MOHAMAD SAZWAN ISMAIL BACHELOR OF CHEMICAL ENGINEERING 2013 UMP

EXTRACTION OF BETA-CAROTENE FROM CARROT VIA SOXHLET EXTRACTION METHOD

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EXTRACTION OF BETA-CAROTENE FROM CARROT VIA SOXHLET EXTRACTION METHOD

MOHAMAD SAZWAN BIN ISMAIL

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JUNE 2013

SUPERVISOR'S DECLARATION

"I hereby acknowledge that the scope and quality of this thesis is qualified for the award of the Bachelor Degree of Chemical Engineering".

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STUDENT'S DECLARATION

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Dedication

In the name of ALLAH, Most Gracious, Most Merciful

To my beloved my father, mother, sisters and brothers.

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Firstly, my highest gratitude goes to God because of His mercy and kindness my final year project has successfully finished within the time provided. Many things I have learned throughout a year especially in extraction process. These experiences gained will be very useful for my future career and endeavours. I hope that all information gained from my final year project may benefit others especially Universiti Malaysia Pahang (UMP) student and staff for reference and research.

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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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LIST OF ABBREVIATIONS

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).

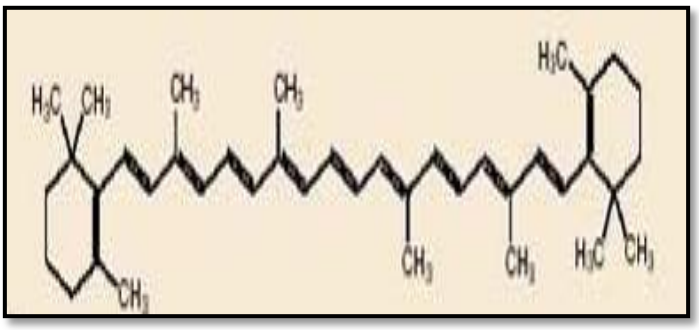


Figure 1.1: Chemical structure of beta-carotene
(retrieved from http://openlearn.op en.ac.uk)

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.

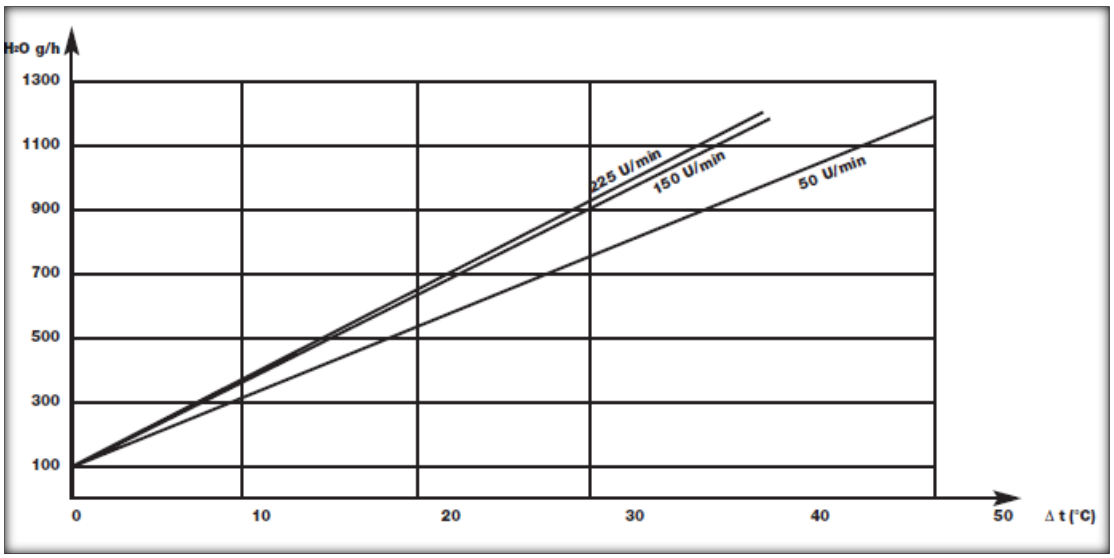


Figure 2.4: Graph of relationship between rate of rotation and rate of distillation (retrieved from BUCHI Labortechnik AG)

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.

3 MATERIALS AND METHODS

3.1 Overview

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.

3.2 Reagent and material

Analytical grade of acetone, ethanol, and 2-propanol are used in this research. While, for HPLC mobile phase, acetonitrile, methanol, and dichloromethane are used. For the purposed of HPLC, the grade of all the mobile phase chemicals are HPLC grade. Fresh carrot is used as sample in this research.

Figure 3.1 : Carrot (Daucus Carota.L)

3.3 Apparatus

Oven is used to dry the chopped carrot. For the purposed of extraction, Soxhlet extractor had been used to extract beta-carotene from carrot for laboratory scale. Rotary evaporator is used

to separate beta-carotene and solvent. Lastly, HPLC unit (Supercosil LC-18 column) is used to analyse the beta-carotene yield.

3.4 Experimental work

3.4.1 Sample preparation

First of all, the sample which was carrot needs to be prepared to produce a kind of powder. The fresh carrot is chopped into smaller size before it was dried in an oven. The temperature of microwave was make up to 60°C. The carrot was dried for 24 hours. The dried carrot is then blended into powder by using blender. Figure 3.1 shows the carrot become powder after blended it by using blender.

Figure 3.2: Carrot powder

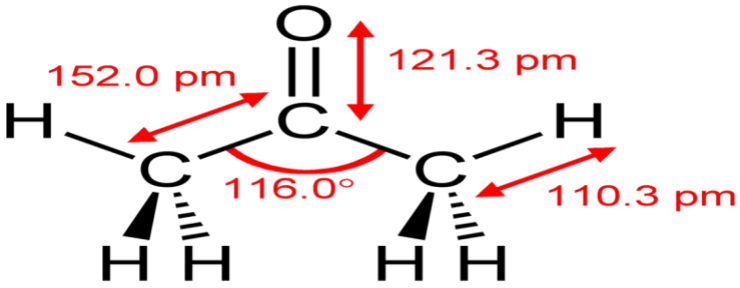
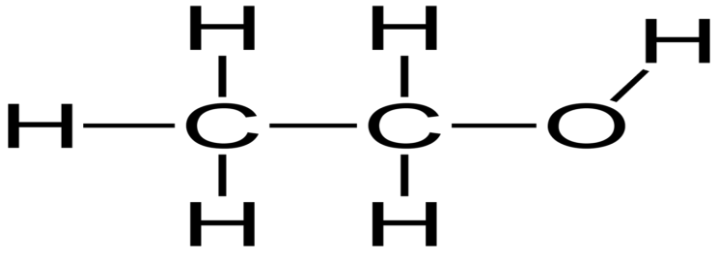
3.4.2 Procedure

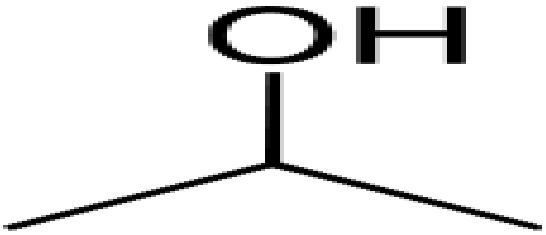
Extraction was performed using Soxhlet extraction method as shown in Figure 3.3. 25g of carrot powder was weight by using an electronic balance and was placed in the porous cellulose thimble. The thimble was placed in the extraction chamber, which was suspended above the flask containing the solvent and below the condenser. 150ml of solvent was placed

in the round bottom flask which is located below the extraction chamber. Acetone, ethanol, and 2-propanol were used as solvents in this study. The operating temperature was depends on the boiling point of the solvent. Table 3.1 shows the chemical formula and boiling points for each solvent. The extractions time are 2hr, 4hr, 6hr, and 8hr.

