EXTRACTION OF BETA-CAROTENE FROM CARROT VIA SOXHLET EXTRACTION METHOD

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ABSTRACT

Extraction of carotene from carrot (Daucus Carota.L) using Soxhlet extraction method was done by using different solvent and time. Beta-carotene is the highest constituents in carotene. Fresh carrots are used as sample in this study. The effect of different parameters, which are type of solvents and duration of extraction time. Three types of solvents are used in this research which are acetone, ethanol, and 2-propanol. While, the duration of extraction time used are 2, 4, 6, and 8 hours. The overall process of this research involves four major steps which are sample preparation, extraction of carotene compound by using Soxhlet extraction, solvent separation of extracted compound by using rotary evaporator and analysis of carotene yield by using HPLC. When separated the desired compound from solvent, set the evaporator temperature at the boiling point of the solvent. The samples is then need to be filter first for the purpose of HPLC. The LC-18 is use as our HPLC column. The peak area for acetone is 19.7714%, 11.7953% for ethanol and 11.9622% for 2-propanol. The optimum extraction time for acetone is 6 hours. So, as a nutshell acetone is the best solvent with the optimum extraction time of 6 hours.

ABSTRAK

Pengekstakan kerotene daripada lobak merah (Daucus Carota. L) dengan menggunakan kaedah Soxhlet telah dilakukan dengan menggunakan pelarut yang berbeza dan masa pengekstrakan yang berlainan. Beta-kerotene adalah sebatian yang mempunyai nisbah paling besar di dalam kerotene. Di dalam penyelidikan ini lobak merah segar digunakan sebagai bahan ujikaji. Kajian ini mengakji kesan pelarut dan jangka masa pengekstrakan terhadap hasil beta-kerotene. Pelarut yang digunakan di dalam ujikaji ini adalah aceton, etanol, dan 2-propanol. Sementara, jangkamasa pengekstrakan adalah 2,4,6, dan 8 jam. Terdapat empat proses yang merangkumi ujikaji ini iaitu penyediaan bahan ujikaji, pengekstrakan menggunakan Soxhlet, pengasingan pelarut dengan menggunakan penyejat berputar dan menganalisis kuantiti beta-kerotene di dalam sebatian dengan menggunakan HPLC. Bagi aceton; kuantiti beta-kerotene adalah 19.7714%, 11.7953% bagi etanol manakala 11.9622% bagi 2-propanol. Jangkamasa pengekstrakan terbaik apabila aceton digunakan sebagai pelarut adalah 6 jam. Kesimpulannya, aceton adalah pelarut terbaik dan jangkamasa pengekstrakan optimumnya adalah 6 jam.
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CH_3-C (=O)-CH_3	-	Acetone
CH_3- CH_2- OH	-	Ethanol
CH_3- C(=OH)-CH_3	-	2-propanol
HPLC	-	High Performance Liquid Chromatograph
Hr	-	Hour
°C	-	Degree Celcius
g	-	gram

1 INTRODUCTION

1.1 Research Background

Nowadays in the era of globalization, where new diseases almost come frequently and continuously, people now are really concerning about their safety and health. Many efforts are done such as practicing healthy life, eat right food, have sufficient sleep and many other beneficial things. According to (The Star, March 2012), one in five Malaysian are diabetic, and surprisingly diabetic patients has increased two-fold from 1.5 million in 2006 to three million in 2011.

Due to the statistics stated above, people now searching for every single healthy thing especially in food. That is why we often heard the phrase 'we are what we eat'. As a result, the demand for "natural food colorants such as carotenoids is growing due to consumer concerns for food safety and quality. Actually, there are many sources of carotenoids such as plants and vegetables. According to (Watson, 2000), carotenoids also can be contained in algae, bacteria, moulds and yeasts. The colour of fruits, roots, flowers and vegetables are usually can be caused by carotenoids which provide the pigments.

Beta-carotene is the most abundant carotenoids. This is proved by (Jeszka, 1997), 80% of carotenes are beta-carotene. It can be found notably in fruits, orange colour vegetables, dark green leafy vegetables including pumpkin, carrots, winter squash, sweet potatoes, apricots, mangoes, kale, spinach and collard greens (Steinmetz and Potter, 1996). Carrots are one of the best sources of beta-carotenes with range 300mg/100 g (Velisek, 1999).

