannava et al., 2008). These unique properties predispose them to form molecular microcapsules, namely inclusion complexes or host-guest complexes, as the displacement of included water molecules by apolar substrates represents a thermodynamically favored process. The binding between guest molecules and host cyclodextrins is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the host-guest complex fits together and on specific local interactions between surface atoms (Jiang et al., 2008). The ability of the CDs to form inclusion complexes with a large variety of molecules has made them suitable for numerous applications in food, cosmetic, agriculture, pharmaceutical, packing and textile industries. It was suggested that the complexation can considerably increase stability, solubility enhancement and bioavailability of guest molecules (Del Valle, 2004).

This research proposes the fluorescence studies on the inclusion properties of natural dyes with β-cyclodextrin. The effected factors will be investigated. The formation constant can be calculated using Hildebrand-Benesi equation (double reciprocal plot), while the thermodynamic parameters will be measured. Infra Red (IR), Thermal studies, Powder X-ray Diffractometer and UV-Vis studies are carried out in order to provide a more detailed description of the interactions. Finally, the inclusion complex was characterized.

1.2 PROBLEM STATEMENT

Before synthetic dye existed, all dyes were extracted from plants, animals or minerals. However after synthetic dyes were discovered, textile producers found that synthetic dyes were cheaper, easier to apply, more colorfast, and could be produced in a wider and brighter range of colors. Since then, natural dyes quickly fell out of favor, being replaced by synthetic dyes for most applications. interactions. Finally, the inclusion complex will be deduced.

Synthetic dyes can also be extremely toxic to manufacture, and harmful to dye workers. Textile factories (where fabrics are dyed), consume vast quantities of water to dissolve the dyes. Once the fabric is dyed, the dye-contaminated water is treated to some extent, and then expelled into rivers. Therefore, health hazards and pollution due to the use of synthetic dyes have become more apparent, more attention has now been given to natural dyes. interactions. Finally, the inclusion complex will be deduced.

However, dying with a natural dye can be difficult and there are the downsides: the dyes are harder applied onto the fabrics, and the process is more of an art form than a science. The final color is affected by many variables which can be part of the charm of natural dyes, but it can also lead to challenges in making them consistent or desirable interactions.

For commercial applications, consistency is very important and is a major reason natural dyes are rarely produced commercially. For certain applications, natural dyes require mordant to fasten the colour on the fabrics. Where safety is concerned, mordant such as alum, tannic acid, certain salts of aluminium, chromium, copper and others are basically hazardous and toxic. This is not good for the environment, nor to the workers who work in with the dyeing process.

1.3 SIGNIFICANT OF THE RESEARCH

From this research, the synthetic dyes can be replaced with natural dyes as interests in natural dyes have been increasing day by day. At the same time, it is economically favorable since the raw material of the natural dye was collected from the waste skin of the pitaya fruit. The problem of waste from the skin pitaya of the fruit can be solved. Furthermore, the waste can be utilized into a valuable product.

Recently, the investigations about natural dyes in textile have been widely proposed by research groups in terms of improving the stability and consistency when applied to fabrics. The major problem when dealing with natural dyes is the color does not last long on textile. Some additives are needed to improve the property of dyes such as the using of β-cyclodextrin. In recent studies, cyclodextrins are widely used to encapsulate and bind with other molecules. Basically, cyclodextrins are used in pharmaceutical, cosmetic, agriculture and food industries. Yet, it is not widely used in textile application. This gap of knowledge can be filled with this study.

This research proposed the fluorescence studies on the inclusion properties of natural dyes with cyclodextrins. The effected factors were investigated. Through this research, the possibility for production of natural dye from the waste skin of pitaya was focuses as future source for red dye due to it's contains rich betacyanins. Furthermore, the factors that are important in influencing the kinetics and properties of the natural dyes during the encapsulation can be identified. At the same time, some essential understanding of CD-natural dye complexes, and their important characteristics were determined. This will lead to facile and successful modifications in dyes' properties for more promising applications.

