EFFECTS OF VARIOUS TREATMENTS ON THE STABILITY OF PIGMENTS FROM BEETROOT (BETA VULGARIS) AND DRAGON FRUIT (HYLOCEREUS POLYRHIZUS)

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ABSTRACT

Betalains are of great taxonomic significance in higher plants and water-soluble nitrogenous pigments. Peel, crown, flesh of beetroot and peel, flesh of dragon fruit were separately analyzed in the present study. The overall aim of the study was to identify suitable conditions for the extraction and storage of betalains from this plant species for further detailed biochemical studies. Major compounds found in beetroot and dragon fruit were betacyanins and betaxanthins by using thin-layer chromatography. Water was more effective for optimum extraction of betalains compared to ethanol: water (80:20) extraction. Beetroot peel had the highest betalains concentration which was 84.97 ± 0.266 mg/100g fresh weight while dragon fruit peel had the lowest betalains concentration which was 11.81 ± 0.280 mg/100g fresh weight for water extraction. The highest pigment loss was observed at pH 8.0 while the lowest was at pH 6.0 in 24 hours. For temperature effect, pigment loss was minimum at 4°C and maximum at 100°C for 3 hours. Betalains extracts showed highest pigment retention when stored at -4 ± 2°C compared to 30 ± 4°C (ambient temperature) in the dark for 4 weeks. Overall, the results showed that betalains extracted from by-product of beetroot and dragon fruit can be used as potential colorants in food.
ABSTRAK

PENGASINGAN PIGMEN DARI UBI BIT DAN BUAH PITAYA DAN KESTABILAN DALAM FIZIKAL PARAMETER

Betalain adalah satu kumpulan pigmen kemerahan larut air yang tersebar luas di dalam tumbuhan dan mengandungi nitrogen. Kulit, umbi, isi dari ubi bit dan kulit, isi dari buah pitaya dianalisis dalam kajian ini. Tujuan penyelidikan ini adalah untuk mengenalpasti keadaan yang sesuai untuk mengesktrak dan menyimpan betalain dari spesies tumbuhan tersebut supaya dapat menyelidik biokimianya. Major komponen betalain yang berjaya diasgingkan dan dikenalpasti melalui kromatografi kertas adalah betasianin dan betaxanin. Air adalah lebih cekap daripada ethanol:air (80:20) untuk mengekstrak pigmen betalain dari ubi bit dan buah pitaya. Dengan pengekstrakan air, kulit ubi bit mempunyai jumlah kandungan betalain yang paling tinggi iaitu 84.97 ± 0.266 mg/100g berat kasar manakala kulit buah pitaya mempunyai jumlah kandungan betalain yang paling rendah iaitu 11.81 ± 0.280 mg/100g berat kasar. Kehilangan kepekatan pigmen yang paling tinggi didapati dalam pH8.0 manakala paling rendah adalah dalam pH6.0 selepas 24 jam. Kehilangan kepekatan pigmen adalah paling minima pada suhu 4°C dan maxima pada suhu didih 100°C selepas 3 jam. Ekstrak betalain menunjukkan kestabilan yang tinggi apabila berada pada suhu rendah -4 ± 2°C berbanding dengan suhu ambien 30± 4°C dalam keadaan gelap selama 4 minggu. Keseluruhan, keputusan menunjukkan bahawa betalain ekstrak dari hasil sampingan ubi bit dan buah pitaya berpotensi digunakan sebagai pewarna dalam makanan.
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<td>LDL</td>
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<tr>
<td>H₂SO₄</td>
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<td></td>
</tr>
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<td>I</td>
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<td>DP</td>
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<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
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<td>rpm</td>
<td>Revolution per minute</td>
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<td>N</td>
<td>Normality</td>
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<td>nm</td>
<td>Nanometer</td>
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<tr>
<td>μm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<td>a</td>
<td>Alpha</td>
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<tr>
<td>A</td>
<td>Absorption value</td>
</tr>
<tr>
<td>DF</td>
<td>Dilution factor</td>
</tr>
<tr>
<td>Vd</td>
<td>Solution volume (mL)</td>
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<tr>
<td>Wd</td>
<td>Sample weight (g)</td>
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<td>L</td>
<td>Path-length (1 cm) of the cuvette</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
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<tr>
<td>E_{\text{con}1%}</td>
<td>Ideal extinction coefficient</td>
</tr>
<tr>
<td>ε</td>
<td>Molar extinction coefficient</td>
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3.1 \[BC \,[\text{mg/g}] = [(A(DF)(MW)Vd / \varepsilon L Wd)]\]