Figure 3.3: Soxhlet extractor unit

Table 3.1: Chemical structure/formula and boiling point for each solvent

Solvent	**Chemical structure/chemical formula**	**Boiling point (°C)**
Acetone		56-57
Ethanol		78.37

2-Propanol		82.5

After finished the extraction process, the extracted compound should be separated from the solvent used. Firstly, the extracted oil was filtered by using filter paper to removed suspended solid. After that, put the extracted mixture in rotary evaporator as shown in Figure 3.4. The extracted mixture was placed in the rotary flask. The solution in the flask is filled no more than about half full. If the solution in the flask is more than half full, the solution is more likely to "bump", a term when the solvent evaporates too quickly, causing the liquid to splash up into the bump trap. So, the type of the flask is something that should be considered. The flask was attached to the rotary evaporator. The flask is attached to the bump trap by using a Keck clamp. The bump trap is designed to capture any of the solvents that splashes up. Then, the bath temperature is set. The temperature used are depends on the boiling point of the solvent used. After that, the bottom of the flask is immersed in the bath by turning the height adjustment lever and gently lowering the flask into the bath. Then, the rotation is turned on by using the knob. The oil obtain after separation method was weighted using analytical balance and placed in the sample bottle.

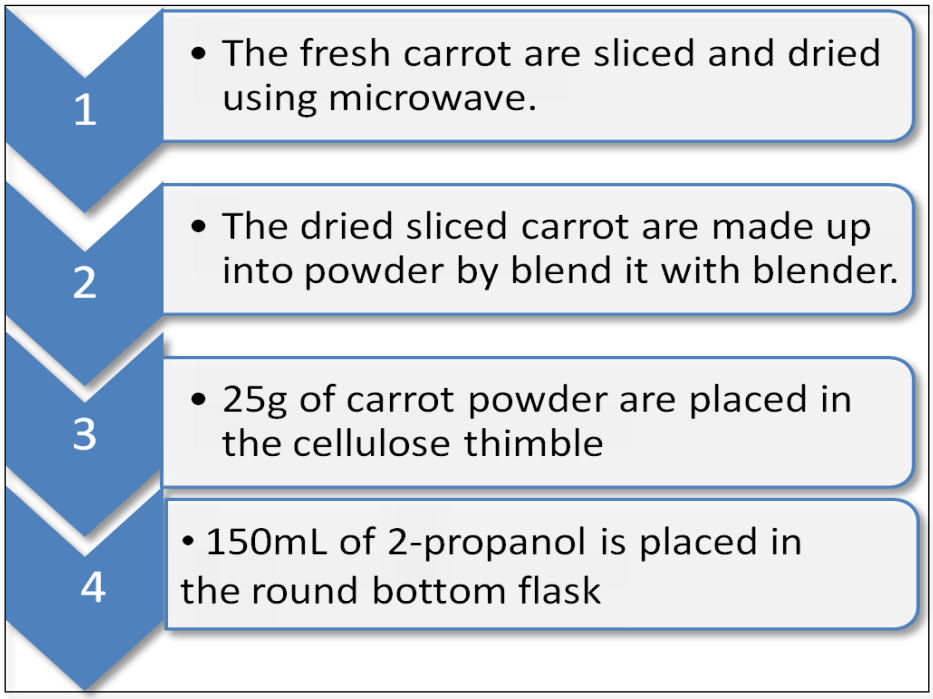
Figure 3.4: Rotary evaporator

Beta-caroetene, which is the extracted compound, will be analyzed by using HPLC system. The identification of beta carotene in the extract samples are performed according to (Mendes et al., 1999). HPLC system used is equipped with a Supercosil LC-18 column ($15cm \times 4.6cm \times 5\mu m$). Before transferred the extracted samples into HPLC vial the extracted sample are filtered by using nylon filter first. This is to remove any particle in the samples to avoid damage occurring in the HPLC system. Note that, the syringe should be changed if the extracted samples are in different type of solvents. This is to preserve the accuracy of the result. After that, the mobile phase is prepared. The chemicals use for mobile phase in this analysis are dichloromethane, methanol and acetonitrile. According to (Ahamad M.N. et al., 2007), the mobile phase consisted of acetonitirle, dichloromethane and methanol by the ratio 70:20:10. So, 700ml of acetonitrile and 200ml of dichloromethane and 100ml of methanol are filled in 1L Schott bottle. The mobile phase is then degas by using pump. The peak for beta-carotene are monitored by a UV detector at 452nm. The flow rate is 2mL/min.

Figure 3.5: High performance liquid chromatography located in FKKSA lab

3.5 Summary

The entire steps involved in these experimental procedures can be simplified in Fig. 3.6:



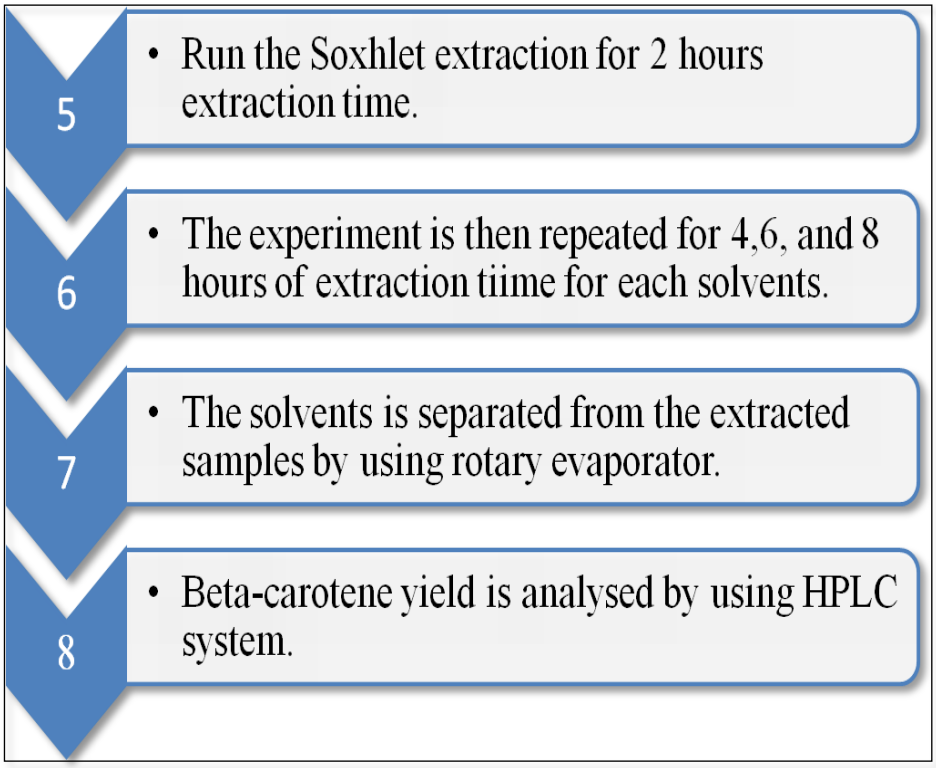


Figure 3.6: Flow chart of methodology

4 RESULTS AND DISCUSSIONS

4.1 Overview

In this experiment, the solvents used are 2-propanol, ethanol, and acetone. This chapter is discussed about which solvent is the best for the extraction of beta-carotene among the three solvents that are being tested. The extraction time also is examined to determine the optimum extraction time which will bring the best beta-carotene yield. This is to avoid excessive extraction time and the worst decreasing of beta-carotene yield.

4.2 Results

4.2.1 Effect of type of solvent

Three types of solvents which are acetone, 2-propanol, and ethanol are used in this research. Table 4.1 shows the oil weight after undergoes the separation process between solvent and oil. The yield of beta-carotene oil throughout this study is calculated by using the formula below:

$$\text{Percentage yield (\%)} = \frac{\text{oil weight (g)}}{\text{sample weght (g)}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots (1.0)$$

From equation 1, the oil weight is obtain by measuring the weight of the oil after undergoes separation process. The sample weight for all analysis is 25g.