1.4 OBJECTIVES

The objectives of this research are:

i. to characterize the encapsulation between the natural dye and β-CD.
ii. to determine the kinetic interactions between the β-CD and natural dye in terms of ionic strength, pH and temperature.
iii. to investigate the favorable of thermodynamics effects such as enthalpy, entropy and Gibbs energy in different temperature ranges upon the inclusion complex formation.

1.5 SCOPES

To achieve the objectives, the following scopes have been identified:

i. to characterize the inclusion complex formation between natural dye and β-CD by using FTIR, SEM, XRD, TGA and DSC.
ii. to investigate the complexation of natural dye with β-CD in the ionic strength range (0.1 to 0.8) mol/dm^3 of sodium chloride.
iii. to investigate the influence of differential electrokinetics (pH values which ranged between 1 to 8) and concentrations of CDs (0 until $7x10^{-3}$mol/L).
iv. to determine the formation constant of the inclusion complex formation by using the Hildebrand-Benesi Equation.
v. to investigate the thermodynamic parameters (ΔG^o, ΔH^o, ΔS^o) of inclusion process in different ranges of temperature. The temperatures were set at 25^{o}C ,35^{o}C, 45^{o}C , 55^{o}C and 65^{o}C.
vi. to investigate the possibility of inclusion complex between the natural dye and β-CD.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Encapsulation in general is the inclusion of one thing within another so that the included thing is not apparent. Instead, decapsulation is the removal or the unmaking apparent thing previously encapsulated. Usually, encapsulation technology is applied in some applications such as food technology, pharmaceutical, textile, biotechnology, agriculture and environmental field. In the context, the encapsulation is used to protect compounds from oxidation, evaporation and off-flavor (Blanch et al., 2007). Basically, encapsulation techniques involved physical processes, chemical processes, high-pressure processes and molecular encapsulation (Irfan and Seiler, 2008). Physical methods are referred to mechanical technique in which an active substance is either coated or physically trapped in a carrier film. The most common physical processes for microcapsulation include spray coating (Re, 1998), fluidized bed coating (Dewettinck et al., 1999), spray chilling (King et al., 1995), melt dispersion (Bodmeier et al., 1992) and pan coating (Thies, 1996). The resulting microparticles are in the size which range is of 10-1000 μm and can either have a core-shell or a matrix structure, depending on the encapsulation technique, as well as the properties of active and carrier substance (Irfan and Seiler, 2008).

Chemical processes in which a chemically induced shell or matrix is formed around the active substance as referred to as "chemical encapsulation processes" and they usually employ emulsification or polymerization techniques (Thies, 1996). In most approaches, the carrier substance is dissolved in a solvent that forms a shell around the suspended active substance. The shell is then stabilized via cross-linking, polymerizing or by inducing changes in temperature or pH of the solution. The microcapsules or microparticles formed by chemical methods usually range from submicrometer to 500µM. Chemical processes include simple or complex coacervation (100), micromulsion (Lawrence and Rees, 2000), miniemulsion polymerization (McDonald et al., 2000, and Asua, 2002) and solvent evaporation (Watts et al., 1990).

The other method that is used for encapsulation is with high-pressure processes. The high-pressure process is developed from the organic-solvent-free methods. The combination concerned is of volatile organic compound (VOC) residual with thermodymically driven product developments in the microcapsules product has led to the development of using high-pressure processes (Irfan and Seiler, 2008). Supercritical solvents have many advantages with respect to thermodynamic, heat and mass transfer issues (Fages, 2004). The well-established high pressure processes include methods such as particles from gas-saturated solutions (PGSS) (Weidner, 1996), gas antisolvent method (GAS) (Schmitt, 1989), precipitation with compressed fluid antisolvent (PCA) (Reverchon, 1999) and rapid expansion of supercritical solvents (RESS) (Smith, 1986).

