DEVELOPMENT OF BETACYANIN PIGMENT EXTRACT AND MICROENCAPSULATED POWDER FROM RED PITAYA FRUIT (*HYLOCEREUS POLYRHIZUS)*

RURI ADITYA SARI

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by

RURI ADITYA SARI

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Norziah, M.H., & Ruri, A.S. (2006). Compositional studies on high dietary fruit fibre obtained from local Malaysian fruits. Proceedings of the *National Conference on Food Science & Nutrition.* 13-14 December, 2006. Kota Kinabalu, Sabah, Malaysia, pp 249-255.

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PEMBANGUNAN EKSTRAK PIGMENT BETACYANIN DAN SERBUK MIKROENKAPSULASI DARIPADA BUAH PITAYA MERAH (*HYLOCEREUS POLYRHIZUS*)

ABSTRAK

Pigmen betacyanin dari buah naga merah (*Hylocereus polyrhizus*) boleh menjadi sumber menarik pewarna merah semula jadi untuk aplikasi makanan. Pigmen betacyanin telah diekstrak daripada isi dan kulit buah naga merah tempatan di Malaysia dengan menggunakan aseton, metanol dan air sebagai pelarut. Didapati bahawa kadar pigmen betacyanin di dalam isi lebih tinggi berbanding dengan kulit (10.14 ± 0.57 dan 6.69 ± 0.21 mg setara betanin, (BE)/ 100g, masing-masing). Berdasarkan kandungan pigmen betacyanin, ekstrak pigmen daripada isi buah naga merah yang diekstrak menggunakan air dipilih untuk kajian seterusnya. Kestabilan larutan ekstrak pigmen dikaji pada pH yang berbeza (2 -10), suhu yang berbeza (25 ˚C - 75 ˚C), kepekatan ion logam (Cu^{2+} dan Fe^{2+}) yang berbeza (0 - 150 ppm) dan penambahan asid askorbik (0 - 1.6 %). Kandungan fenolik dan flavonoid daripada ekstrak pigmen didapati meningkat lebih banyak setelah mengalami proses klarifikasi. Dapatan kajian daripada analisis HPLC dan LC-MS mengesahkan kehadiran betanin dan phylocactin juga isoformnya termasuk hylocerenin dalam ekstrak pigmen betacyanin. Ekstrak pigmen buah naga telah dikeringkan menggunakan teknik pengeringan semburan untuk meningkatkan kestabilan dan jangkahayat ekstrak pigmen yang diperolehi. Dua jenis maltodekstrin (10 DE dan 25 DE) dan campuran (10 DE + 25 DE) digunakan sebagai agen lapisan dalaman bagi teknik pengeringan semburan untuk memberikan pepejal terlarut total (TSS) berjulat di antara 20 % - 30 %. Serbuk pigmen pitaya yang dihasilkan pada suhu pengeringan (200 ˚C) dicirikan kepada kandungan pigmen betacyanin, perolehan pigmen, warna, kandungan air, keterlarutan dan higroskopisiti. Pengaruh penambahan asid askorbik (0.1 % dan 1.0 % b/b, masing-masing) ke dalam ekstrak pigmen buah naga sebelum pengeringan semburan untuk mengkaji kestabilan ekstrak pigmen buah naga juga diselidiki dengan kehadiran cahaya dan kelembapan pada suhu bilik. Penggunaan campuran maltodekstrin (10 DE dan 25 DE) ke dalam serbuk pigmen buah naga sebagai agen pelapis dalaman meningkatkan higroskopisiti dan kestabilan selama penyimpanan berbanding dengan 25 DE dan 10 serbuk DE secara berasingan. Penggunaan campuran maltodextrin 10 DE dan 25 DE pada perbandingan 1:2 terpilih sebagai agen pelapis dalaman. Setelah pigmen disimpan pada relatif kelembapan 0 % RH (25 ˚C) selama 24 minggu dalam gelap, degradasi pigmen yang rendah (21.9 ± 0.26 %) diperhatikan pada serbuk pigmen buah naga yang ditambah dengan 0.1% (b/b) asid askorbik. Dalam kajian ini, serbuk pigmen buah naga dan pewarna komersil diaplikasikan dalam sistem model makanan iaitu jeli untuk menentukan ciri-ciri warna dan kestabilan pada suhu yang berbeza. Keputusan kajian menunjukkan bahawa betacyanin diperolehi daripada isi buah naga (*Hylocereus polyrhizus*) merupakan pewarna semula jadi yang berpotensi untuk digunakan dalam aplikasi makanan.

DEVELOPMENT OF BETACYANIN PIGMENT EXTRACT AND MICROENCAPSULATED POWDER FROM RED PITAYA FRUIT (*HYLOCEREUS POLYRHIZUS*)

ABSTRACT

Betacyanin pigments from red pitaya fruit (*Hylocereus polyrhizus*) could be an attractive source of natural red colourant for food application. The extraction of betacyanin pigment was extracted from the flesh and peels of red pitaya fruits grown locally in Malaysia by using acetone, methanol and water as the extracting solvents. Both the flesh and peels were investigated and it was found that the flesh had higher of pigment contents compared to the peels (10.14 ± 0.57 and 6.69 ± 0.21 mg of betanin equivalents, (BE)/100 g, respectively). Based on the betacyanin content, the pigment extract with water extraction obtained from red pitaya fruit flesh was selected for further study. The stability of aqueous pigments extract was investigated at different pH (2 -10), different temperatures (25 ˚C - 75 ˚C) and in presence of varying concentrations (0 - 150 ppm) of metal ions (Cu^{2+} and Fe^{2+}) and ascorbic acid (0 - 1.6 %). The phenolic and flavonoid contents were effectively increased by further optimising of the clarification process on the pigment extracts. The HPLC and LC-MS studies confirmed the presence of betanin and phyllocactin and their isoforms including hylocerenin in the betacyanin pigment extract. In order to increase the stability of pitaya pigment extract, Spray-drying technique was also performed on the pigment extracts to increase the stability and shelf life of the pigment extracts obtained. Two types of maltodextrin with different DE's (10 DE and 25 DE) and mixtures (10 DE + 25 DE) were used as coating agents in the spray drying technique to give a total soluble solid (TSS) ranging from 20 % - 30 %. The quality attributes of the pitaya pigment powders produced at drying temperatures