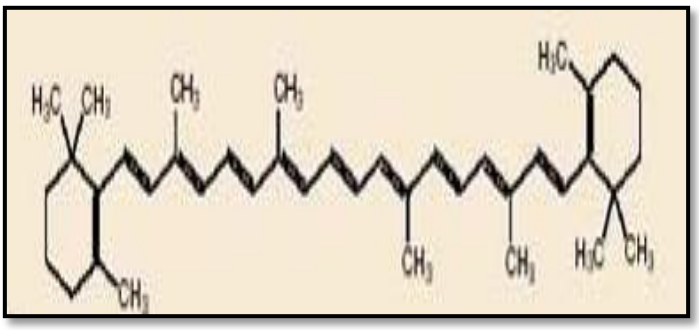

Figure 1.1: Chemical structure of beta-carotene
(retrieved from http://openlearn.op en.ac.uk)

Carotenoids are commonly known as provitamin A. Other than that, it has been prove that carotenoids play a role as antioxidants (Bohm et al., 2002). Surprisingly, beta-carotene has the ability to act as anti-cancer activity as well as protection against cardiovascular disease and cataract prevention (Dietmar & Bamedi, 2001). Since it has large benefits to human being, many experimental tests have been done in order to extract carotenoids from it sources.

In order to obtain carotenoids from plants, many extraction methods have been introduced to fulfill the requirements such as hydro-distillation, supercritical fluid extraction (SFE), solvent extraction, microwave-assisted distillation and soxhlet extraction. Extraction is the best way to keep maintains the sufficient nutrients in the sources. The simple definition of extraction is a kind of separation process by using a solvent in which the desired substance dissolves in while the undesired substance does not dissolve in. Among the techniques used for implementation, Soxhlet extraction has been choose to complete this project. It has been used for a long time and this assertion has been supported by the fact that it is standard technique during more than one century (Castro & Ayuso, 1998). The Soxhlet extraction is the most conventional of all methods and consists of a simple distillation process repeated a number of times. Furthermore, this method is straight forward and inexpensive.

In this project, carrot is use as a source of carotenoids where it will be extracted by using Soxhlet extraction. This project will investigate the optimum condition for extraction by

varying the type of solvents and duration of extraction time. The solvents are acetone, ethanol, and 2-propanol. While, the duration of extraction time are 2hr, 4hr, 6hr, and 8hr.

1.2 Problem Statement

One of the major public health nutritional problems in Malaysia as a developing country is Vitamin A inadequacy. This is because most people in developing countries do not really know the function of carotenoids. They rarely include fruits and vegetables into their diet. Preventable blindness is responsible due to deficiency of Vitamin A. According to (V.S.Ekam et al., 2006), diseases such heart diseases, cancer, cataracts, and macular degeneration can be minimized if sufficient carotenoids are taken into the diet.

Carotenoids had known of it's attributed to health benefits when consumed as part of human diet. Carotenoids consumprion can reduced the risks of cancers, variety of diseases, eye-disease (cataract), and age-related macular degeneration (luteinlab.unh.edu). So, it can be said that carotenoids is very usefull as it can act as diseases prevention.

This project proposes to extract carotenoids from carrot. Fortunately, carotenoids is believed to have derived their name from the fact that they constitute the major pigment in the carrot root, Daucus Carota (Tee, 1995). Carrot is one of the major source of carotenoids. In Malaysia, these kinds of fruits are can be easily obtain. But the problem is people do not know the use of carotenoids and the importance of it. From the early discovery based on carrot, it is worth to extract the carotenoids from carrot that is very usefull for human health in term of the role carotenoids play as provitamin A, antioxidant, food colourant and disease prevention.

Due to the multifunctioning of carotenoids, another method of extraction process was being developed such as supercritical fluid extraction (SFE). Actually, there are many method of extraction process available such as supercritical fluid extraction (SFE), hydrodistillation, and microwave-assisted distillation. However, there are still lack of detail information regarding this process, especially for the purpose of beta-carotene extraction. Thus, Soxhlet extraction

has been identified as one of the most economical method and is widely used in Malaysia since it required simple apparatus and safe.