The encapsulation of guest molecules in synthetic or natural macromolecules to form molecule-in-molecule assemblies is referred to as molecular encapsulation (Inoue, 2000). It is the macromolecular assemblies on the basis of two fundamental aspect, for example self-assembly and the subsequent encapsulation of the guest molecules (Hoff et al., 2002). Between the guest and host molecules, they occurred in the presence of the functional group that are capable of noncovalent forces such as hydrogen bonding, hydrophobic forces, Van der Waals, metal coordination, π- π interactions or electrostatic forces (Reinhoudt and Crego, 2002). In fact, the subsequent encapsulation of the guest molecule is dependent on the size and shape of the guest molecule as well as on its chemical interaction with the macromolecule and the solvent (Kim and Webster, 1990, and Newkome, 1992). In the past few years, the size of the guest and carrier molecules as well as host-guest interactions, have been intensively studied to understand the phenomenon of the molecular encapsulation.

2.2 APPLICATION OF ENCAPSULATION TECHNOLOGY IN INDUSTRY

Encapsulation technology has been widely applied in many industries. The encapsulation of small guest molecules such as flavors in cyclodextrin molecules was first demonstrated by Szejtli et al. (1979). Then, Newkome et al. (1992) employed unimolecular micelles based on dendritic molecules to encapsulate small guest molecules. The application of encapsulation technology in industries was summarized in Table 2.1.

Table 2.1: Application of Encapsulation Technology (Source: Biwer et al.2002)

Application	**Industry**	**Reference**
Stabilization of volatile or unstable compounds	Food, pharmaceutical	Horikoshi et al., 1981; Bender 1986; Hedges 1992; Maggi et al., 1998;Liese et al., 2000
Reduction of unwanted tastes and odour	Food, pharmaceutical	Bender 1986; Hashimoto 1996; Nagai and Ueda 1996; Liese et al., 2000; Buschmann et al., 2001
Gelling and thickening agent	Food	Hashimoto, 1996
Protection from decomposition induced by light, temperature and air	Food	Hedges, 1992; Pederson et al., 1995; Hashimoto, 1996; Buschman et al., 2001

Dietary fibre and calorie substitute for weight control	Food	Lee et al., 1992
Perfuming fabrics or loading with antiseptic substances	Textile	Denter et al., 1992
Auxiliary material in production processes (e.g fatty acids, benzyl alcohols, antibiotics)	Biotechnology	Bar, 1996
Improvement of bioavailability and reduction of side effects (e.g Ibuprofen)	Pharmacuetical	Nagai and Ueda, 1996; Uekama and Irie, 1996; Brunet et al., 1998
Intermediate in drug production	Pharmaceutical	McCoy, 1996
Separation of isomers	Chemistry	Hedges, 1992; Szejtli 1996; Brunet et al., 1998
Solution of water-insoluble compounds	Chemistry, food	Bender, 1986; Hedges, 1992; Pedersen et al., 1995; Hashimoto, 1996; Buschman et al., 2001
Additives in pesticides	Agriculture	Szente and Szejtli, 1996
Control of plant growth	Agriculture	Brunet et al., 1998
Immobilization of toxic compounds (e.g heavy metals, tricholoroethane)	Environmental protection	Bar, 1996; Szejtli, 1996; Wilson, 1999
Improving decomposition of stable compounds (e.g tricholorfon) and sewage sludge	Environmental protection	Szejtli, 1996

2.3 ENCAPSULATION TECHNOLOGY

In general, encapsulation is the inclusion of one thing within another thing so that the included thing is not apparent. While in the supramolecular chemistry, encapsulation is an interaction between host and guest molecules in which host-guest chemistry describes complexes that composed of two or more molecules or ions that are held together in unique structural relationships by chemical forces. It is known as inclusion complex formation. The inclusion complex formation or host-guest a complex is a displacement of included water molecules by apolar substrates. The binding between host and guest molecules are not fixed or permanent (Jiang et al., 2008).