(200 ˚C) in the spray drying technique were characterized by their betacyanin pigment content, pigment recovery, colour, moisture content, solubility and hygroscopicity. The stabilising effect of addition ascorbic acid (0.1 % and 1.0 % w/w, respectively) into feed pitaya pigment extract prior to spray drying was also investigated under the presence of light and moisture at room temperature. The use of mixtures maltodextrin (10 DE and 25 DE) in pitaya pigment powders as coating agent enhanced the hygroscopicity and the stability during storage compared to the 25 DE and 10 DE powders separately. The use of mixtures 10 DE and 25 DE at ratio 1:2 was selected as coating agent. After pigment being stored at 0 % RH (25 ˚C) for 24 weeks in the dark, lower pigment degradation (21.9 ± 0.26 %) was observed in pitaya pigment powder supplemented with 0.1% (w/w) ascorbic acid. In this study, pitaya pigment powders and commercial colourants were evaluated to determine colour characteristics and stability at different temperatures in jelly. The results showed that betacyanin obtained from pitaya (*Hylocereus polyrhizus*) could be a potential natural colourant use in food applications.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Colour is the first characteristic of a food and often predetermines or "colours" our expectation. We use colour as a way to identify a food and a way to judge the quality of a food. Now health-conscious consumers are taking the more seriously. They want appropriate colour, but they want it "natural." Consumers are concerned about the foods and beverages they consume and how it affects their health and the health of their children.

According to Heller (2009), there is an ongoing review of colourants within Europe. The Food Standards Agency (FSA) in UK has published a list of food products that have been reformulated to remove six food colours associated with hyperactivity in young children. In 2008, the FSA proposed a voluntary ban to phase out six food colourants (Tartrazine (E102), Quinoline Yellow (E104), Sunset Yellow (E110), Carmoisine (E122), Ponceau 4R (E124) and Allura Red (E129)) from food products by 2009, which is in line with the action by the European Union (EU). This new legislation on food additives that states a requirement for food products in the market containing any of the six colours should carry additional labeling information that states "Consumption may have an adverse effect on activity and attention in children".

Hylocereus polyrhizus mostly known as pitaya, pitahaya or dragon fruit were native to central South America. For many years efforts have been made to develop the cultivation of pitayas, vine species of the genus *Hylocereus.* The colour of the fruits depended on the species, some of them contained pulp of red and or/ purple colours in various hues (Mizrahi et al., 1997; Mizrahi & Nerd, 1999).

In pitayas the most important pigments are betalains which consist of the betacyanins and betaxanthins (Gibson & Nobel, 1986). The most important betalain sources for natural red colouring is from a variety of red beetroot, commercial preparation of which are mainly composed of the red-purple betanin and its C_{15}-isomer isobetanin.

Red beetroot or *Beta vulgaris* which is commercially prepared in powder forms or juice concentrates and listed as E162 in Europe, is extensively used in the food industry worldwide as a red colourant. However, because of the unfavourable earthlike flavour characteristics caused by geosmin and pyrazine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines, there is a demand for alternative compounds (Castellar et al., 2006; Stintzing & Carle, 2004). Red pitaya exhibits a pigment spectrum consisting of non-acylated (i.e., betanin = betanidin 5-*O*-β-glucoside) as well as acylated betacyanins (i.e., phylocactin = betanidin 5-*O*-β-malonyl-glucoside), (Stinzing et al., 2002b; Wybraniec et al., 2001). Hence, fruits from the Cactaceae family have been proposed as a promising betalain source (Stintzing et al., 2001, 2003). Fruits from *Hylocereus polyrhizus* produce a deep purple-coloured flesh comparable to red beetroot (Stinzing et al., 2000) or amaranth (Cai et al., 1998a). It is known that *Hylocereus* cacti are the third richest betacyanin source for food colouring agents after *Beta vulgaris* and *Amaranthus* species. Betacyanin pigments from red purple pitaya fruit could be an attractive source of red colourant for food application.

In previous studies (Wybraniec et al., 2001; Stintzing et al., 2002b; Wybraniec & Mizrahi, 2002; Wu et al., 2006), efforts have been made to quantify and determine the identity of betacyanin pigment in pitaya fruits cultivated in Taiwan and Israel. Wu et al. (2006) also studied on total phenolics and flavonoids in the fruits.

Though efforts have been made to characterize the pitaya fruits grown in Taiwan and Israel, both places are of different climate compared to Malaysia. Since the plants are able to tolerate drought, heat, poor soil and cold, they are able to grow under most of the climates in different parts of world. In Malaysia, there is an alternating wet and dry seasons of the tropical climate and this suitable for the growth of pitaya fruits. From a survey conducted by Department of Agriculture, Malaysia (Anonymous, 2006a), about 435.8 ha of land located separately in Malaysia has been established for the cultivation of pitaya fruits, particularly in parts of Kluang, Johore. Even though it was just introduced few years ago, the fruit cultivation had increased substantially over the years due to the high demand from local and overseas market.