1.3 Research Objective

The main objective of this research is to determine the best condition of extraction, purposely to get the highest yield of beta-carotene.

1.4 Scopes of project

There are some important tasks to be carried out in order to achieve the objective of this project. The important scopes have been identified for this research in achieving the objective:

i. Extraction of carotenoids via Soxhlet extraction from fresh carrot
ii. Study the effect of different solvents (acetone, ethanol, and 2-propanol) on extraction yields.
iii. Investigate the effect of different extraction duration on extraction yields for 2hr, 4hr, 6hr, and 8hr.

1.5 Rational and Significant

Currently, there are lot of disease contributes to the lack of vitamin A in body. With high level of carotenoids in carrots which means high vitamin A, carotenoids can be used as an alternatives to medicine that are need to consume by patients who suffer diseases caused by malnutrition of vitamin A. This is useful to human as medicine from hospital usually contain drugs where carotenoids on the other hand are natural, provitamin A. Other than that, carotenoids have the anti-cancer activity that can be used to fight cancer.

Based on the knowledge gain from this project, it will enable to obtain the best condition for obtaining the highest yields of carotenoids. Thus, the knowledge gain will enable for the development and technology transfer to the local producers.

1.6 Organisation of this thesis

The structure of the thesis is outlined as followed:

Chapter 2 provides a description of the raw materials which is carrot. A general description on the nutritions contain in the carrot are presented. This chapter also provides a brief discussion of the carotenoids in term of types of carotenoids in carrot. Apart from that, the function and uses of carotenoids are also presented. Three stages of experimental work which are Soxhlet extraction, rotary evaporation and analysed by HPLC is also discussed in general.

Chapter 3 gives a review of the procedure for this experiment. This includes the extraction of beta-carotene from carrot by Soxhlet extractor, separation of solvent from beta-carotene by rotary evaporator and analyzed beta-carotene contained in the solution by HPLC. Apart from that, the pre-treatment method for the sample preparation is also presented.

Chapter 4 contains result related to this experiment. From this result, the best solvent and extraction time can be discovered. The result data is tabulated in table and graph form. Other than that, discussion also been made regarding the result obtain.

Chapter 5 provides anything possible that can be done in order to improve this experiment.

2 LITERATURE REVIEW

2.1 Overview

Chapter 2 provides a description of the raw materials which is carrot. A general description on the nutritions contain in the carrot are presented. This chapter also provides a brief discussion of the carotenoids in term of types of carotenoids in carrot. Apart from that, the function and uses of carotenoids are also presented. Three stages of experimental work which are Soxhlet extraction, rotary evaporation and analysed by HPLC is also discussed in general.

2.2 Carrot

Carrot is a type of fruit which is usually red in colour when it is in rape condition. People are commonly use it in cooking and as a type of drinking called carrot juice. It taste is not so delicious for eat it rawly but it turns opposite way when make it as juice to drink. Apart from delicious and refreshing drinking, it is very nutritious. Carrot is known to be the major source of carotenoids.

2.3 Carotenoid

Ekam et al. (2006) explained, carotenoids are largely naturally distributed occurring pigments responsible for the yellow, orange and red colour of fruits, roots and flowers. Examples of carotenoids are alpha-carotene, beta-carotene, and lutein. Carrot which is the major source of carotenoids, containing beta-carotene (60-80%), alpha-carotene (10-40%) and lutein (1-5%) respectively (Chen et al., 1995). 80% of carotenes in carrot are beta-carotene (Jeska, 1997). Carotenoids is dominated by beta-carotene and is found widely in orange colored vegetables and fruits and in dark green leafy vegetables such as carrots, pumpkin, winter squash, sweet potatoes, apricots, mangoes, kale, spinach and collard greens (Steinmetz and Potter, 1996).

