ISOLATION AND THE EFFECT OF THE PHYSICAL PARAMETERS ON THE STABILITY OF CAROTENOIDS FROM CARROT

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ABSTRACT

Carotenoids are isoprenoid molecules that are mostly found in colored fruits and vegetables. Imported and local carrots were analyzed in the present study. The relative efficiency of acetone and methanol based solvent for the extraction of pigments from carrots was compared, together with the comparison of total carotenoid content between imported and local carrot. The total carotenoid content in acetone solvent was found to be 21.81% while for methanol solvent it was 13.05%. In general, the levels of carotenoids were higher in imported than in local carrot; the levels of total carotenoid content were about 12% (Australia) and 10% (China) higher in the imported than the local carrot, respectively. Major compound found in carrot were β-carotene and α-carotene by using Thin Layer Chromatography (TLC). Investigation was also conducted on the stability of carotenoid under different pH and temperature range. The highest pigment loss was observed at pH 3.0 while the lowest was at pH 7.0 for 4 hour. For temperature effect, pigment loss was minimum at 0 ± 4°C and maximum at 96 ± 4°C. Stability of carotenoid under different conditions during storage was studied for 4 week. All pigments were stored at light, dark condition at ambient temperature for storage condition. Besides, carotenoid pigments were stored under 0 ± 4°C and ambient temperature 30 ± 4°C. Results showed that the total carotenoid content decreased with increasing temperature. On the other hand, visible light in any form resulted in the destruction of carotenoid.

ABSTRAK

PENGASINGAN PIGMEN DARI LOBAK DAN KESTABILAN KAROTENOID DALAM FIZIKAL PARAMETER

Karotenoid merupakan molekul isoprenoid yang kebanyakan boleh didapati dalam buah-buahan yang berwarna–warni dan sayur-sayuran. Lobak merah import dan tempatan dari Kundasang Sabah akan digunakan untuk analisis pigment karotenoid. Kecekapan mengekstrak keluar pigmen karotenoid dari lobak merah di antara larutan methanol dan acetone akan dibanding berdasarkan peratusan jumlah perolehan karotenoid. Di samping itu, jumlah kandungan karotenoid antara lobak merah import dan tempatan juga akan dibanding. Dalam larutan acetone, jumlah peratusan perolehan yang berjaya diekstrak keluar adalah sebanyak 21.81% manakala untuk larutan methanol hanya berjaya mengekstrak keluar sebanyak 13.05%. Secara umumnya, jumlah kandungan karotenoid adalah lebih tinggi di lobak merah import di mana lobak merah Australia (12%) dan China (10%) lebih tinggi berbanding dengan lobak merah tempatan. Major komponen karotenoid yang berjaya diasing dan diidentiti melalui *Thin Layer Chromatography (TLC)* adalah beta-karoten dan alpha-karoten. Penyelidikan terhadap kestabilan pigmen karotenoid juga akan dijalankan dalam beberapa lingkungan pH dan kesan suhu yang berlainan. Kehilangan kepekatan pigmen karotenoid yang paling tinggi telah didapati dalam pH 3.0 manakala kehilangan kepekatan pigmen karotenoid paling rendah adalah dalam pH 7.0 selama 4 jam. Penurunan kepekatan adalah paling minima dalam suhu rendah 0 ± 4°C dan maxima pada suhu didih 96 ± 4°C. Penyelidikan kestabilan penyimpanan pigmen juga dijalankan dalam beberapa keadaan penyimpanan selama 4 minggu. Untuk menguji ketahanan pigmen dalam keadaan simpanan yang berbeza, pigmen akan diuji di antara keadaan gelap dan keadaan bercahaya. Di samping itu, pigmen akan disimpan pada suhu rendah 0 ± 4°C dan suhu bilik 30 ± 4°C dalam keadaan gelap selama 4 minggu. Keputusan menunjukkan kadar kehilangan pigmen meningkat dengan peningkatan suhu. Di samping itu, keadaan cahaya akan menyebabkan pembinasaan of carotenoid.

CONTENT

LIST OF TABLE

LIST OF FIGURE

APPENDIX LIST

SYMBOL AND SHORT FORM LIST

°c	celsius degree
%	percentage
nm	nanometer
mm	millimeter
g	gram
ml	milliliter
UV	ultra violet
TLC	thin layer chromatography
SPSS	statistical package for the social sciences

CHAPTER 1

INTRODUCTION

1.1 Natural Colour

Natural colours have been a part of man's normal diet and have, therefore, been safety consumed for countless generations. The desirability of retaining the natural colour of food is self-evident, but often the demands of industry are such that additioned colour is required. Contrary to many reports, natural sources can provide a comprehensive range of attractive colours for use in the modern food industry.