Table 4.1: Percentage yield of oil for each solvent

Solvent	Oil weight (g)	Sample weight (g)	Percentage yield (%)
Acetone	**12.905**	**25**	**51.62**
Ethanol	8.05	25	21.47
2-propanol	5.37	25	32.18

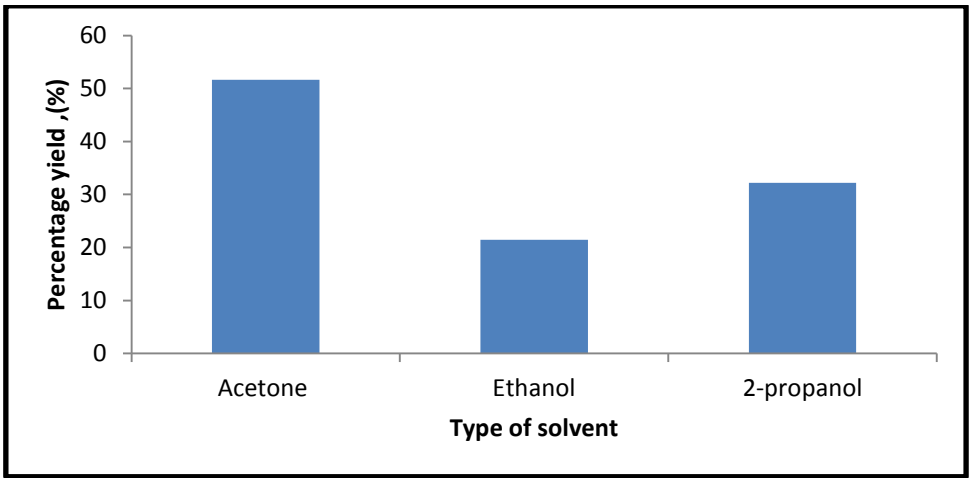


Figure 4.1: Graph of percentage yield versus type of solvent

Based on Figure 4.1, it is shown that the highest percentage yield of beta carotene is discovered when using acetone with 51.62%. Followed by 2-propanol with 32.18% and ethanol with 21.47%.

4.2.2 Effect of extraction time

Effect of extraction time towards extraction yield is also being studied. The range of extraction time is 2.0, 4.0, 6.0, and 8.0 hours. Table 4.2, 4.3, and 4.4 below shows the oil weight after undergoes separation process for acetone, ethanol, and 2-propanol.

Table 4.2: Percentage yield for oil at different extraction time using acetone as solvent

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	11.9	25	47.6
4	12.45	25	49.8
6	**12.91**	**25**	**51.62**
8	11.43	25	45.7

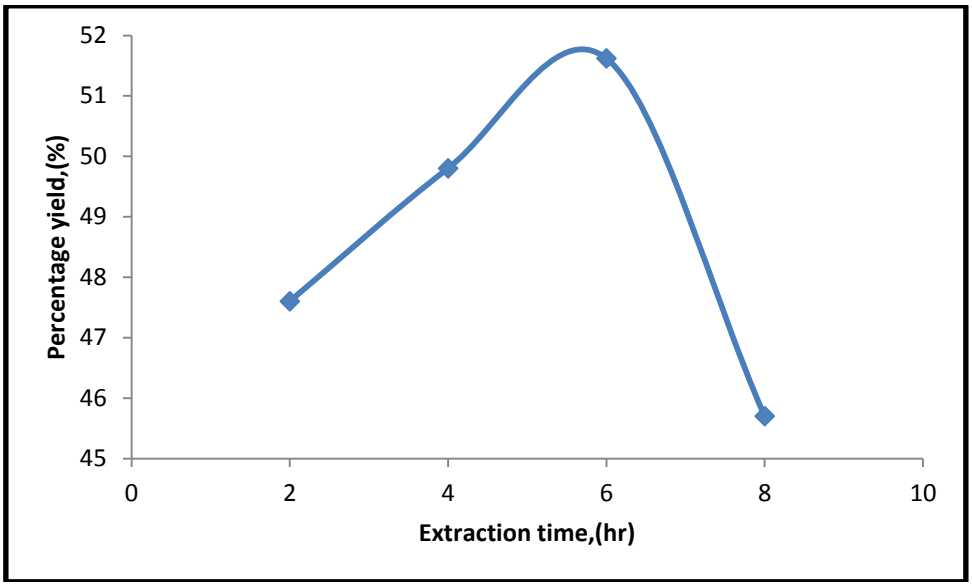


Figure 4.2: Graph of percentage yield versus extraction time using acetone as solvent

From Figure 4.2, at 2 hours of extraction time the percentage yield is 47.6%. While at 4 hours of extraction time the percentage yield is 49.8%. It is shown that the highest percentage yield for acetone is obtained to be 51.62% when the extraction time is 6 hours. After that, the percentage yield starts to decrease. The lowest percentage yield for acetone is discovered to be 45.7% at 8 hours of extraction time. In the extraction time from 2-6 hours, the percentage yield is increasing. When the extraction time exceed 6 hours, the percentage yield start to decrease.

Table 4.3: Percentage yield for oil at different extraction time using ethanol as solvent

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	7.09	25	28.34
4	8.92	25	35.67
6	**9.86**	**25**	**39.42**
8	9.75	25	39.01

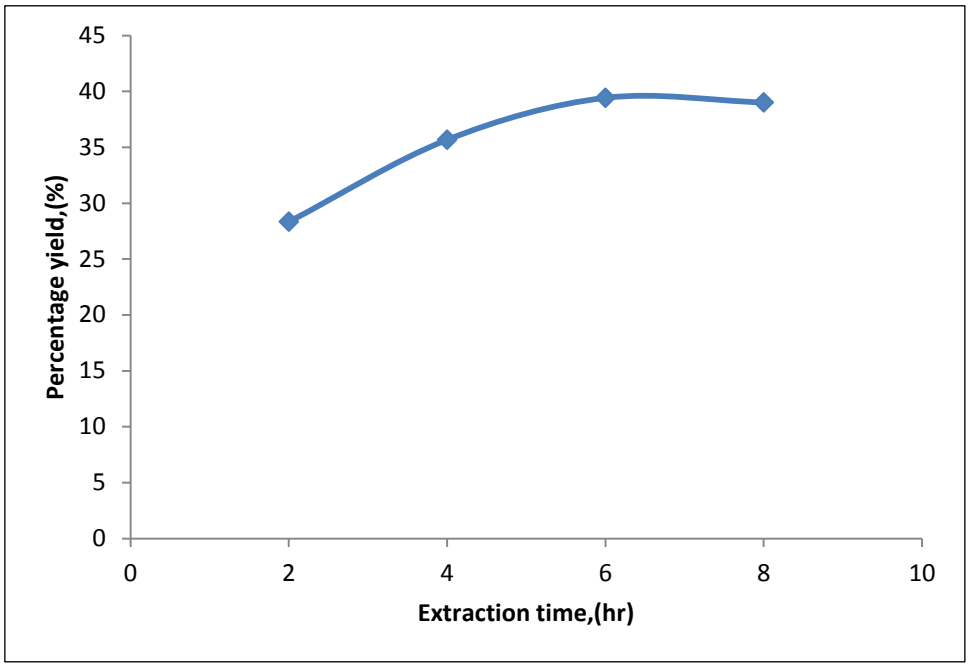


Figure 4.3: Graph of percentage yield versus extraction time using ethanol as solvent

From Figure 4.3, it can be said that the highest percentage yield is obtained when extraction time is 6 hours where the percentage yield is 39.42%. While at 2 hours of extraction, the percentage yield is 28.34%. 35.67% of percentage yield is obtained when the extraction time is 4 hours. The lowest percentage yield is discovered at 8 hours of extraction time. In the extraction time from 2-6 hours, the percentage yield is increasing. When the extraction time exceed 6 hours, the percentage yield start to decrease.