2.3.1 Importance of carotenoids

Carotenoids are widely known as a provitamin A. Carotenoids also has possibility as antioxidants (Bohm et al., 2002). Other than that, beta-carotene providing anti-cancer activity will give the protection against cardiovascular disease or cataract prevention (Dietmar & Bamedi, 2001). Nutrients in carotenoids such as beta-carotene, alpha-carotene, beta-cryptoxantin and other constituents are good for human body. Many research have been made and the result obtain indicate that higher intake of nutrients in carotenoids will help in asthma and allergy (Devereux, 2006). Hung et al. (2006) mentioned, in the studies that have been done lately, carotenoids give the shield effects on bladder cancer. Nowadays, lot of people died caused by cancer. Cancer is known as a disease that very tough to be cured unless that disease is detected earlier. So, the best way to prevent cancer is reduced the risk to be affected by cancer. Carotenoids rich in antioxidants activity, which could lead to lower cancer risk (Steinmetz & Potter, 1993).

Oxidative metabolism in the human body by-products and exogenous sources derived may generate free radicals. Carotenoids are potent antioxidants enabling them to neutralize free radicals. Consequently, free radicals can destruct RNA and DNA in cells as well as inactive proteins and enzymes by reactions with amino acids. Test on rats had been made and the result obtains shows carotenoids function as anticarcinogens and this may be the same for human (Singh and Lippman, 1998). Constituents in carotenoids which are alpha-carotene, beta-carotene, canthalxanthin, lutein, and lycopene are discovered to up-regulate gap junctional intracellular communication via differs in gene expression (Zhang et al., 1992).

Furthermore alpha- carotene and beta-carotene may inhibit cell proliferation and may enhance immune function. It may aid the body system to fight against diseases and infections. Virus may spread widely just in a blink. Human in this world always expose to virus that may cause disease. Things will become worst if that contacted person hove low or weak antibody. Carotenoids are needed in human body as they will fight the infections and enhance immune function.

2.3.2 Function and uses of Carotenoids

Below are some of the function and uses of carotenoids:

a) Food colours

Colour in food is very important because colour determine the acceptability. Food with good colour may attract or initiated the appetie. Colour also may define the processing of the food either it is processes well or poorly processes. An important use of carotenoids is in food colouring.

Carotenoids have been use widely as food colourant and most of the countries accept the use of carotenoids. According to (Bauernfeind, 1972), beta-carotene is allowable in 40 countries; the other carotenoids constituents are allowable in the other 20 countries. Actually, it is for about centuries ago the world has used natural extracts containing carotenoids for colouring food. Borenstein & Bunnel (1966) explained that, the natural extracts containing carotenoids that use as food colouring are annatto with bixin, saffron with derivatives of crocetin and other carotenoids, paprika containing the two pigments capsanthin and capsorubin, xanthophylls extracts from leaves, and red palm oil.

Several synthetic carotenoids are presently available, making it possible for them to be used widely in colouring processed and fabricated foods (Bauernfeind, 1972). Among all the constituents of carotenoids, beta-carotene was the first synthetic carotenoids to be marketed in 1954 (Bauernfeind, 1972). Beta-carotenoid nowadays becomes the most widely used carotenoids for colouring foods. Carotenoids are used in food colouring in variety of fat or water-based foods including butter, cheese, margarine, cheese, ice cream, wheat products, vegetable oils, cake mixes, candy, soups, desserts, fruit juices, and beverages.

b) Precursor of vitamin A

Vitamin A which also known as retinoid cannot be synthesized within the body (Underwood, 1984). The main source of retinoid for man are the carotenes apart from pre-formed vitamin A contained in food such as milk, eggs, fish liver oil, liver and of course synthetic vitamin A. Beta-carotene is the vital vitamin A precursor compare to other carotenes. This is due to its

concentration in food and feed ingredients greatly exceed that of the other vitamin A active compounds (Bauernfeid et al., 1971).