1.1.1 Natural Pigment

By definition, a natural pigment in biological systems is one that is synthesized and accumulated in, or excreted from, living cells. In addition, certain pigments, such as oxidized phenols and more simple phenolic derivatives such as the anti-coagulant coumarins, may be formed by the dying cell. Natural colorant includes "pigments formed in living or dead cells of plants, animals, fungi or microorganisms, including organic compounds isolated from cells and structurally modified to alter solubility, solubility or colour intensity. According to Benoit (2004), a large number of vegetables harbour shining colours. These colors are conferred by the pigments contained in the cells.

"Plant pigments" is a generic expression used to designated a large number of coloured molecules (Benoit, 2002). Plants are predominantly characterized by the presence of chlorophylls that are crucial for photosynthetic activity. Pigmentation other than green is important to create contrasting hues for the attraction of animals both for pollination and seed dispersal. Furthermore, pigment synthesis in plants has been proven to be a consequence of exogenic stress or senescence and of an ecological adaption to changing environments, respectively (Gould, 1995). In this sense, chlorophylls, carotenoids and flavonoids contribute to maintain a balanced physiological state in the respective tissues (Blokhina, 2003).

1.2 Colour

Colour is described by the wavelength (λ) of the maximum absorption (A_{max}) in the visible part of the electromagnetic spectrum, expressed in nanometers (nm). White light is seen as the simultaneous incidence of the full range of the visible spectrum (wavelength between 380 – 730 nm approximately) at the same relative intensities. Any object that lessens the intensity of one part of the spectrum of white light by absorption will bring about the perception of colour in the residual transmitted light, as well as a reduction in irradiance or more simply a darkening. The human eye only be able to sense six hues: red at around 700 nm, orange at 625 nm, yellow at about 600 nm, green at 525 nm, blue at around 450 nm, and violet at and below 400 nm. The key element in the special context of food colorants, however, is the hue perceived by the eye of the normal, healthy and average adult.

1.3 Food Colorant

According food laws of Malaysia which state in regulation 21, "colouring substance" means any substance that, when added to food, is capable of imparting colour to that food and includes colouring preparation.

Because the consumption of fresh food has decreased, while that of processed foods has increased, food colours have become an important aspect of the food formulation process (Britton *et al.*, 1995). Colour can be introduced into several ways such as raw material – the fruit, vegetables, meat, eggs (have their own intrinsic colour), the processing conditions may generate colour or a colour may be added. Some food products have little or no inherent colour and rely on added colour for their visual appeal.

Colourants are added to many foods to make them appear healthier and more appealing; reinforcing the fact that colour plays an important role in consumer choice. What is important is that the colours that are applied are safe, and that colours should not be used to mask unhealthy products (Remi and Christoph, 2004).

To the consumer, appearance is one of the most important attributes affecting choice of purchase. For food, colour is ranked alongside freshness as one of the main criteria governing selection (Remi and Christoph, 2004). The colour of food will therefore influence not only the perception of flavour, but also that of sweetness and quality. It is also important not to underestimate aesthetic value.

The best food with a perfect balance of nutrients is useless if it is not consumed. Consequently, foods need to be attractive. Humans are not much different in their use of colour. Food packaging is often coloured (after all, which is often what attracts consumer to buy a certain brand) at the end of the day, the food left on supermarket shelves tends to be discoloured produce (from vegetables to meat).

Natural dyes/colourants have been used historically throughout the world. The use of natural dyes/colourants has decreased to a large extent due to the advent of synthetic dyes. Recently, dyes derived from natural sources have emerged as important alternatives to synthetic dyes, which have been reported to have carcinogenic effects (Sewekow, 1988). With the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest (Eom *et al.*, 2001). The plant kingdom offers a vast source of natural dyes/colourants which can be obtained from many plant parts e.g., leaves, fruits, seeds, flowers, barks and roots.

Although it is common practice to refer to plant pigments and food dyes, the distinction between these in the food literature is not always clear. According to DeMann (1980), he refers pigments as 'a group of natural colorants found in animal and vegetable products'. In colour chemistry texts related to the textile industry, however, pigments are defined as being practically insoluble in the media in which they are applied (Herbs, 1992) whereas dyes are soluble or partially soluble in the liquid in which they are applied. It is evident from this that the majority of our plant 'pigments' are water soluble. The term colourant, however, is a collective term for all soluble or solubilized colouring agents, as well as insoluble pigments.

1.4 Objective

Main objective of this research are list in below:

1. Determine the knowledge of total carotenoid content of imported carrot and local carrot.
2. Determine the most effective of solvent for the extraction on the total carotenoid.
3. Separation and qualitative identification of total carotenoid through Thin Layer Chromatography.
4. Determine the effect of pH, temperature, and storage condition on stability of carotenoid pigment.