Where,
- \(A\) = absorption value
- \(DF\) = dilution factor
- \(Vd\) = solution volume (mL)
- \(Wd\) = sample weight (g)
- \(L\) = path-length (1 cm) of the cuvette
- \(MW\) = molecular weight
- \(\varepsilon\) = molar extinction coefficient

3.2 Pigment retention (\%) =
\[
\frac{\text{concentration of pigment at specified storage time} \times 100}{\text{concentration of pigment at zero storage time}}
\]

3.3 Retardation factor, \(R_f\)
\[
R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}
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CHAPTER 1

INTRODUCTION

1.1 Background

Color becomes the most sensitive part of any commodity not only for its appeal but also it enhances consumer acceptability (Clydesdale, 1993). In addition, the color of a food substance is important to indicate its freshness and safety that are also indices of good aesthetic and sensorial values (Newsome, 1986; Frick & Meggos, 1988; Clydesdale, 1993; Murano, 2003; Pritam et al., 2008). In understanding the chemical basis of food color, the food technologist can address quality assurance issues regarding color stability, important in both unprocessed foods and processed foods during storage (Murano, 2003).

The consumption of fresh food has decreased, while that of processed foods has increased, food colors have become an important aspect of the food formulation process. In order to restore the nature level of pigments in the processed products, extracted pigments are incorporated into the final food products (Garcia-Viguera et al., 2000). Similarly the preparation of fortified products requires addition of pigments are incorporated either under their natural occurrence or under a chemical modified form. As a consequence of these additional pigment needs, the demand in isolated natural colorants has increased as compared with synthetic dyes, which have been reported to have carcinogenic effects (Sewekow, 1988).

Natural food dye is gaining popularity especially in the food and beverage sectors due to strong demand for more natural products by health-conscious consumers (Rebecca et al., 2008) and interest in natural colorants has increased considerably, mainly because of the apparent lack of toxicity and eco-friendliness.
In addition, natural colors and pigment from fruits and vegetables may contribute additional nutritional value to food colored. An increasing number of studies indicates that plant pigments have positive roles on the human health (Benoit, 2004). Natural colors are found to be nutritional antioxidants and their presence in diet can reduce the risk of cardiovascular disease, cancer and diseases associated with ageing (Delgado-Vargas et al., 2000).

Although natural pigments are generally less stable and have higher cost than synthetic colorants, their development and utilization is attracting more and more attention due to strong consumer demand for more natural product which is more safety and health benefit (Sasson, 1991; Jackman & Smith, 1996; Boyd, 1998). Natural colorants have a vast economic significant because the dye trade has a world market worth £2.5 billion/year (Harivaindaran et al., 2008). Some examples of natural food colorants which have already been used in the food industry include anthocyanin, curcumin, beetroot red, caramel, lycopene, paprika extract and chlorophyll (Rebecca et al., 2008). However, this need of natural pigments can not always be satisfied due to the limitation in the supply of raw material because the production of pigments using conventional plant cultivation methods is influenced by climatic conditions, plant cultivars and varieties (Rodriguez-Amaya, 2000). Consequently part of plant pigment research is oriented in finding new sources of pigments. This quest is not only directed in finding natural alternatives for synthetic dyes, but also with the aim to discover new taxons and new procedures for the pigment production.