Table 4.4:Percentage yield for oil at different extraction time using 2-propanol as solvent

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	**10.00**	**25**	**40.01**
4	9.61	25	38.43
6	8.81	25	35.24
8	7.53	25	30.11

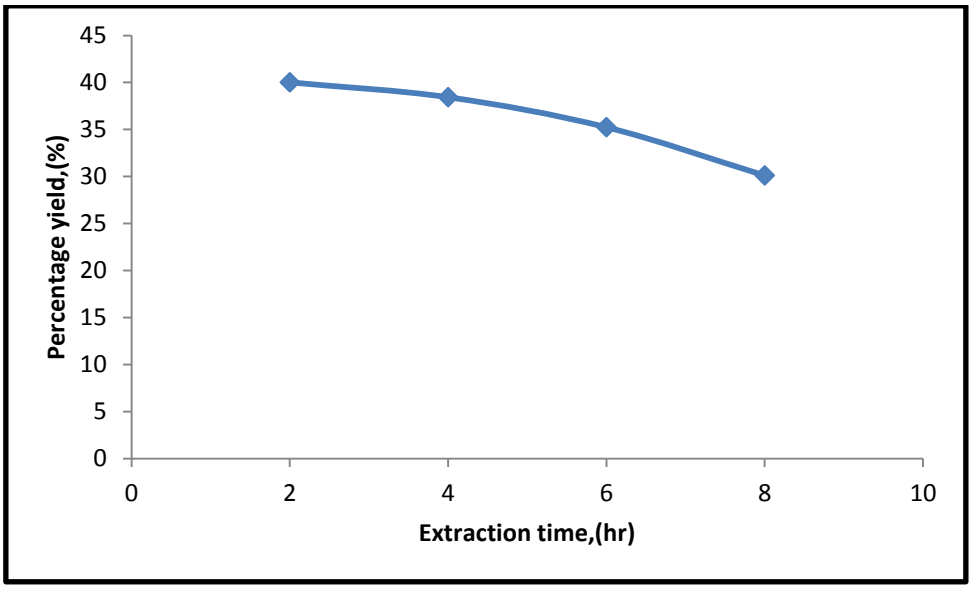


Figure 4.4: Graph of percentage yield versus extraction time using 2-propanol as solvent

According to Figure 4.4, at 2 hours extraction time the percentage yield is the highest with the percentage yield of 40.01%. At 4 hours of extraction time the percentage yield is 38.43%. 35.24% of percentage yield is obtained when the extraction time is 6 hours. The lowest percentage yield is 30.11% at 8 hours of extraction time. When the extraction time longer than 2 hours the percentage yield is decreasing.

4.2.3 HPLC analysis

This research is completed by analyzing the beta-carotene yield in the extracted oil by using HPLC unit. In this research beta-carotene is identified at retention time of 4.897 min. Table 4.5, 4.6, and 4.7 shows the peak area for acetone, ethanol, 2-propanol for different extraction time.

Table 4.5: Peak area of beta-carotene for acetone at different extraction time

Acetone		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	15.6849
4	4.897	16.7980
6	**4.897**	**19.7714**
8	4.897	14.4280

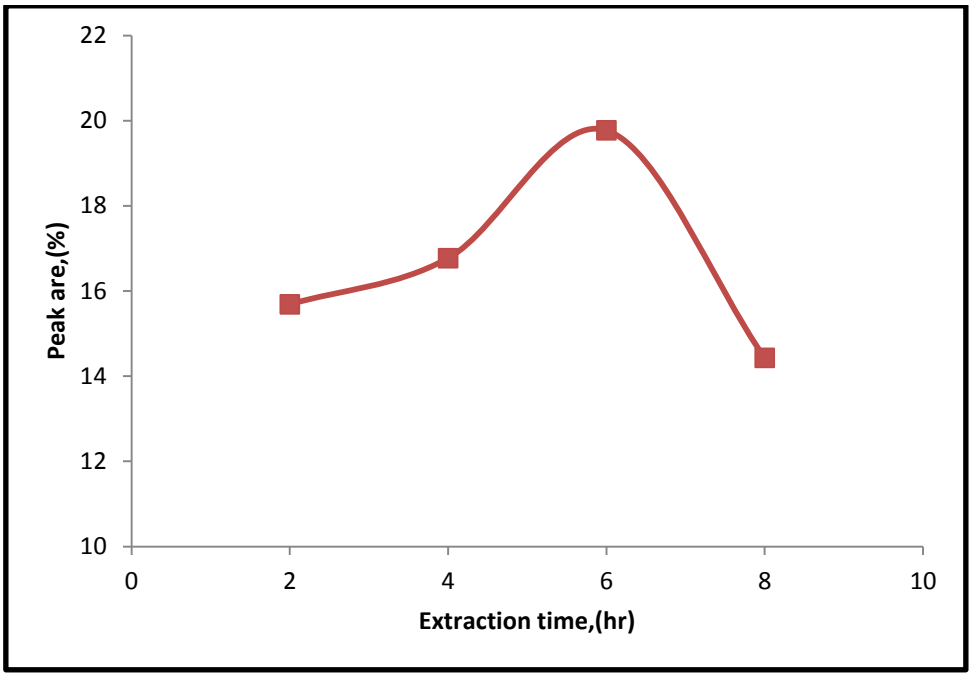


Figure 4.5: Graph of extraction time versus peak area using acetone as solvent

From Figure 4.5, for two hours extraction the peak area is 15.6849%. While at extraction time 4 hours the peak area is 16.7680%. The highest peak area is obtained at extraction time of 6 hours with the peak area 19.7714%. The lowest peak area is discovered at 8 hours of extraction time. Extraction longer than 6 hours will lead in decreasing of peak area for beta-carotene.

Table 4.6: Peak area of beta-carotene for ethanol at different extraction time

Ethanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	5.2255
4	4.897	9.1473
6	**4.897**	**11.7953**
8	4.897	11.5686

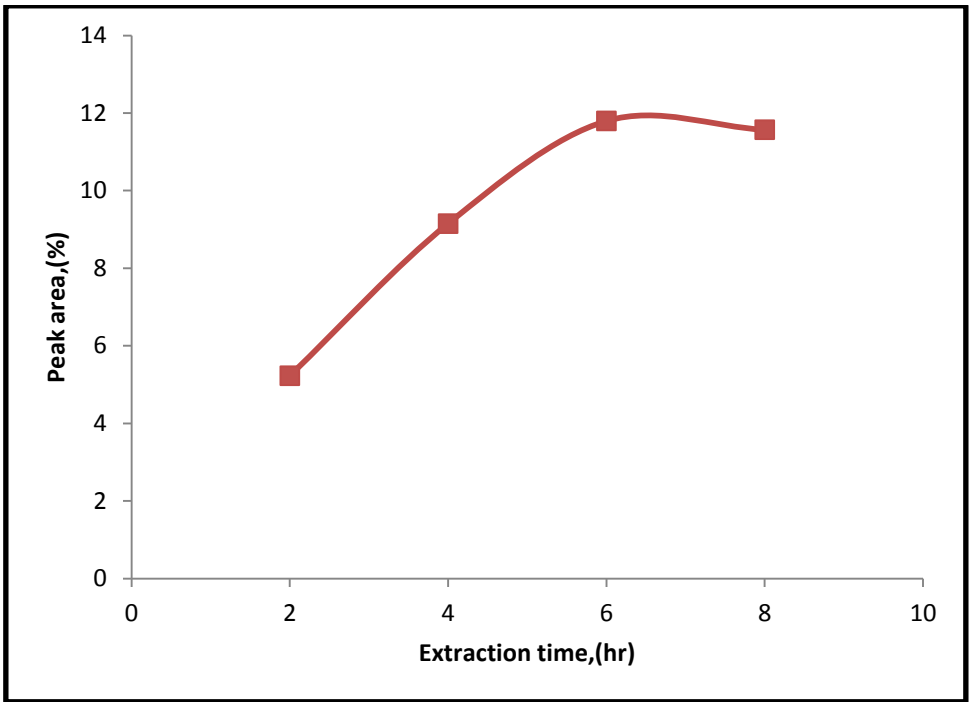


Figure 4.6: Graph of extraction time versus peak area using ethanol as solvent

Based on Figure 4.6, the peak area is 5.2255% for 2 hours of extraction. When the extraction time is 4 hours, the peak area is 9.1473%. The highest peak area is found to be 11.7953% at 6 hours of extraction time. After that, the peak area is starting to decrease to 11.5686% when the extraction time is 8 hours.