1.5 Justification

Carotenoids, a fat soluble compound are still being actively studied all over the world because of their commercially desirable properties. However, data on carrot are lacking around Sabah concerning the relevant study about the carotenoid content. Carrot root are being choose because of wide consumption by Malaysian in every meal such as carrot juice and due to its health benefit especially the high content of vitamin A as preventable blindness. Besides, carrot is also easily obtained in everyway supermarket, as Kundasang is the main plantation of carrot. The main aspect that has been emphasized in this study was to determine the knowledge of total carotenoid content of imported carrot and local carrot.

This study was carried out to develop a simple system, workable to detect routine determination of carotenoid content in carrot whether imported or local to build a suitable effective solvent and for extraction of carotenoids. Besides, this study was develop a simple and selective chromatographic system for resolution of the major, as well as minor, carotenoids from carrot roots. Silica plate was selected for these experiments because it has successfully been employed as a component of adsorbent mixtures for TLC separation of the carotenoids, primarily carotenes. Then, we can identify the health benefits that contribute by each compound in carrot root.

The studies on carotenoid behavior and its stability during storage are relatively less than processing effects. The stability of carotenoids during storage is a very important factor to make the final product attractive and acceptable. The main problems associated with working with carotenoids come from the inherent instability of pigments. Being highly unsaturated molecules, the pigments are subjected to isomerisation, which causes color loss, and oxidation. This study was done 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.

CHAPTER 2

LITERATURE REVIEW

2.1 Carrot (*Daucus carota* var *sativus)*

Carrot (*Daucus carota* var *sativus)* belongs to family Umbelliferae which consists of chiefly biennial or perennial herbs of north temperate regions. Carrots are grown as a cool season vegetable.

Of all root crops, carrots is considered the most important vegetable because of its high vitamin A, mineral, dietary fiber content and thus act as a major food crop utilized in human diet. Uniform, bright orange color is a major quality attribute for fresh market and 'fresh-cut' carrots in both retail consumer markets and foodservice outlets and institutions (Suslow *et al.*, 1999).

2.1.1 Cultivation, Distribution and Production

Carrot can be grown in a wide variety of deep, loose, friable soils at cool to moderately warm temperatures. Seeds are planted in raised beds or flat rows. The fertility program should pay close attention to maintaining high levels of phosphorus and potassium

during the growth cycle. Weed control is very important throughout growth. Soil moisture should be held constant throughout the growth cycle, with at least 1½" water per week and more if grown in an arid region or in a sandy soil.

Carrot is a rustic plant that best develops in moderate climates. The optimum growth temperature is 16°C to 18°C. Temperatures above 28°C accelerate the root aging process and causes discoloration, thereby decreasing the commercial quality of the product. In Malaysia, this crop is come from the highland such as Cameron Highland, Genting Highland in West Malaysia, and Kundasang in Sabah. These areas have the correct temperature, type of soil and water for the production of good quality carrots during, practically, the entire year (FAMA, 1992).

2.1.2 Nutritional Value

Carrots are by far one of the richest source of carotenoids - just one cup provides 16,679 IUs of beta-carotene, more than 250% of the RDA, and 3,432 REs (retinol equivalents), or roughly 686.3% the RDA for vitamin A.

The carrot is an herbaceous plant containing about 87% water, rich in mineral salts and vitamins B, C, D, and E. Raw carrots are an excellent source of vitamin A and potassium; they contain vitamin C, vitamin B6, thiamine, folic acid, and magnesium. Cooked carrots are an excellent source of vitamin A, a good source of potassium, and contain vitamin B6, copper, folic acid, and magnesium. The high level of beta-carotene is very important and gives carrots their distinctive orange color.

Carrots also contain, in smaller amounts, essential oils, carbohydrates and nitrogenous composites. They are well-known for their sweetening, antianaemic, healing, diuretic, remineralizing and sedative properties.

Table 2.1: Nutritional Profile of Raw Carrot.

Nutrient	Amount	Daily Value (%)
Beta - carotene	34317.40 IU	686.3
Vitamin K	176.90 mcg	221.1
Vitamin C	11.35 mg	18.9
Dietary fiber	3.66g	14.6
Potassium	394.06 mg	11.3
Vitamin B6 (pyridoxine)	0.18 mg	9.0
Manganese	0.17 mg	8.5
Molybdenum	6.10 mcg	8.1
Vitamin B1 (thiamin)	0.12 mg	8.0
Vitamin B3 (niacin)	1.13 mg	5.6
Phosphorus	53.68 mg	5.4
Magnesium	18.30 mg	4.6
Folate	17.08 mcg	4.3
Raw Carrot **1 cup contain 52.46 calories**		

(Sources: World's Healthiest Foods, 2006)

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