2.3.1 Guest Molecule in Encapsulation

As mentioned, encapsulation consisted of host and guest molecules when they bind the interaction between them. Therefore, guest molecule is important to be considered during encapsulation. Guest molecule can be dyes, pharmaceuticals, catalyst, cosmetic, and aromatic hydrocarbons (Irfan and Seiler, 2008). The size of guest molecule must be compatible with the diameter of the host molecule. Hence, the guest molecule can be included into the host molecule cavity. The guest molecule can be presented either with spherical geometry (single-particle structure) or with irregular geometry (aggregated structure) (Shahida and Han, 1993). The potential guest list for molecular encapsulation is quite varied and includes compounds such as straight or branched chain aliphatics, aldehydes, ketones, alcohol, organic acids, fatty acids, aromatics, gases and polar compounds such as halogens, oxyacids and amines (Schmid, 1989)

2.3.1.1 Natural Dye as a Guest Molecule

There is a demand for new method of protecting natural dyes from aggregation effects and photochemical degradation. Natural dyes have been encapsulated inside inorganic matrices such as molecular sieves and molecular containers such as cyclodextrins, cucurbitulrils, dendrimers and self-assembled gels. Natural dye has a few drawbacks such as it has the tendency to aggregate, which induces multichromophoric interactions that can alter the color quality and quench the photoluminescence. Also, natural dye has long-wave-length absorption bands which are susceptible to chemical and photochemical degradation. Somehow, these limitations can be improved by molecular encapsulation (Arunkumar et al., 2005).

Table 2.2: Number of papers and patents application on color encapsulation found in CYCLOLAB's database in August 1997

Key words	No of papers	No of patents	Total No
Color	100	96	196
Dye	188	68	256
Carotene	14	17	31
Anthocyanine	9	2	6
Curcumin	4	2	6
Betalains	1	0	1
Chloropyll	1	4	5
Xanthophylls	1	0	1

Source: Lajos et al. (1998)

Based on the Table 2.2, there are only several studies that used dye or colorant as a guest molecule.

2.3.1.2 Natural Dye from the Pitaya Waste

One of the most recent newcomers in the Malaysian fruit industry is dragon fruits (*Hylocereus polyrhizus*). After many trials and errors, dragon fruit cultivation has made its way in the Malaysian fruit industry. Unlike Vietnam and Thailand which have a long history of dragon fruit growing, Malaysia is fast keeping pace leading its niche market due to high local demand. Ministry of Agriculture stated that the scale for dragon fruit cultivation is very small which is about 300 hectares compared to others crops. However the rate of expansion is increasing tremendously fast. The dragon fruit growing areas are in the states of Johor, Perak, Negeri Sembilan, Pahang, Pulau Pinang and Sabah. There are three species of dragon fruit in the genus of *hylocereus* which are *hylocereus guatemalensis*, *hylocereus polyrhizus* and *hylocereus undatus*. The flesh can be red, white or magenta, as the varying degree is dependant upon the variety. The pitaya or dragon fruit has become a famous crop due to its unique shape, attractive red colour and high functional properties (Phebe et al., 2009). *Hylocereus polyrhizus* and its related species belong to the vine cacti from the subfamiliy Cactoideae of the tribe Cacteae and family of Caryophyllales (Raveh et al., 1993). It is also known by its common name pitaya or 'the scaly fruit' since it contains scales on its fruit skins (Wybraniec, 2001). The peel is usually red, and the pulp varies from red to purple various hues to white. The flesh is delicate and juicy and contains soft black seeds.

2.3.1.3 Characterisation of Natural Dye from Pitaya Waste

Since natural colorant is receiving growing interest, many researchers started to investigate the potential of this crop due to its colour contents and functional properties. Previous research has investigated that pitaya contains betalains pigment. The red-violet betacyanins and the yellow betaxanthins belong to betalains pigments (Cai et al., 2001). The red colour of pitaya is attributed to betacyanins, which is a class of water-soluble pigments (Wybraniec et al., 2007). Alternatives to pitaya are betacyanins from the genus *Amaranthus* (Corke et al., 2005), red beet root (Pavlov et al., 2002) or *Opuntia ficus-indica* (Stintzing et al., 2005)

Betalains are a class of red and yellow indole-derived pigments found in the plant of the Caryophyllales, where they replace anthocyanin pigments. Betalains have never been found together with anthocyanins in the same plant. Unlike three main classes of plant pigments (chlorophyll, carotenoids, flavonoids), betalains have limited distribution.