The other sources of pre-formed vitamin A are milk, eggs or liver. But, in the diets of population groups in the tropical world rarely contain this source. Thus, carotenoids become a great deal since it is the other source of vitamin A to these communities.

c) Carotenoids in photosynthetic tissues

Other than contribution to human, carotenoids also had its own function to the plants as well. Carotenoids own the ability to absorb visible light. The role of carotenoids in the case of photosynthesis tissues are:

1- Help in photosynthesis
2- Protect the photosynthesis tissue against photosensitized oxidation

The mechanism is carotenoids act as accessory light-absorbing pigments in the photosynthesis process. According to Mathews-Roth (1981), carotenoids absorb light at wavelength lower than that absorbed by chlorophyll. So, carotenoids have extended the wavelength of light that can be used in photosynthesis. Carotenoids have been discovered to protect organisms against the seriously damaging effects of photooxidation by their own endogeneous photo-sensitizers, chlorophyll. This is then being applied to human, where carotenoids have been used for treatment of patients with photosensitivity disease. This disease is where the person affected is sensitive to the sunlight known as light-sensitive porphyria, the porphyrins produced resemble the porphyrin ring of chlorophyll and act as photo-sensitizers in the patient. Carotenoids will protect the patient from the photosensitizers.

d) Antioxidants

Various proposals have been put forth to explain the protective function of carotenoids against harmful photosensitized oxidations discussed in the above section. Krinsky (1979) discussed the major mechanisms whereby these pigments exert this function: (1) quenching of triplet sensitizers; (2) quenching of singlet oxygen ($^1O_2$); (3) inhibition of free radical reactions. The

photochemical reactions that can induce photodamage were reviewed, and the possible mechanism of action of carotenoids on the reactive chemical species produced was discussed. In photochemically induced oxidations, carotenoid pigments have been shown to have the capacity to quench the first potentially harmful intermediate, the triplet sensitizer, at a significant rate. The remaining triplet sensitizer species could then continue to initiate a series of reactions, depending on the availability of oxygen and the nature of other potentially reactive species in the environment, with the production of singlet oxygen and free radicals.

The ability of carotenoids to deactivate reactive chemical species such as singlet oxygen, triplet photochemical sensitizers and free radicals have been actively studied in recent years, with the main focus on beta-carotene. Some insight into the antioxidant activities of other naturally occurring carotenoids have also been reported (Terao, 1989; Di Mascio *et aL,* 1989).

It has been suggested that reactive oxygen species and free radicals may play an important role in cancer development. These species are continually being formed in human tissues and their safe sequestration is an important part of antioxidant defence.

Thus, the protective effects of carotenoids against the harmful effects of oxidation would be expected to have a protective effect against cancer.

2.4 Sohlet Extraction

Soxhlet extraction is one of the common method uses in extraction of essential oil. Soxhlet extraction is conducted by using Soxhlet extractor. Soxhlet extractor is a piece of laboratory apparatus (Laurence, p. 122-125) and is invented by Franz von Soxhlet in 1879. Actually, Soxhlet extractor is design for the extraction of a lipid from a solid material. Although it is designed for that function but it is not limited for the extraction of lipids. When the desired compound has a limited solubility in a solvent and the impurity is insoluble in the solvent, Soxhlet extractor is then needed. Otherwise, simple filtration can be used if the desired

compound has a significant solubility in a solvent. Figure 2.3 shows the example of Soxhlet extractor.

Figure 2.3: Soxhlet Extractor

The concept of the Soxhlet extraction is organic compound is extracted by washing it continuously with an organic solvent under reflux in special glassware. In general, the setup consists of round bottom flask containing the solvent, and extraction chamber and a condenser.

In this method, the sample is dried, chopped into small size and is placed in a porous cellulose thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the

solvent running back down to the distillation flask. This cycle may be allowed to repeat for many times.

2.5 Rotary Evaporator

A rotary evaporator is a specially designed instrument for the evaporation of solvent (single-stage or straight distillation) under vacuum. The evaporator consists of a heating bath with a rotating flask, in which the liquid is distributed as a thin film over the hot wall surfaces and can evaporate easily. The evaporation rate is regulated by the heating bath temperature, the speed of the rotary, the size of the flask and the pressure of distillation.

This is how heating bath temperature, the speed of the rotary, the size of the flask and the pressure of the distillation affect the separation process:

- Pressure of the distillation

Unlike a substance's melting point, its boiling temperature depends greatly on the ambient pressure. The higher the ambient pressure, the higher the boiling point temperature; the lower the ambient pressure, the lower the boiling point temperature. As example, the boiling point of water is 100°C at sea level, but 84°C at 4810 meter altitude. This indicates that high-boiling substances can be distilled at lower boiling temperature if the ambient pressure is reduced.