Betalains show good stability in the pH range from 3 to 7, thus have a great potential in colouring a broad array of food. However, these pigments are generally considered heat-labile, and are also affected by pH, light, air and water activity (Jackman & Smith, 1996a) and are highly instable compared to synthetic food colourant like Amaranth and FD&C Red #3 (Cevallos-Casals & Cisneros-Zevallos, 2004). Thus alternatively these colour pigments could be encapsulated and spray dried to produce pigment powder to enhanced storage stability. There are many studies on spray drying of betacyanin pigments from *Amaranthus* (Cai & Corke, 2000), *Opuntia ficus-indica* (Saénz et al., 2009), *Opuntia stricta* (Obón et al., 2009) and Red beetroot (Azeredo et al., 2007). However, no reports were found on production of spray dried betacyanin pigment from red pitaya fruit (*Hylocereus polyrhizus)* grown in Malaysia. Thus the evaluation of spray drying condition to produce pigment powder needs to be studied.

The research study comprised of three phases. The first phase was extraction of betacyanin pigment from red pitaya (*H. Polyrhizus*) fruits with various solvents to obtain the most suitable solvent to be used. These pigment extracts were characterized further for the phenolic, flavonoid and antioxidant capacity. This phase included stability studies against selected factors (temperature, pH, metal ions and additive) and investigated the effects of two pectinase enzymes for clarification of the aqueous pigment extracts. The second phase involved microencapsulation study to produce pigment powder using spray drying technique. The third phase investigates the colour stability of pigment powder in food model system.

1.2 OBJECTIVES

The main objective of this research is to produce natural colourant from red pitaya fruit. The specific objectives of this research work are:

a) To produce and characterize the aqueous betacyanin pigment extract.
b) To prepare and characterize microencapsulated betacyanin in a powder form using spray dried technique.
c) To evaluate the colour stability in a model food system in comparison with commercial red colourants.

CHAPTER 2

LITERATURE REVIEW

2.1 Red pitaya (*Hylocereus polyrhizus*)

2.1.1 Classification

Red pitaya is native to Central South America and the tropical forest regions of Mexico (Mizrahi et al., 1997). The pitaya (*Hylocereus* sp.), known as strawberry pear, thany loy (in Vietnam), pitahaya roja (in Spanish) and la pitahaya rouge (in French), grows on tropical climbing cacti. In Malaysia, pitaya is alternatively known as dragon fruit, 'huo long guo' or 'buah naga' in various languages. Currently, they are being grown commercially in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia and USA. For the past few years, extensive efforts have been made to develop the cultivation of pitayas commonly known as the 'dragon fruit' in Malaysia.

The classification of pitaya (*Hylocereus polyrhizus*) is shown below (Danial, 2008);

Red pitaya (*Hylocereus polyrhizus*)

Order : Caryophyllales

Family : Cactaceae

Genus : Hylocereus

Species: Polyrhizus

There are three types of pitaya, the normally white-fleshed (*H. Undatus*), red-purple fleshed (*H. Polyrhizus*) and white-fleshed with yellow skin (*Selenicereus megalanthus*) (Plate 2.1), varying degrees dependent upon variety. All of these species are sprinkled with tiny edible black seed. Species of *Hylocereus* included *H.*

Costaricensis, *H. Purpusii* (all red flesh species) and the white flesh species *H. Undatus* (Wybraniec & Mizrahi, 2002) among which *H. Polyrhizus* having a glowing deep red-purple fruit flesh is more commercially cultivated in Malaysia.

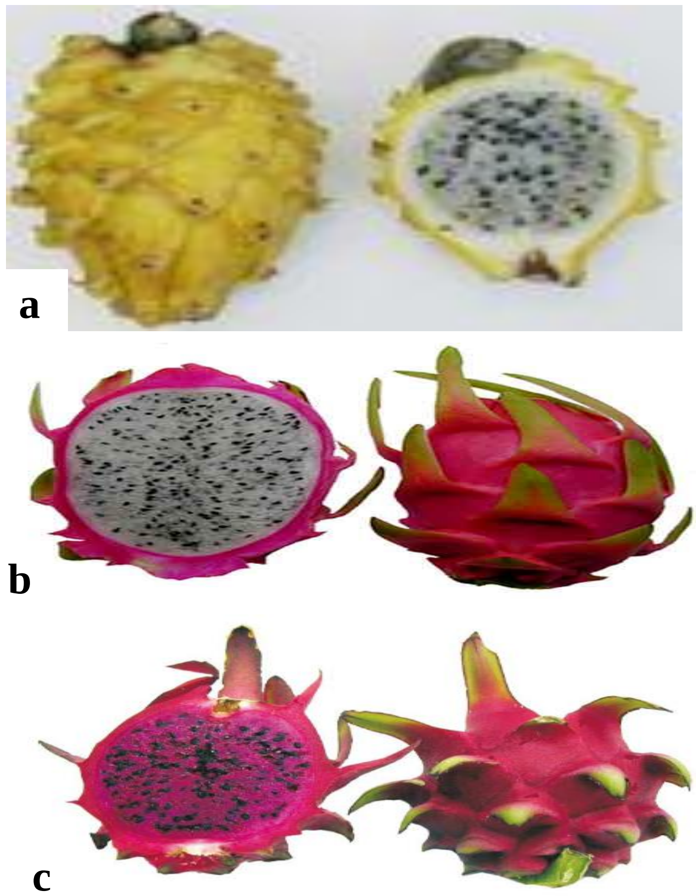


Plate 2. 1 Types of Pitaya fruits ((a) *Selenecerius Megalanthus*, (b) *H. Undatus*, (c) *H. Polyrhizus*) (Source: Danial, 2008)

Nerd et al. (1999) studied the optimum date of harvest in relation to colour development of pulp and peel and postharvest behaviour of *Hylocereus undatus* (Haworth) Britton & Rose and *H. Polyrhizus.* Nerd and Mizrahi (1998) reported that yellow pitaya (*Selenicereus megalanthus*) showed the duration of fruit development depends on seasonal temperatures and that the fruits reach the optimal flavour close to full colour stage. However, little research has been done on red pitaya fruit development and on the behaviour of the fruit during or after storage.