They are only found in the petals of flowers, but the color of the fruits, leaves, stems and roots of plants may also contain them. The name “betalain” comes from the Latin name of the common beet (*Beta vulgaris*) from which betalains were first extracted. The deep red color of the beets, *bougainvillea*, *amaranth* and many cacti are results from the presence of betalain pigments. They are the nitrogenous vacuolar pigments of 13 families within the plant kingdom also accumulating by some members of the Basidiomycetes (Gill, 1994, and Clement and Mabry, 1996). They comprise two subgroups, betacyanins and betaxanthins (Steglich and Strack, 1990, and Strack et al., 2003). Betacyanins include the reddish to violet betalains pigments, while betaxanthins appear to be yellow to orange.

Betalains are synthesized from the amino acid tyrosine through L-DOPA into two subclasses, betacyanins (reds and purples) and betaxanthins (yellows). Recently, researcher had investigated about betacyanin and it is found in the fruit pulp of *Hylocereus polyrhizus* (Wybraniec et al., 2001, and Stintzing et al., 2004). Mizrahi et al. (1997) claimed that *Hylocereus polyrhizus* has already been produced commercially for its glowing deep red-purple flesh fruit. Betalains have great potential in colouring a broad array of food because it shows a stable appearance in the pH range of 3 to 7. In this view, betacyanins from *H.polyrhizus* are most not promising, only as a coloring agent but also in processing radical potential (Escribano et al., 1998). Structural study that has been done by Wybraniec et al. (2001) reported that there are three main betacyanins (betanin, phyllocactin and hylocerenin), with corresponding C-15 diastereoisomers.

While, Stintzing et al. (2002) reported that 10 betacyanins could be separated by using HPLC-PDA. Eight of the betacyanins could be identified, but the other two remains unknown. They were unequivocally assigned to bougainvillein-r-I, betanin, isobetanin, phyllocactin, iso-phyllocactin, iso-betanidin-5-O-(6'-O-3-hydroxyl-3-methyl-gulatryl)-β-glucoside, betanidin-5-O-(6'-O-3-hydroxyl-3-methyl-gulatryl)-β-glucoside and betanidin-5-O-(6'-O-3-hydroxyl-butyryl)-β-glucoside.

2.3.1.4 Other Current Uses of Natural Dye

Dragon fruit is among the most nutritious and wonderful exotic fruits. It is a favorite to many, particularly people of Asian origin. It features a mouth watering, slightly sweet taste with an intense shape and color, not forgetting its outstanding flowers. In addition to being tasty and refreshing, this beautiful fruit boasts a lot of water content and other vital minerals with varied nutritional ingredients. Due to its vital nutrients, the pitaya fruit is suitable for all diets as it supplements fiber which is best for laxative purposes and the liver. Similarly, people who suffer from high blood pressure, diabetes and obesity have attested to the fruit's goodness. Apart from that, dragon fruit also has the potential to become food and textile colorant.

There has been much interest in the development of natural colorant to be used in the food industry. It is apparently so because of a strong consumer demand for more natural products. Food coloring also becomes a popular way to achieve the desired hues because application does not require certification (Stintzing et al., 2004). In addition, coloring from fruit or vegetables extract may contain additional ingredients of nutritional vales as demonstrated recently by pitaya or *H.polyrhizus*. In Israel, the pulp of *H.polyrhizus* is already being used for red-violet ice-cream (Wybraniec et al., 2001). In other parts, red colorants from the red beet root (*Beta vulgaris* L.) are approved additives for the use in foods in the United State (Title 21 of the Code of Federal Regulation, 21 CFR 73.40) and in the European Union (E-162) and commercially they are exempted from batch certification (Castellar et al., 2003). This statement shows that the natural colorant from the plants has a great potential to replace the former coloring agent in the food industry.