In practice, distillations are performed at reduced pressure (vacuum distillation) in order to prevent damage to temperature-sensitive substances. Substances with the boiling point of 100°C or higher are often distilled in vacuum in order to enable a water bath to be used as heat source.

- Size of the flask

The bigger the size of flask, the higher the rate of distillation.

- Rate of rotation

The distinctive characteristic of a rotary evaporator is the rotating evaporating flask. Its rotation should be selected to produce maximum turbulence in the bath as well as inside the flask. This turbulence depends on the amount of substance filled in the flask, the viscosity of the substance and the flask size.

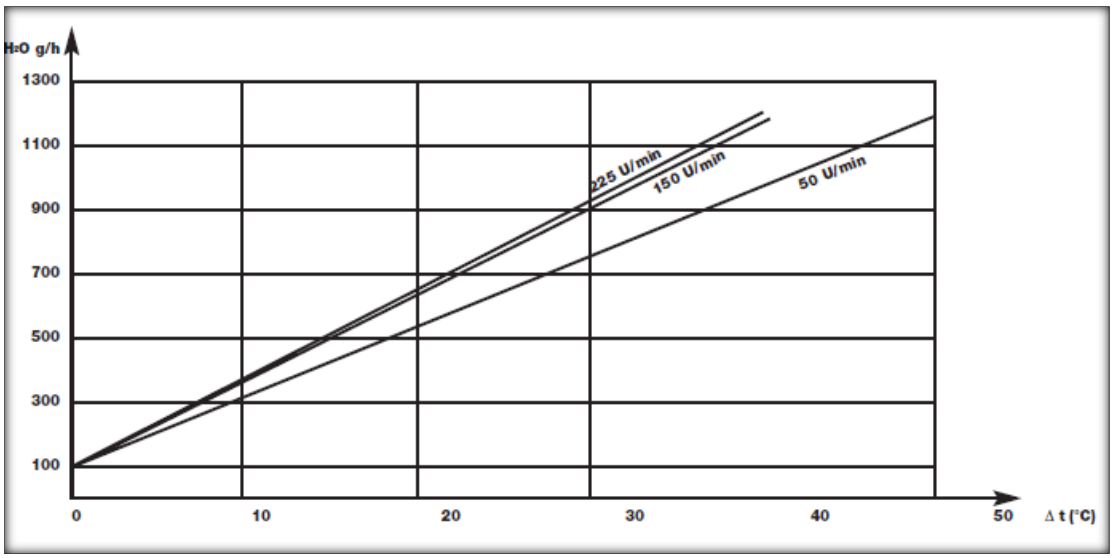

Figure 2.4: Graph of relationship between rate of rotation and rate of distillation (retrieved from BUCHI Labortechnik AG)

Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents from the compounds. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation (azeotropic behavior), and a sufficient difference in boiling points.

Basically, if the solvents is high in boiling point (>100°C) oil based rotary evaporator is used. This is because oil has higher boiling point compare with water.

2.6 High Performance Liquid Chromatography (HPLC)

HPLC (as shown in Figure 2.2) is a technique most commonly used for the quantitation of drugs in pharmaceutical formulations. HPLC involves the simultaneous separation and quantitation of compounds in a sample matrix that has been introduced onto a chromatographic column, packed with a stationary phase. Separation is achieved by the use of a stationary phase and a solvent, termed the mobile phase, that is allowed to flow through the stationary phase at a set flow rate, for isocratic chromatography (Dumortier et al., 2001 and Meyer et al., 2002).

During analysis, the sample components partition to differing degrees between a stationary and mobile phase, based on their inherent physico-chemical properties (Meyer et al., 2002). The nature of the physico-chemical interaction between the mobile and stationary phase allows solute molecules to emerge from the column in individual component zones or bands, which are then monitored as a function of an appropriate detector response versus time.