Pitaya have a climbing growth habit, reaching 10 m or more in height if suitable supports available (Plate 2.2). Seeds grow well in compost or potting soil mix - even as a potted indoor plant. Pitaya cacti usually germinate between 10 and 14 days after shallow planting (Danial, 2008). As they are cacti, overwatering is a concern for home growers. As their growth continues, these climbing plants will find something to climb on, which can involve putting aerial roots down from the branches in addition to the basal roots. Once the plant reaches a mature 4.5 kg weight, the flower of the plant could be grown. The plants can flower between three and six times in a year depending especially on growing conditions. Pitaya cacti flower overnight, usually wilting by the morning (Plate 2.3). They rely on nocturnal creatures such as bats or moths for fertilization by other pitaya. Self fertilization will not produce fruit. This limits the capability of home growers to produce the fruit.

Like other cacti, if a healthy piece of the stem is broken off, it may take root in soil and become its own plant. This is a much shorter route to reproduction. The plants handle up to 40 ^{o}C and very short periods of frost, but do not survive long exposure to freezing temperatures (Danial, 2008). Pitaya is large in size, average of 300 - 500 g for each fruit. It is oblong in shape with a red peel and large green scales. The scales turn yellow upon ripening. For the *H. Polyrhizus*, the colour of the fruit skin begins to change 25 to 35 days from flowering. At the same time, flesh firmness approaches a minimum and the eating quality approaches a maximum 33 to 37 days after flowering.

As the fruit matures, acidity reaches a peak just as the skin colour change occurs, then declines 25 to 30 days after flowering (Nerd et al., 1999). Fruits can be harvested from 25 to 45 days after flowering. The size of the fruit depends on seed number in fruit (Castellar et al., 2003). The recommended storage temperature for pitaya is around 10 ˚C since lower temperatures induce microbial growth and chilling injury. Fruit must be kept moist; either by misting, or by storage in a sealed box or plastic bags to prevent desiccation. If the skin removed, the inner flesh will keep well for up to a month in the refrigerator; or can be frozen for later use.

Plate 2.2 Pitaya's plant (Source: Danial, 2008)

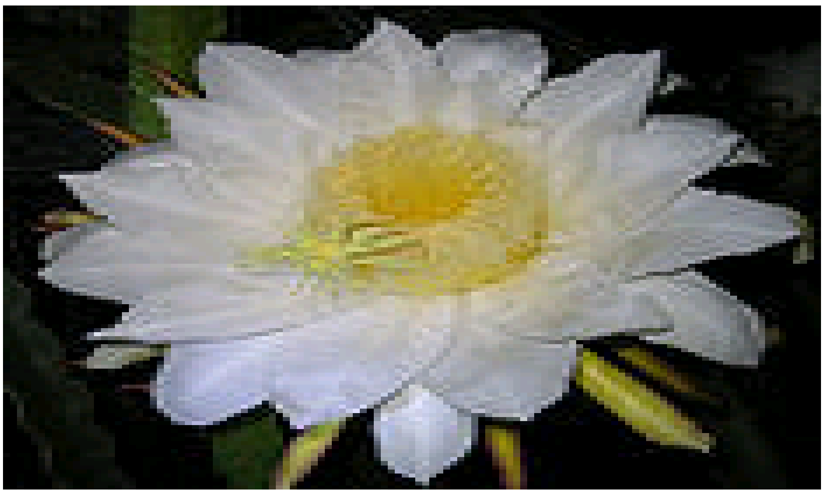


Plate 2.3 Pitaya's bloom (Source: Danial, 2008)

2.1.2 Potential in food application

There are many studies stated that pitaya is a good source of fibres, which gave the juice a favourable mouth feel and helps to reduce blood sugar and plasma cholesterol levels (Fernández et al., 1992; Munoz-de-Chavez et al., 1995; Trejo et al., 1995). Beside, pitaya has significantly high amount of vitamin C and have high nutritional value and medicinal value, it helps our body especially in digestion, preventing colon cancer and diabetes. Antioxidants in the diet reduce the risk of cardiovascular disease and cancer (Pedreno & Escribano, 2001; Esribano et el., 1998; Kanner et al., 2001). Antioxidant activity and health improving capacity of pitaya has been reported in the previous studies (Lim et al., 2007; Wu et al., 2006; Mahattanatawee et al., 2006). The pitaya fruit has a stronger antioxidant activity than most vegetables (Schliemann et al., 1999).

Pitaya is very particular for the presence of betalains, a widely used natural colourant in the food industry. Recently, Pitaberry Sdn. Bhd. was applied the pitaya fruit to beverages. They claimed that there are no artificial colouring added due to the natural occurring of red-purple colour impart provided by itself. In addition, it also can be made into a nutritious fermented “enzyme” drink which is sold in Malaysia as a health food supplement.

There are some studies conducted on mucilages of Cactaceae family fruit which consists of complex polysaccharides, mainly composed of arabinose, galactose, rhamnose, and galacturonic acid (Lee et al., 1998; Saénz et al., 1992). The fruit’s mucilages have a high water-holding capacity, so they could serve as thickening or emulsifying agents and form viscous or gelatinous colloids, these properties are needed especially in food systems like jam, jellies and ice-cream production (Piga, 2004). Furthermore, Arriffin et al. (2009) and Rui et al. (2009)

have been studied the extraction and characterization of seed oil obtained from pitaya fruit.