Betalains, the red-violet betacyanins and the yellow betaxanthins, which is a class of water soluble nitrogenous pigments (Robinson, 1963; Salisbury et al., 1991) deserve intense research as they offer hues and stability characteristics uncommon to anthocyanic sources (Stintzing & Carle, 2007). Trejo-Tapia et al. (2007) reported that betalains are considered as food-safe colorants, water soluble and do not need chemical modification for dispersion in a wide range of products.

Betalains is approved as food colorant (E-162) by the European Union and that enlarges the specific weight of these compounds in the diet (Diego et al., 2008). Red beet color extracts are currently under development as alternatives to certain of the
FD&C (Food, Drug and Cosmetic) red food color dyes for application in food systems. A number of processing modifications including conversion of beet juice to powders and concentrates and fermentation procedures for concentrating the colorants have enhanced the potency and applicability of beet extract as a food colorant (Kent & Robert, 1978). Specifically, there is a growing interest in betalains because of their antiradical and antioxidant activity. Also, betaxanthins can be use as a mean of introducing essential amino acids in the diet (Kanner et al., 2001).

With a focus on food, the scare attention towards betalains may be due to the fact that red beet has long been considered the only edible betalainic source (Diego et al., 2008). Nowadays, red beetroot (Beta vulgaris) are the main commercial source of betacyanins which available in the concentrated and powder form. However, red beetroot contains geosmin and pyrazines that are responsible for the unpleasant peatiness of this crop as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines, there is a demand for alternative compounds (Tesoriere et al., 2004; Moßhammer et al., 2005; Rebecca et al., 2008). Thus, it stimulated scientists to study other sources of betalains and their possibility of use as food colorant from the nutritional and technologically related analytical tissues (Stintzing & Carle, 2007a; Herbach et al., 2006).

Red-fleshed pitaya fruit is choosen because red-fleshed pitaya fruit does not have this negative sensorial impact. Beside, the red-fleshed pitaya or dragon fruit (Hylocereus polyrhizus) is becoming popular in Malaysia due to its unique shape, attractive red color and high functional properties. It belongs to family of Cactaceae and order of Caryophyllales (Phebe et al., 2009). The peel and flesh of this species are red in color. The red color of pitaya fruit is attributed by betacyanins (Wybraniec et al., 2007; Phebe et al., 2009). Alternatively, betacyanins from red-fleshed pitaya fruit may be a potential source on top of red beetroots. Additionally, data on beetroot and dragon fruit are lacking around Sabah concerning the relevant study about the betalains content. Therefore, this study is to evaluate the suitability of pigments from beetroot and dragon fruit in Sabah for the development of natural colorants.
Another aspect that has been emphasized in this study is to determine the knowledge of total betalains content of in the different part of local beetroot and local dragon fruit. Kujala et al. (2000) and Tytti et al. (2002) reported that the distribution of betacyanins in red beetroot was found to be distributed mostly towards the outer parts of root, decreasing in their order peel, crown and flesh. There is little report on profiles and total contents of betacyanins and betaxanthins in the different parts of local beetroot and local dragon fruit. If the by-products of beetroot and dragon fruit also contain high betalains content, it can be developed into beauty and health products and food natural colorant as its flesh and hence reduce the food wastage. This study use spectrophotometry and chromatographic to detect and quantify betalains and the results are expressed in terms of ‘total betacyanins and total betaxanthins contents’.

This study is carried out to develop a simple system and workable to detect routine determination of betalains content in beetroot and dragon fruit to build a suitable effective solvent for extraction of betalains. Besides, this study is develop a simple and selective chromatographic system for resolution of the major, as well as minor, betalains from beetroots and dragon fruits. Cellulose plate is selected for these experiments because it has successfully been employed as a component of adsorbent mixture for thin layer chromatography separation of the betalains, primarily betacyanins and betaxanthins.