Table 4.7: Peak area of beta-carotene for 2-propanol at different extraction time

2-propanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	**4.897**	**11.9622**
4	4.897	11.0787
6	4.897	9.5225
8	4.897	7.8413

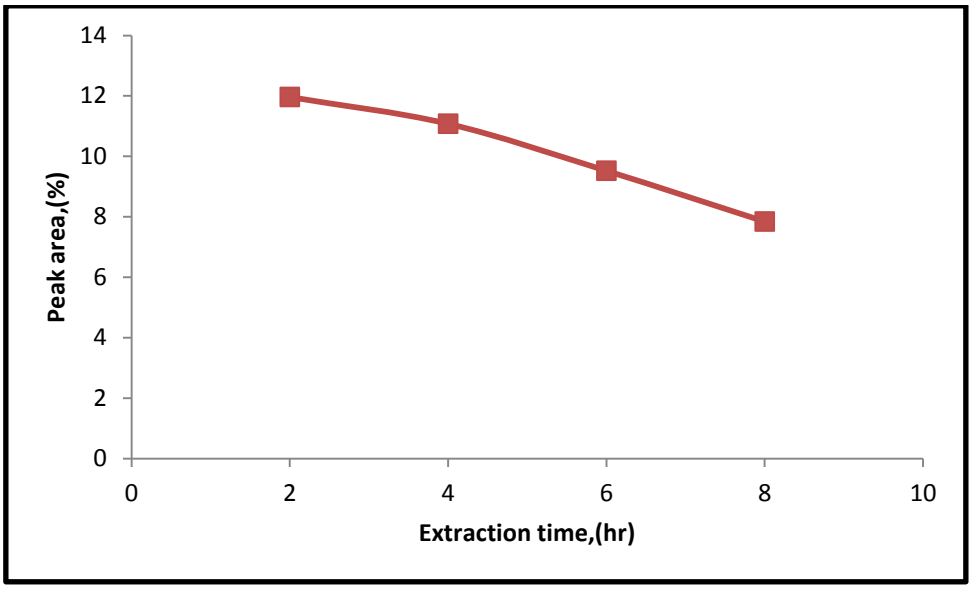


Figure 4.7: Graph of extraction time versus peak area using 2-propanol as solvent

By referring to Figure 4.7, the highest peak is obtained just at the moment for the lowest time of extraction in this analysis which is 2 hours. The peak area at this extraction time is 11.9622%. The peak area is continuously decreasing for extraction time longer than 2 hours. At 4 hours of extraction time, the peak area is 11.0787%. The peak area equal to 9.5225% is obtained at 6 hours of extraction time. While at 8 hours of extraction time, the peak area is 7.8413.

4.3 Discussions

4.3.1 Effect of type of solvents

In this analysis, three chemicals are used as solvents which are acetone, ethanol, and 2-propanol. This analysis is to determine which type of solvent will give the highest amount of beta-carotenes. First of all, the amount of beta-carotene yield in each solvent is compared. As we can see from HPLC result table, the highest peak area for acetone is 19.7714%, while for ethanol is 11.7953% and 11.9622%. So, by comparing the value of peak area it can be said that acetone is the best solvent. According to (Martina.F. et al., 2003), 2-propanol will give higher yield of beta-carotene than ethanol.

Beta-carotene is a non polar solvent due to (Pavia D.L. et al., 1999). Beta carotene is a hydrocarbon, making it quite non-polar. According to (Belitz et al., 2004), carotenoids are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, petroleum ether because carotenoids are liposoluble.

Table 4.8: List of solvents' polarity (retrieved from www.erowid.org)

Relative Polarity	Compound Formula	Group	Representative Solvent Compounds
Nonpolar	R - H	Alkanes	Petroleum ethers, ligroin, hexanes
	Ar - H	Aromatics	Toluene, benzene
	R - O - R	Ethers	Diethyl ether
	R - X	Alkyl halides	Tetrachloromethane, chloroform
	R - COOR	Esters	Ethyl acetate
	R - CO - R	Aldehydes and ketones	Acetone, methyl ethyl ketone
	R - NH_2	Amines	Pyridine, triethylamine
	R - OH	Alcohols	Methanol, ethanol, isopropanol, butanol
	R - $COHN_2$	Amides	Dimethylformamide
	R - COOH	Carboxylic acids	Ethanoic acid
Polar	H - OH	Water	Water

Increasing Polarity

Based on Table 4.8, we can say that ethanol is the most polar, followed by 2-propanol and the less polar solvent in this analysis is acetone. So, the sequence of increasing in polarity among three solvents in this analysis is acetone < 2-propanol < ethanol.

Ethanol is the most polar solvent among all the solvents. Ethanol is the polar protic solvent. Protic refer to a hydrogen atom attached to an electronegative atom. In ethanol, oxygen is an electronegative atom. In other words polar protic solvents are compounds that can be represented by the general formula ROH. The polarity of the polar protic solvents come from the bonding of O-H bond. The large difference in electronegativities of the oxygen and the hydrogen atom, combine with the small size of the hydrogen atom, warrant separating molecules that contain an OH group from those polar compounds that do not. While, on the other hand, acetone with the general formula RCOR is less polar compare to ethanol and 2-propanol. This is because the bonding is weaker than 2-propanol and ethanol. Beta-carotene is substance which is quite non-polar because it is hydrocarbon. That is why acetone has the

higher yield of beta-carotene because less polar compound is best to be extracted by the usage of less polar solvent too.

4.3.2 Effect of extraction time

In this study, the extraction time those being examined for beta-carotene yield are 2.0, 4.0, 6.0 and 8.0 hours. In any extraction process has their optimum extraction time. This optimum extraction time indicates the highest yield of desired compound. For example, in this analysis the optimum extraction time for acetone is 6 hours with the yield of 19.7714% beta-carotene. Extraction that exceeds this optimum extraction time which is 6 hours will decrease in the yield of beta carotene. So, it will be useless if the extraction time exceeds the optimum extraction time. That is why it is important to obtain the optimum extraction time. For ethanol, the highest yield of beta-carotene is 11.7953% of peak area. This is at 6 hours of extraction time. So, the optimum extraction time for ethanol is 6 hours. While for 2-propanol the highest yield is 11.9622% peak area with 2 hours of extraction time. So, the optimum extraction time for 2-propanol is 2 hours. Based on the peak area among all three solvents, it can be concluded that acetone is the best solvent with the optimum extraction time 6 hours.

The reason of the decreasing in the yield of beta-carotene due to exceeding in the extraction time is relatively due to isomerisation. Beta-carotene actually, has been known to be relatively sensitive compounds is also the reason for the degradation of beta-carotene. The most predominant degradation for beta-carotene is oxidative degradation (Fikselova, M., et al., 2008).

4.4 Summary

Three solvent are being analyzed to determine the solvent that will extract highest yield of beta-carotene. Furthermore, extraction time also being studied to determine the optimum extraction time. Based on the analysis method that had been done, it can be concluded that acetone is the best solvent and the optimum extraction time for acetone is 6 hours.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The study on beta-carotene extraction using Soxhlet extraction method has been conducted. It was discovered that the optimum yield of beta-carotene was obtained using acetone as solvent with 6 hours of extraction time. Apart from that, it is can be said that Soxhlet extractor is one of the oldest and easiest methods being used for the extraction of essential oil commonly. Thus, Soxhlet extraction has found to be economically viable and safe to operate. In this research, the affecting parameters such as type of solvent and extraction time can be identified by analyze the carotene yield.

5.2 Recommendations

In order to improve this study, there are some action that should be taken as future improvement as listed below:

1) The beta-carotene yield is studied by using non-polar solvent. This is because the nature of the beta-carotene itself is less polar. Less polar compound would be extracted better by using less-polar solvent.
2) Increase the extraction time. This is to get more data and hence will provide accurate analysis.
3) The extraction is conduct by using another method such as pressurized fluid extraction (PFE). This method of extraction utilizes conventional solvents at elevated temperature and pressure, and it requires less solvent which will reduce environment hazardous.