In addition, the consumers concern about side effect of artificial colourant applied in food system, thus there was increasing demand for natural colourant in drink, dairy products and many food productions. In cacti, the most important fruit pigments are the betacyanin and betaxanthins (Wybraniec et al., 2001). The known betacyanin pigments of *Hylocereus polyrhizus* are betanin, phylocactin (6′-*O*-malonylbetanin), and a recently discovered betacyanin, hylocerenin (5-*O*-[6′-*O*-(3″-hydroxyl-3″-methyl-glutaryl)-β-$_{D}$-glucopyranoside) (Wybraniec & Mizrahi, 2002; Wybraniec et al., 2001).

2.2 Food colourants

The first characteristic of food that is noticed is its colour and this predetermines our expectation of both flavour and quality. Food quality is first judged on the basis of colour and we avoid wilting vegetables, bruised fruit, rotten meat and overcooked food. Numerous tests have demonstrated how important colour is to our appreciation of food. When foods coloured, the colour and flavour are matched, i.e. green for lime, yellow for lemon and the flavour is correctly identified on most occasions. However, if the flavour does not correspond to the colour then it is unlike to be identified correctly (Henry, 1996).

Colour level also affects the apparent level of sweetness. The colour of a food will therefore influence not only the perception of flavour, but also that of sweetness and quality. The best food with perfect balance nutrients is useless if it is not consumed. Consequently, food needs to be attractive. During this century, the use of synthetic colour has steadily increased at the expense of these products of natural origin, due principally to their ready availability and lower relative price. Generally two types of organic food colours are recognized in the literature: synthetic colours and natural colours.

2.2.1 Food colourants in Malaysia

According to Malaysian Food Acts and Regulations (Anonymous, 2006b) as shown in Table 2.1, all the additives were added in food which gave colour are considered as colourants. Colourants which added in food should be contained more than 4 percent of approved colourants that listed in Food Act. Futhermore, liquid colourants could be contained benzoate acid (below 400 ppm) as the preservatives.

Table 2.1 Colourants listed in Malaysian Food Acts and Regulations

Colourant	Index number
Allura Red AC	16035
Amaranth	16185
Brilliant Black PN	28440
Brilliant Blue FCF	42090
Carmoisine	14720
Chocolate Brown HT	20285
Erythrosine BS	45430
Fast Green FCF	42053
Green S	44090
Indigotine	73015
Ponceau 4R	16255
Red 2G	18050
Sunset Yellow FCF	15985
Tartrazine	19140
Quinoline Yellow	47005

Source: Anonymous (2006b)

2.2.2 Synthetic colours

Colour is a major contributor to flavour anticipation and is often perceived before aroma. It is known to play a major role in the acceptability of food products. Indeed there are many product types which would be greatly curtailed without the ready availability of safe colourants. For example, soft drinks, puddings, gelatins, reconstituted and imitation fruits, candies, jelly, pastries, ice-cream, syrups, sauces and snacks. Some studies have been shown that food does not taste ‘right’ when it does not have the proper colour (Johnson & Lichtenberger, 1980). Since the synthetic colours have high tinctorial power, very small amounts of them are adequate for practically all applications. Use level customarily needed range from 20 or 30 ppm to about 300 ppm in foods ready for consumption (Francis, 1996).

These are colourants that do not occur in nature and are produced by chemical synthesis. The development of food laws in the USA followed those of European are governed by the Food and Drug Act. The 1938 Act established the term Food Drug and Cosmetic (FD & C) Colours and stated that colourants, presumably held to higher specifications for purity, would be allowed in foods, drugs and cosmetics. Francis (1996) reported that the first group comprises seven synthetic FD & C colour additives which are certified to comply with the purity specifications required by the FDA. The principles of food law are similar throughout the world but the specifics vary considerably between countries. The EU (European Union) has three main principles: protection of health of the consumer, prevention of fraud and removal of non-tariff barriers to intra-community trade. The EU Colours Directive lists colourants deemed to be suitable for food use and specifies the limits of impurities. Each colourant is identified by a specific E number. There are some synthetic colourants which are permitted in USA but not permitted in UK, and vice versa (Table 2.2).

Table 2.2 Comparisons of UK and USA food colour permitted

Colour	E Number	FDA Number
Tartrazine*	E102	Yellow No.5
Quinoline Yellow*	E104	-
Sunset Yellow FCF*	E110	Yellow No.6
Ponceau 4R*	E124	-
Carmoisine*	E122	-
Amaranth	E123	-
Erythrosine	E127	Red No.3
Patent Blue V	E131	-
Indigo Carmine	E132	Blue No.2
Brilliant Blue FCF	-	Blue No.1
Green S	E142	-
Black PN	E151	-
Allura Red AC*	E129	Red No.40
Fast Green FCF	-	Green No.3

Source: Walford (1968)
*Synthetic colourants which are still in question by Food Standard Agency, UK (Heller, 2009)

2.2.3 Natural colourants

Organic colourants are derived from natural edible sources using recognized food preparation methods, such as cucurmin (from turmeric), bixin (from annatto seeds), anthocyanin (from red fruits) and betalains (betacyanin and betaxanthin). This description of a natural colour would exclude caramels manufactured using ammonia and its salts and also copper chlorophyllins, since both of these products involve chemical modification during processing using methods not normally associated with food preparation (Henry, 1996).

Natural colours are widely permitted throughout the world. However, there is no universally accepted definition of this term and many countries exclude from their list of permitted colours those substances that have both flavouring and a colouring effect. The trend towards natural ingredients in foodstuffs is continuing and this is evidenced by consumer acceptance of 'natural' foods and the various regulations which completely or selectively ban artificial colours from food.

The EU permits a wide range of colours, some of which are of natural origin and these are listed in Table 2.3. From table 2.3, lycopene is not widely available commercially and four of the colours are only available commercially as nature-identical products. Nature-identical colours are manufactured by chemical synthesis so as to be identical chemically to colourants found in nature (Henry, 1996). The USA has a different set of 'natural' colours and those currently permitted by the Food and Drug Administration (FDA) which listed in Table 2.4; these not require certification and permanently listed.