HPLC separations, to a large extent, include liquid-liquid chromatography (LLC), liquid-solid chromatography (LSC), size exclusion chromatography, normal, RP-HPLC, ion exchange and affinity chromatography. In reversed-phase chromatography, the stationary phase is usually a hydrophobic bonded phase, such as an octadecylsilane or octylsilane and the mobile phases are usually polar solvents such as water or mixtures of water and water-miscible organic solvents such as methanol, acetonitrile, THF or isopropanol. Nonionic, ionic and ionisable compounds can be separated using a single column and mobile phase, with or without added buffer salts, using bonded-phase columns that are reproducible and relatively stable (Wysocki, 2001).

Only one solute was being investigated, thus the use of an isocratic system was deemed appropriate for the development of an HPLC analytical method. For samples in which different solutes are present, it may be advantageous to use gradient elution where the composition of the mobile phase is altered during the separation, usually by blending two or more solvents with different eluting powers in continually changing proportions (Paul, 1991)

whereas in isocratic systems, a mobile phase of constant composition is used to effect a separation.

In the case of an HPLC system that may not be accurate or precise, the use of an internal standard improves accuracy, by correcting for variable injection volumes of a test solution. A solution containing a fixed amount of internal standard is added to the sample in a precisely measured volume. Any subsequent losses of the analyte sample are accounted for, since losses of the analyte will be mirrored by losses of the internal standard. A chemical substance may be used as an internal standard if it is related to the analyte of interest, is stable and elutes as close as possible to the analyte of interest whilst is still adequately resolved from the analyte and any possible excipients that may be present in the sample matrix being analysed (Wilson, 1990).

There are two major things should be highlighted in HPLC which are:

1) Mobile phase selection
2) Flow rate selection

2.6.1 Mobile phase selection

In addition to the stationary phase, the mobile phase composition plays a significant role in the elution or retention of the compounds of interest. HPLC is a multi-faceted process with the appropriate interplay of various parameters being vital during analysis, to produce a desired separation. Parameters such as the physico-chemical properties of an analyte of interest, the type of stationary phase chosen in addition to the mobile phase selected for analysis play a combined role in effecting a suitable separation, and manipulation of one or all of these factors to optimize a separation and improve the chromatographic behaviour of the compound under investigation, may be necessary.

The important characteristics of solvents for use in HPLC analysis include the need for high purity, immiscibility with the stationary phase, absence of reactivity towards an adsorbent, low boiling point and low viscosity (Skoug et. al., 1996). Mobile phases of extreme pH must also

be avoided, *i.e.*, pH<3 and pH>9, as these may damage the bonded phase of the silica backbone or lead to dissolution of the silica. However newer stationary phases are reported to be more resilient to extreme pH conditions. The solvents used in reversed-phase chromatography with bonded non-polar stationary phases are generally polar solvents or mixtures of polar solvents, such as acetonitrile, methanol and/or water (Shah et al., 1992). Of particular importance, is the fact that the mobile phase should be pure and free from impurities, dust, particulate matter and dissolved air (Paul, 1991and Skoug et al., 1996). Particulate matter can interfere with the pumping action of the solvent delivery module or pump and this can cause damage to the seals and/or check valves, collect on the top of the column causing subsequent column blockages thereby promoting chromatographic anomalies such as changes in retention time and poor peak resolution (Gent, 2002).

2.6.2 Flow rate selection

Flow rates ranging from 0.5-1.5 ml/min have been used for the analysis of CBZ (Al-Zein et al., 1999 and Etman, 1995). Lower flow rates minimize any potential deleterious effects on the pump and column and conserve solvents. A flow rate of 1.0 ml/min was chosen for use in these studies since adequate peak resolution was observed at this flow rate without the extreme effects of high flow rates and associated high back pressures on equipment. Furthermore, this flow rate enabled a compromise in terms of conserving solvents and retention time, as the slower the flow rate, the longer the resultant retention time.

2.7 Summary

This chapter give the complex picture on this topic. Starting from the raw material and its nature. Next, the nutrition contain carrot especially beta-carotene and it’s function. After that, some idea on the way Soxhlet extractor works. Then, what factor affecting rotary evaporator process is also discussed. Lastly, the general idea on HPLC is also provided.