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APPENDICES

Appendix A

Gantt Chart for Undergraduate Research Proposal 1

MONTH	SEPTEMBER				OCTOBER				NOVEMBER				DISEMBER			
FRAMEWORK	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
Research Title Given																
Discussion with Supervisor																
Literature Review																
Preparation of Proposal Report																
Chemical Order																
Submission Report Draft																
Presentation URP 1																
Submission of Final Report																

Appendix B

Gantt Chart for Undergraduate Research Proposal 2

ACTIVITY	MONTH																				
	February				March				April				May					June			
	W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4	W 5	W 1	W 2	W 3	W 4
Consult supervisor																					
Literature Review																					
Methodology																					
Experimental Work:																					
1) Part 1: Soxhlet Extraction																					
2) Part 2: Rotary Evaporation																					
3) Part 3: HPLC Analysis																					
Analyze Data																					
Thesis Writing																					
Draft Submission																					
Seminar Presentation																					
Final Report Submission																					

Appendix C

Data collection:

Percentage yield of oil for different type of solvent:

Solvent	Oil weight (g)	Sample weight (g)	Percentage yield (%)
Acetone	**12.905**	**25**	**51.62**
Ethanol	8.05	25	21.47
2-propanol	5.37	25	32.18

Percentage yield of oil for different extraction time for acetone:

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	11.9	25	47.6
4	12.45	25	49.8
6	**12.91**	**25**	**51.62**
8	11.43	25	45.7

Percentage yield of oil for different extraction time for ethanol:

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	7.09	25	28.34
4	8.92	25	35.67
6	**9.86**	**25**	**39.42**
8	9.75	25	39.01

Percentage yield of oil for different extraction time for 2-propanol:

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	10.00	25	40.01
4	9.61	25	38.43
6	8.81	25	35.24
8	7.53	25	30.11

Peak area of beta-carotene for acetone with different extraction time:

Acetone		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	15.6849
4	4.897	16.7980
6	**4.897**	**19.7714**
8	4.897	14.4280

Peak area of beta-carotene for ethanol with different extraction time:

Ethanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	5.2255
4	4.897	9.1473
6	**4.897**	**11.7953**
8	4.897	11.5686

Peak area of beta-carotene for 2-propanol with different extraction time:

2-propanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	**4.897**	**11.9622**
4	4.897	11.0787
6	4.897	9.5225
8	4.897	7.8413

Appendix D

Sample preparation:

Fresh carrot

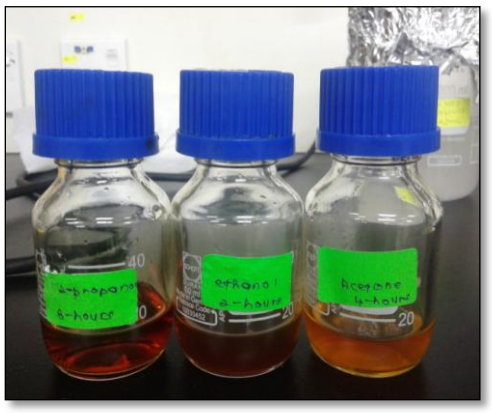
Chopped carrot

Dry carrot

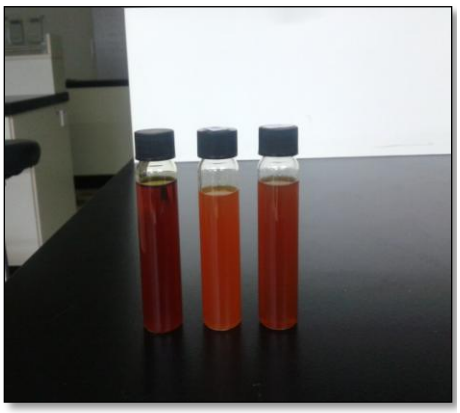
Carrot powder (sample)

Appendix E

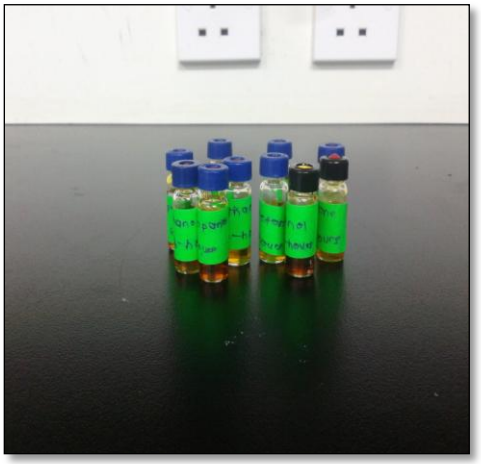
Procedure:



Extracted beta-carotene and solvent



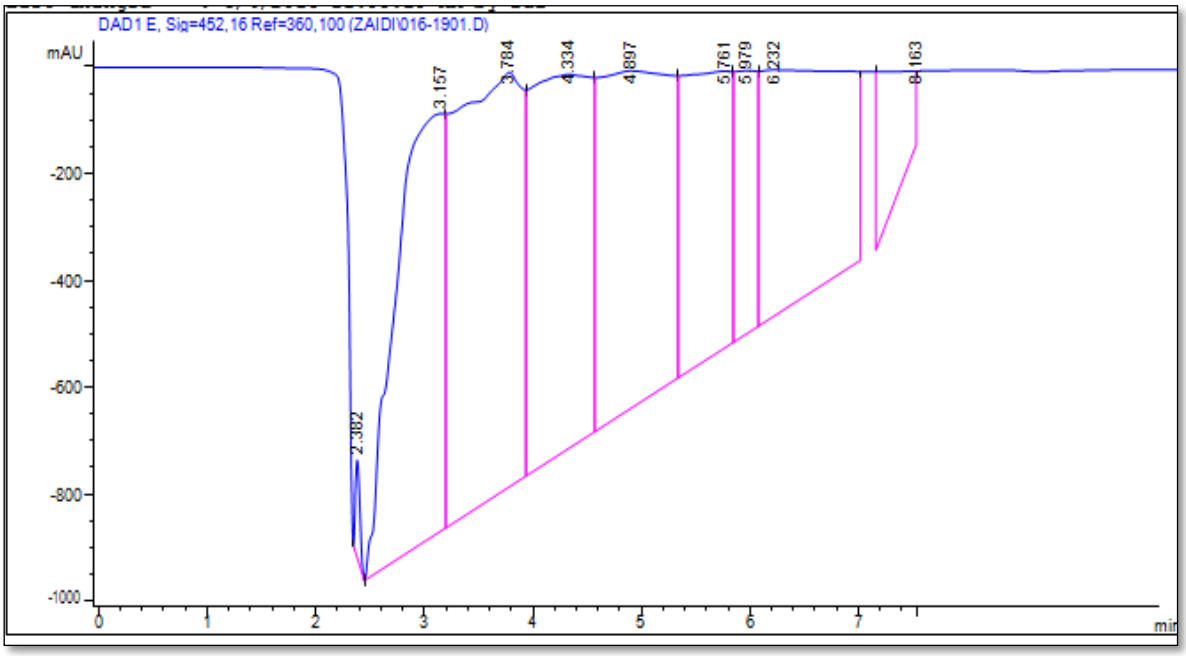
Beta-carotene oil



Beta-carotene oil in vial for HPLC analysis

Appendix F

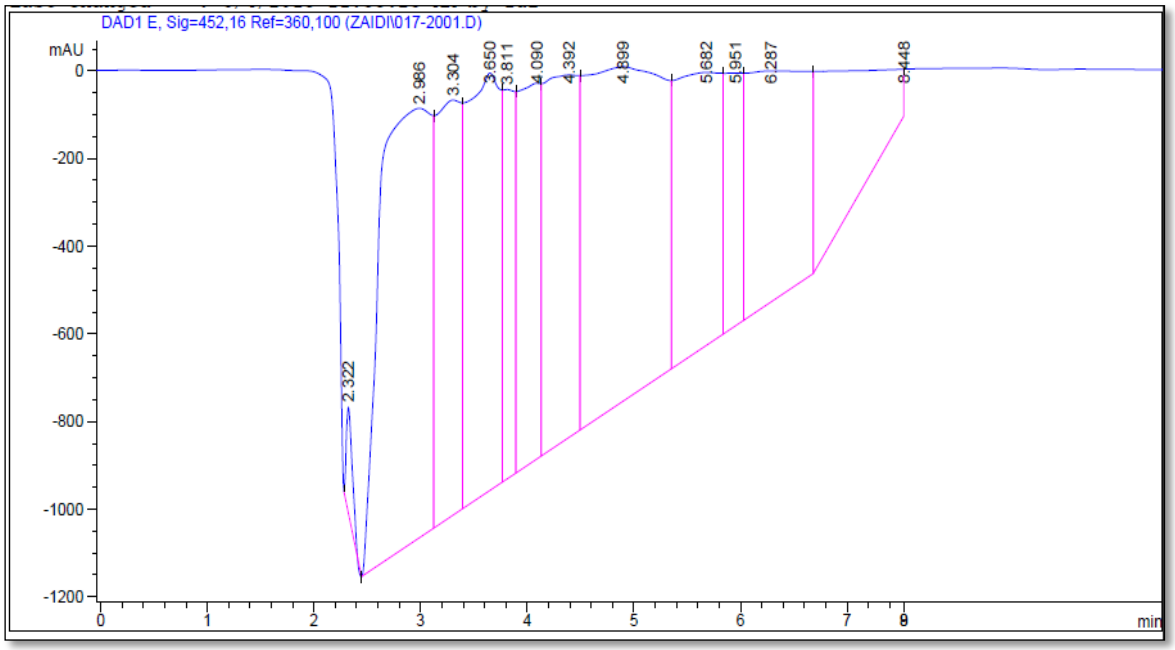
HPLC analysis: Acetone (2 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.382	BV	0.0476	529.87378	184.05511	0.2907
2	3.157	VV	0.4810	2.45516e4	780.54932	13.4712
3	3.784	VV	0.5313	3.37462e4	776.00818	18.5161
4	4.334	VV	0.4730	2.67032e4	700.00818	14.6517
5	4.897	VV	0.5792	2.85863e4	632.61646	15.6849
6	5.761	VV	0.4113	1.63183e4	518.39172	8.9537
7	5.979	VV	0.1989	6854.16602	490.85043	3.7608
8	6.232	VB	0.6365	2.35016e4	459.63794	12.8950
9	8.163	BV	1.2592	2.14621e4	206.04216	11.7760
Totals :				1.82253e5	4748.15950	

Appendix G

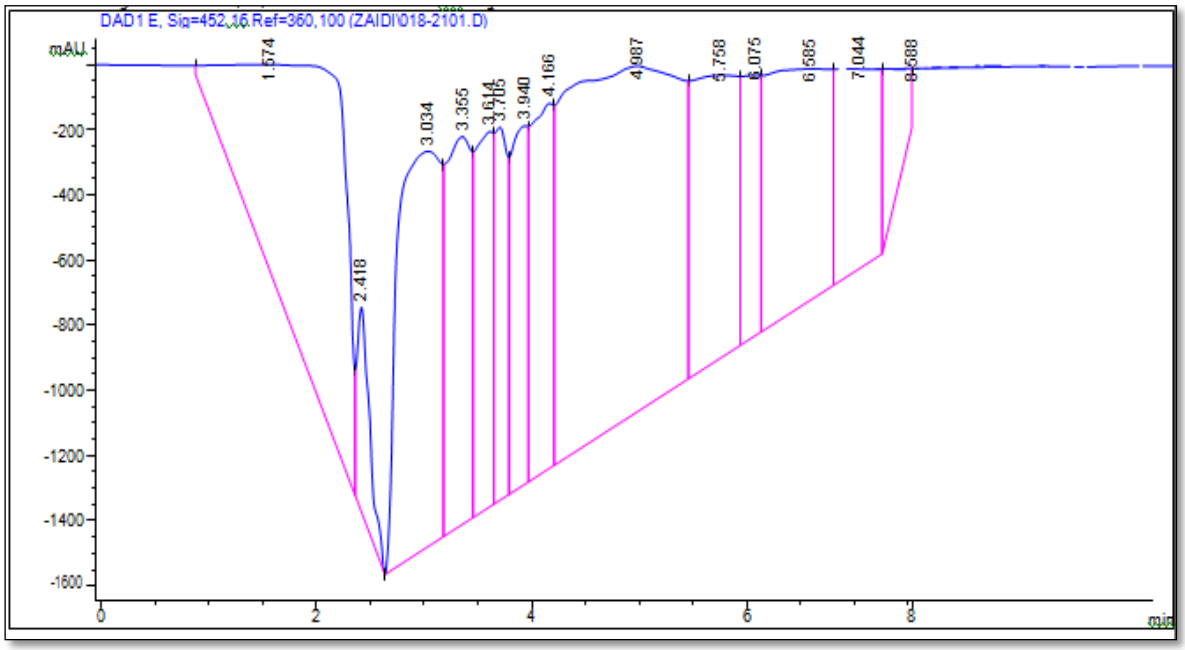
HPLC analysis: Acetone (4 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.322	BV	0.0616	942.63379	241.04637	0.4131
2	2.986	VV	0.5302	3.26050e4	980.00757	14.2889
3	3.304	VV	0.2301	1.52657e4	946.94818	6.6901
4	3.650	VV	0.2796	2.06963e4	952.01605	9.0700
5	3.811	VV	0.1056	6667.81543	888.48456	2.9221
6	4.090	VV	0.1916	1.23118e4	857.23523	5.3955
7	4.392	VV	0.2894	1.82430e4	826.80963	7.9949
8	4.899	VV	0.6200	3.83416e4	763.39465	16.8029
9	5.682	VV	0.4013	1.84196e4	622.36328	8.0723
10	5.951	VV	0.1664	6646.79199	576.98376	2.9129
11	6.287	VV	0.5200	2.01404e4	526.84375	8.8264
12	8.448	VV	2.5190	3.79033e4	181.30638	16.6109
Totals :				2.28184e5	8363.43942	

Appendix H

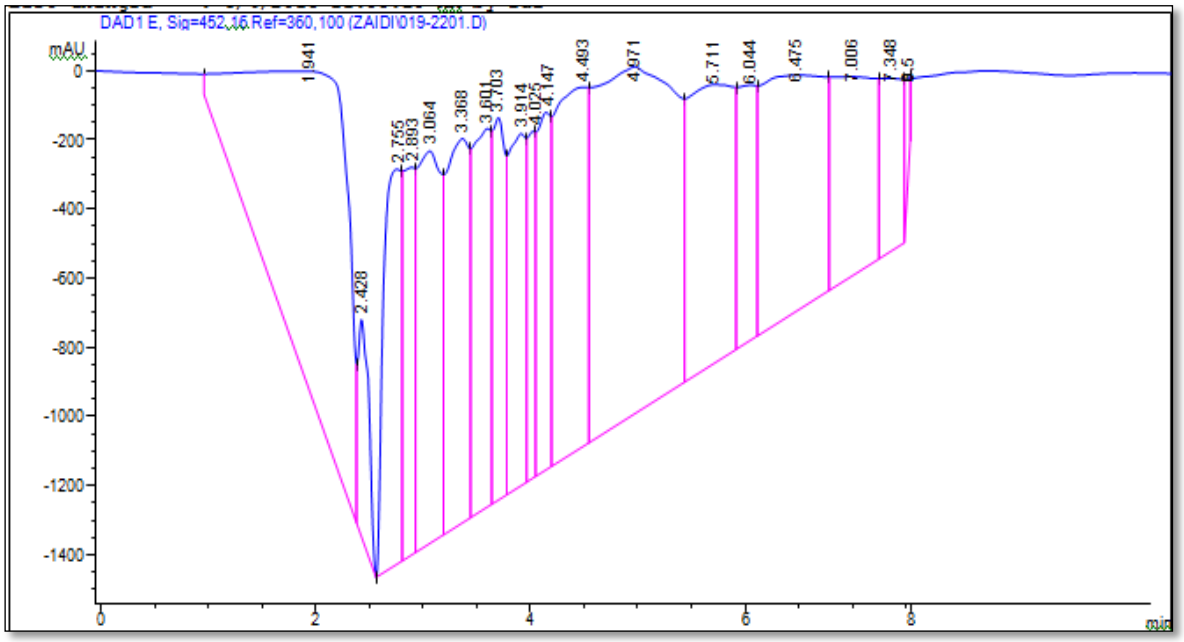
HPLC analysis: Acetone (6 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.574	BV	1.0577	5.59159e4	638.46729	15.3176
2	2.418	VV	0.1137	5159.81152	629.23590	1.4135
3	3.034	VV	0.3852	3.32181e4	1214.28333	9.0998
4	3.355	VV	0.2291	1.90894e4	1190.13171	5.2294
5	3.614	VV	0.1536	1.33723e4	1150.78333	3.6632
6	3.705	VV	0.1166	9280.83594	1144.78015	2.5424
7	3.940	VV	0.1626	1.17409e4	1099.25659	3.2163
8	4.166	VV	0.1871	1.58444e4	1119.66003	4.3404
9	4.987	VV	0.9407	7.91094e4	1059.95007	21.6713
10	5.758	VV	0.4412	2.52262e4	867.53705	6.9105
11	6.075	VV	0.1672	9401.96289	799.53699	2.5756
12	6.585	VV	0.5682	2.94727e4	710.13733	8.0738
13	7.044	VV	0.3824	1.67148e4	612.49268	4.5789
14	8.588	VV	1.6907	4.14952e4	293.62518	11.3672