One of the advantages of using natural colours is that they are generally more widely permitted in foodstuffs than synthetic colours. At present the use of natural colourants in food is limited, due to their instability, poor tinctorial power and the limited range of colours available. Natural colourants produced for use are crude extracts of pigments which are basically unstable, and strongly dependent on condition of storage and processing (Jeszka, 2007). The apparent stability of some food products owes more to the amount of pigment present than to the tinctorial power of the pigment itself. For example red beetroot, even after prolonged cooking, retains an attractive deep red colour, but the extracted pigment is unstable (Taylor, 1980).

Table 2.3 Natural colourants listed by the EU (European Union)

Colour	E Number
Curcumin	E100
Riboflavin, riboflavin-5′-phosphate*	E101
Cochineal, carminic acid, carmines	E120
Chlorophylls and chlorophyllins	E140
Copper complexes of chlorophylls and chlorophyllins	E141
Plain caramel	E150a
Vegetable carbon	E153
Mixed carotenes and β-carotene	E160a
Annatto, bixin, norbixin	E160b
Paprika extract, capsanthin, capsorubin	E160c
Lycopene	E160d
β-Apo-8′-carotenal (C30)*	E160e
Lutein	E161b
Canthaxanthin*	E161g
Beetroot red, betanin	E162
Anthocyanins	E163

Source: Henry (1996)
*available commercially as nature-identical products

Table 2.4 Natural colourants listed by the FDA for food and beverage use

Colour
Annatto extract
β-Apo-8′-carotenal*
β-carotene*
Beet powder
Canthaxanthin*
Caramel
Carrot oil
Cochineal, carmine
Cottonseed flour, toasted
Fruit and vegetable juices
Grape colour extract
Grape skin extract
Paprika and paprika oleoresin
Riboflavin*
Saffron
Turmeric and turmeric oleoresin

Source: Henry (1996)
*available commercially as nature-identical products

Carotenoids are the most widespread and the important group of pigments in nature. It comprises a group of structurally related colourants that are mainly found in plants, algae and several lower organisms. All carotenoid contains a system of conjugated double bonds that influence their physical, biochemical and chemical properties. In principal, each of the polyene chain double bond could exist in a cis or trans conformation, thus creating a number of isomers. It is relatively stable and there is sufficient demand to make complex chemical synthesis of ‘natural-identical’ carotenoids worthwhile (Jeszka, 2007; Sikorski, 2007). Their colour range is limited to yellow/ orange/ red and they are naturally oil soluble although water-soluble forms are available.

While, anthocyanins are among important groups of plant pigments which are present in almost all higher plants and are the dominant pigments in many fruits and flower, which performed in red, violet or blue colour. They play a definite role in attracting animals in pollination and seed dispersal. Anthocyanins are part of very large and widespread group of plant constituents known as flavonoids, which posses the same C_6-C_3-C_6 basis skeleton. They a glycoside of polyhydroxyl and polymetoxy derivatives of 2-phenylbenzopyrilium salts or flavylium cation and are most commonly based on six anthocyanidins: pelargonidin (orange-red), cyaniding, peonidin, delphinidin (blue-violet), petonidin and malvidin. The sugar moiety present is most commonly one of the following: glucose, galactose, rhamnose and arabinose (Figure 2.1). Anthocyanin preparations have found use in some products, but their colour variation with pH has restricted their use, mainly to acidic products (Henry, 1996).

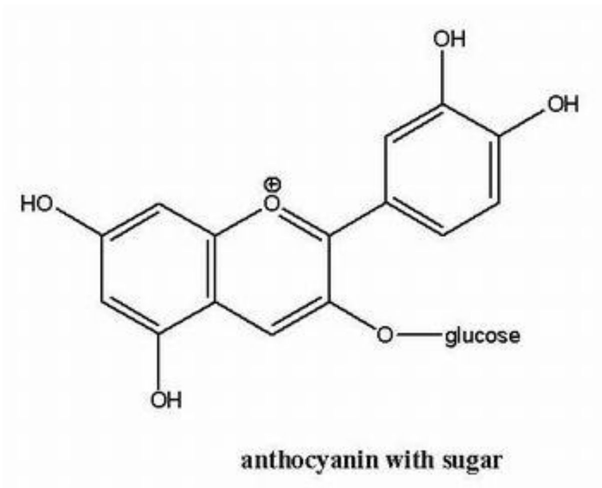


Figure 2.1 Anthocyanin molecule (Source: Francis, 1999).

The degradation of anthocyanin is at pH value above 2, this explained by intramolecular copigmentation which is based on the stacking of the hydrophobic acyl moiety and the flavylium nucleus, thus reducing anthocyanin hydrolysis (Dangles et al., 1993; Stintzing & Carle, 2004). In addition, anthocyanin glucosides are affected by glucosidases resulting in the formation of the highly labile aglycones which in turn oxidize easily and resulted deterioration of colour accompanied by unwanted browning (Stintzing & Carle, 2004). Ascorbic acid, glucose and fructose may even accelerate anthocyanin colour loss catalyzed by high temperature, oxygen and metal ions. The stability of anthocyanin is depending on the co-pigment, high stability of anthocyanin was obtained in an aqueous environment, at pH value between 3.1 and 4.7 at low temperatures. During extraction process of anthocyanin, factors promoting colour loss is promoted by deactivation endogenous and microbial enzymes such as glycosidases, peroxidises and polyphenoloxidases released upon tissue maceration (Stintzing & Carle, 2004). Thus, producing anthocyanin in concentrates and powder form could enhanced and stabilize the colour.