Besides that, analysis of the stabilities of pigment of betalains to pH, temperature and storage condition test is carry out. The stability of betalains during storage is very important factor to make the final product attractive and acceptable. Understanding the chemistry of the molecules responsible for color as influenced by temperature, oxygen, and various elements and molecules has opened up a field of functional ingredient applications. Determining the effects of several treatments on preventing color changes has become a key area in food research and in product development. The main problems associated with working with betalains come from the inherent instability of pigments. This study is conducted to show the method which could be used to identify the stability of pigment and apply it as a natural colorant in future to enhance the nutritional value. Much effort is put into identifying and
quantifying the pigments responsible for the color of red beet root and dragon fruit as well as into studying the main factors affecting its color deterioration.

1.2 Objective

1. To determine the betalains content in different parts of local beetroot (*Beta vulgaris*) and local pitaya (*Hylocereus polyrhizus*).

2. To determine the most effective solvent for the extraction on the total betalains.

3. Qualitative identification of betalains through Thin Layer Chromatography.

4. To determine the effect of freeze drying, pH, temperature and storage condition on the stability of the pigment extraction.
CHAPTER 2

LITERATURE REVIEW

2.1 Background of Beetroot (*Beta vulgaris*)

The beet (*Beta vulgaris*) is a plant in the beet root family. It is best known in its numerous cultivated varieties, the most well known of which is probably the red root vegetable known as the garden beet (Bokern *et al.*, 1991). The garden beet (*Beta vulgaris* subsp. *vulgaris* var. *vulgaris*), also known as the table beet, beetroot, red beet or informally simply as beet, is one of the many cultivated varieties of beets and arguably the most commonly encountered variety in North America and Britain.

*Beta vulgaris* is a herbaceous biennial or rarely perennial plant with leafy stems growing to 1-2 m tall. The leaves are heart-shaped, 5-20 cm long on wild plants (often much larger in cultivated plants). The flowers are produced in dense spikes, each flower very small, 3-5 mm diameter, green or tinged reddish, with five petals; they are wind-pollinated. The fruit is a cluster of hard nutlets. The beetroot is a red colored tuber that is cooked like potatoes and eaten as a vegetable (Nilsson, 1970).

2.1.1 Cultivation

Beetroot grows best in cool weather, but it can be planted throughout the year. The best time to plant is from early spring to early summer (August, September, October) and again late in summer and into autumn (February, March, April). These are the cool periods of the year. Beetroot does not grow well in summer when it is very hot,
neither does it grow well in the middle of winter when it is very cold (Mukundan et al., 1998).

Beetroot will grow on any soil type except acid soil. It grows well on brackish and on alkaline soils, but the soil must be well drained. This means that when plants are watered, the water must soon drain into the ground and not be left as long-standing puddles. If this happens, compost must be worked into the soil (Taya et al., 1992; Mukundan et al., 1998). This will also prevent the soil from forming a crust through which the young plants can emerge only with difficulty. However, compost must not be worked into the soil just before the beetroot is sown, otherwise the beetroot can become hairy and form thick lateral roots (Shimomura et al., 1991).

The most important plant nutrient required by beetroot is nitrogen (N), phosphorus (P), potassium (K) and boron (B). Beetroot requires a great deal of nitrogen, phosphorus and potassium, but only a small quantity of boron (Dilorio et al., 1993; Trejo-Tapia et al., 2001). The soil does not always have enough of this for beetroot, and then the beetroot farmer must supplement it. It is important not to give too few or too many plant nutrients, therefore the soil must first be analysed so that the plants will not be burned, nor show poor growth (Rodriguez-Monray & Galindo, 1999; Bohn & Mack, 2004).

Beets are popular in the home garden because they are relatively easy to grow and practically the whole plant can be eaten. Beets can be grown for their root qualities which include different shapes and sizes as well as red, yellow or white colors (Murashige & Skoog, 1962). The tops or greens, when young, are excellent in salads and when the plant is older, can be cooked. The greens are even more nutritious than the roots.
REFERENCES


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