Another important pigment in nature and is present in all plants capable of photosynthesis is chlorophyll. However, the addition of chlorophyll as a colour to foodstuffs is very limited, principally because of its poor stability. It is an oil-soluble colour that can be extracted from a range of green leaves, but usually grass, nettles or alfalfa is used. Chlorophyll degrades easily, particularly in acidic conditions, losing its magnesium ion to yield phaeophytin, which is yellow-brown in colour. Chlorophyll colours tend to be rather dull in appearance and of an olive green-brown colour. Chlorophyll extracts can be standardized using vegetable oil for oil-soluble products or blended with a food solvent or permitted emulsifier to give a water-miscible form (Delgaldo-Vargas & Paredes-Lopéz, 2003).

Cochineal is described both the dried insects themselves and also the colour derived from them. Coccid insects of many species have been used for thousands of years as a source of red colour. Each insect is associated with a specific host plant and each is the source of a particular colour such as Armenian red, kermes, Polish cochineal, American cochineal. Cochineal extract exhibits shade changes with changes in pH levels. At pH levels of 4.0 and below, it is orange; at 4.0-6.0, it is magenta red colour; and above 6.0 it is a blue-red shade (Henry, 1996).

2.3 Betalains (Betacyanin and Betaxanthin)

Betalains have a limited distribution in the plant world and it would appear that betalains and anthocyanin are mutually exclusive. Plants producing betalains do not contain anthocyanins. Betalains can be divided into two classes, the red betacyanins and yellow betaxanthins. Most varieties of red beetroot contain the red betacyanin, betanin as the predominant colouring compound and this represents 75% to 90% of the total colour present (Henry, 1996).

Betalains are water-soluble nitrogenous pigments that replace the anthocyanins in a small number of taxonomically related plants families (Strack et al., 1993; Clement & Mabry, 1996; Herbach et al., 2006a). Structurally, betalains are immonium derivatives of betalamic acid (Figure 2.2), the lemon-yellow colour of the latter resulting from the 1, 7-diazaheptemethinium resonance system exhibiting three conjugated double bonds (Zrÿd & Christinet, 2004). The betalains comprise a quite modest number of about 55 structures including the red-violet betacyanins and the yellow-orange betaxanthins (Stintzing & Carle, 2007), while up to 550 anthocyanins have been identified in nature thus far (Andersen & Jordheim, 2006). Although not yet being clarified, the co-occuring betacyanin C_{15}-stereoisomers are mainly considered isolation artifacts. In contrast, the analogous C_{11}-isomers for the betaxanthins have not yet been detected as genuine compounds.

The uniqueness of betalains is their N-heterocyclic nature with betalamic acid being their common biosynthetic precursor. In comparison with the anthocyanins (Andersen & Francis, 2004), a much smaller number of substituent have been reported for the betalains: glucose, glucoronic acid and apiose are typical sugar monomers, while malonic and 3-hydroxy-3-methyl-glutaric acids as well as caffeic-, *p*-coumaric, and ferulic acids represent typical acid substituents (Strack & Schliemenn, 2003). Noteworthy, sinapic acid has been rarely reported for betalains (Kugler et al., 2007; Wybraniec et al., 2007), while inversely 3-hydroxy-3-methyl-glutaric acid has never been found as a structural feature in anthocyanins. The yellowish counterparts to the acyanic flavonoids, the so-called anthoxanthins, are the betaxanthins (Kremer, 2002).

Betaxanthins are condensation products of betalamic acid and amino acids or amines respectively. Depending on the particular structure of the amino compound, maximum absorption of betaxanthins varies between 460 and 480 nm (Stintzing et al., 2002a). The most common and frequently addressed betaxanthins (Figure 2.2) are glutamine-betaxanthins (vulgaxanthin I), betaxanthin in red beetroot (*Beta vulgaris* L.) and indicaxanthin (proline-betaxanthin), the predominant pigment in yellow cactus pears (*Opuntia* sp.), respectively.

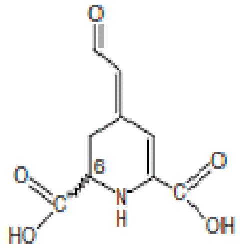
Condensation products of betalamic acid and *cyclo*-Dopa [*cyclo*-3-(3,4-dihydroxyphenylalanine)] are commonly referred as betacyanins due to their deep red violet colour. Their strong bathocromic shift of 50 to 70 nm as compared to betaxanthins is ascribed to the aromatic structure of *cyclo*-Dopa (Zrÿd & Christinet

2004). By glycosylation with one or two monosacharides as well as acylation of the resulting 5-*O*- or 6-*O*-glucosides, a great variety of betacyanin structures is possible.

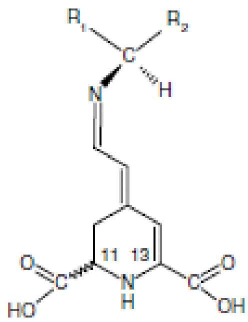
Nevertheless, betacyanin research has mainly focused on betanin (betanidin 5-*O*-β-glucoside; Figure 2.2), the most abundant betalains in red beetroot (*Beta vulgaris* L.). As already stated for betaxanthins, the absorption maximum of the betacyanins is influenced by the particular substitution pattern of the betanidin backbone. Generally, glycosylation of betanidin comes along with a hypsochromic shift of the resulting betacyanin, glucose attached at C_6 being less effective than C_5 glycosylation (Stintzing et al., 2004). While esterification with aliphatic acyl moieties was reported to have little impact on the maximum absorption of betacyanins (Wybraniec et al., 2001; Stintzing 2002b), acylation with aromatic acids leads to a bathochromic shift (Heuer et al., 1992). Schliemann & Strack (1998) was explained by copigmentation like intramolecular association. Moreover, C_6 attachment of acyl-glucosides enhances the bathocromic shift, which possibly results from a more rigid conformation (Heuer et al., 1994).

Besides this biochemically related distinctions, the betalains are more water soluble than the anthocyanins and exhibit a tinctorial strength up to three times higher than the anthocyanins. Betalains possess higher molar absorption coefficients in the visible light spectrum than anthocyanins (Clement & Mabry, 1996), indicating their function in UV protection. Due to the high molar extinction coefficients, the colouring power of betacyanin is competitive to synthetic colourants (Henry, 1996). Apart from that, contrast to anthocyanins, their appearance is maintained and stable over a wide pH range from 3 to 7, making them ideal pigments for colouring low pH acidic foodstuffs (Stintzing & Carle, 2004) also reported that betalains are not as susceptible to hydrolytic cleavage as the anthocyanins.

(A) Betalamic acid

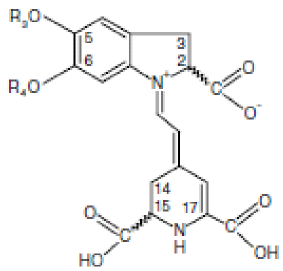


(B) Betaxanthins



Substitution pattern		Trivial name
R_1	R_2	
H	Glutamic acid	Vulgaxanthin I
Proline		Indicaxanthin

(C) Betacyanins



Substitution pattern		Trivial name
R_3	R_4	
β-glucose	H	Betanin
6´-*O*-(malonyl)-*β*-glucose	H	Phyllocactin
6´-*O*-(3´´-hydroxy-3´´-methylglutaryl)-*β*-glucose	H	Hylocerenin
2´-*O*-(*β*-glucuronic acid)-*β*-glucose	H	Amaranthin
H	*β*-glucose	Gomphrenin I

Figure 2.2 Structure of (A) betalamic acid; (B) Betaxanthins and (C) Betacyanins. Source: Herbach et al. (2006a)

2.3.1 Amaranth

Betalains occur only in the plants from 10 families of the order Caryophyllales (old name: Centrospermae), such as the family *Amaranthaceae* which includes several important genera, i.e. *Amaranthus*, *Celosia*, *Gomphrena*, and *Iresine* (Cai & Corke, 2005). Amaranth is still cultivated as a minor crop in Central Asia and Africa. Grain amaranth (*Amaranthus*) has developed worldwide as a new crop over the past 20 years, with good nutritional quality, strong tolerance to stress conditions (drought, salinity, alkalinity, acidic or poor soil), and high biomass yield.

For the last decade, some researchers have been conducting research and development of amaranth, including natural betalains from the plants in the *Amaranthaceae* (Cai et al., 1998a; 1999; 2005; Cai & Corke, 2000; Degaldo-Vagas et al., 2000). Red violet betacyanin pigments from *Amaranthus* genotypes produce particularly high biomass and contain high levels of pigment. Production commercial betalains depends not only on efficient processing techniques (i.e. enzymatic control, extraction, purification, concentration, and drying operations), but also on a continuous availability of highly pigmented sources (Degaldo-Vagas et al., 2000).

The betacyanins in *Amaranthus* tricolour were identified as amaranthin (the 5-*O*-[2-*O*-(β-$_{D}$-glycopyranosyluronic acid)-β-$_{D}$-glucopyranoside] of betanidine) and isoamaranthin (C-15 epimer) (Cai et al., 1998b). Amaranthine has the same basic structure-betanidin (aglycon) as the betacyanins from red beetroot. The distribution of betacyanins in 37 species of eight genera in the *Amaranthaceae* was investigated and the total of 16 kinds of betacyanins and three kinds of betaxanthins were isolated and characterized by Cai et al. (2001). They consist of six simple (nonacylated) betacyanins and 10 acylated betacyanins, including eight amaranthin-type pigments, six gomphrenin-type pigments and two betanin-type pigments. Acylated betanin were identified as betanidin 5-*O*-(2′-*O*-(glucuronosyl) glucoside or betanidin 6-*O*-(-glucoside acylated with ferulic, p-coumaric or 3-hydroxy-3-methylglutaric acids. Three new types of betaxanthins were isolated from three *Celosia* species in the *Amaranthaceae* and identified to be immonium conjugates of betalamic acid with dopa-mine, 3-methoxytyramine and (S)-tryptophan (Schliemann et al., 2001). In *Amaranthus*, the colour of red-violet was performed by betacyanins, like red beetroot betalains, are susceptible to temperature and also affected by pH, light, air and water activity, with better pigment stability at lower temperatures (<15 ˚C) in the dark and in the absence of air over the pH range 5-7, being more stable pH 5.6. The *Amaranthus* pigment powders were very stable at 25 ˚C, with longer half-life ($t_{1/2}$ = 23.3 months) and higher pigment retention (78.2%) compared to aqueous pigment extracts ($t_{1/2}$ = 1.04 months and 18.3%) after 43.5 weeks storage (Cai et al., 2005).

2.3.2 Red beetroot

In the first place, betalains are associated with red beetroot because it is not only rich in betacyanins but also the exclusive commercially exploited betalains crop. Red beetroot was proposed to be included in low-acid food items such as meat and dairy products. The main topics that needed to be addressed were the fast browning through polyphenoloxidase activities and the reduction of the naturally high nitrate content. While the first controlled by heat inactivation and oxygen removal, the latter were reduced by fermentation strategies (Stintzing et al., 2007).

Red beetroot contains abundant amounts of betalain pigments which include two main groups of compounds, namely red betacyanins and yellow betaxanthins. The betacyanins, 75 - 95% consists of betanin together with smaller amounts of isobetanin and prebetanin; the main betaxanthin derivatives are vulgaxanthin I and II.