Appendix I

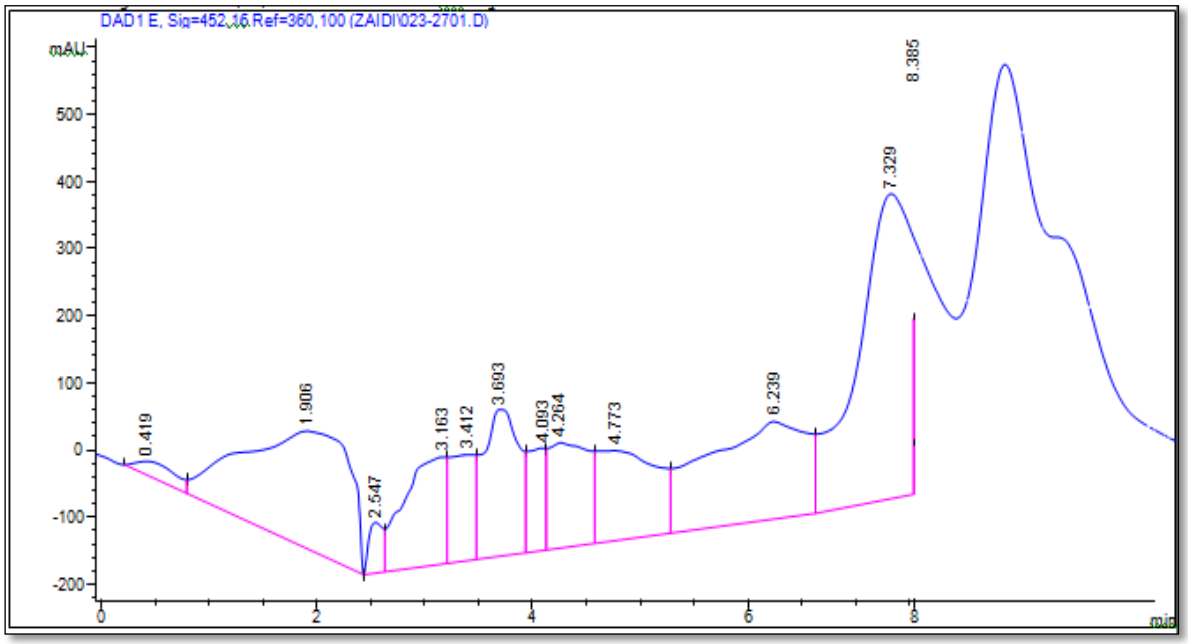
HPLC analysis: Acetone (8 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.941	BV	0.7929	5.42792e4	921.89740	15.4190
2	2.428	VV	0.0952	4477.80566	628.20056	1.2720
3	2.755	VV	0.1838	1.29138e4	1142.69116	3.6684
4	2.893	VV	0.1021	8265.51660	1120.54395	2.3480
5	3.064	VV	0.2161	1.73188e4	1134.57422	4.9197
6	3.368	VV	0.2173	1.63076e4	1110.47253	4.6325
7	3.601	VV	0.1550	1.28446e4	1093.42651	3.6487
8	3.703	VV	0.1144	8939.56055	1105.10474	2.5394
9	3.914	VV	0.1440	1.09830e4	1017.13220	3.1199
10	4.025	VV	0.0714	5100.47998	1001.78064	1.4489
11	4.147	VV	0.1220	9032.52734	1032.64368	2.5659
12	4.493	VV	0.2894	2.16469e4	1037.43188	6.1492
13	4.971	VV	0.6269	5.13355e4	1003.42773	14.5828
14	5.711	VV	0.4033	2.31569e4	805.81140	6.5782
15	6.044	VV	0.1680	8737.06445	738.76575	2.4819
16	6.475	VV	0.5022	2.72786e4	682.67230	7.7490
17	7.006	VV	0.3770	1.58858e4	573.70532	4.5126
18	7.348	VV	0.2046	7072.19482	501.29956	2.0090
19	8.269	VV	1.1178	3.08953e4	342.68192	8.7764
20	9.557	VBA	0.8740	5556.44580	82.47379	1.5784
Totals :				3.52027e5	1.70767e4	

Appendix J

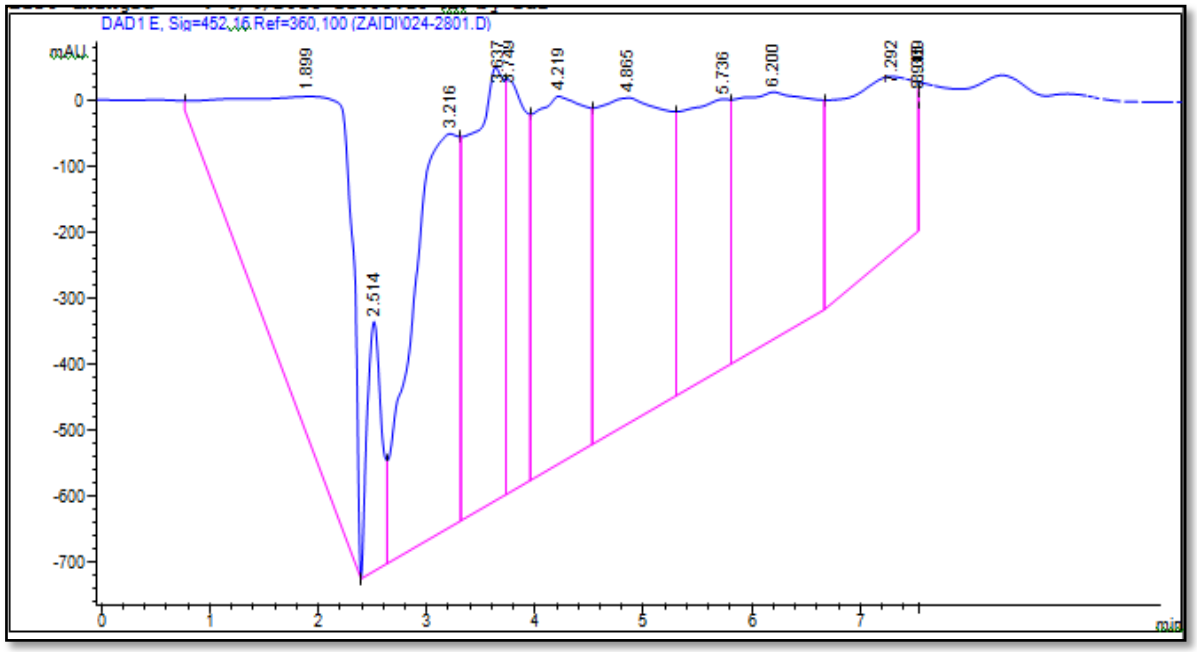
HPLC analysis: Ethanol (2hours extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.419	BV	0.4671	610.83582	19.84617	0.5845
2	1.906	VV	0.8550	1.17643e4	174.85251	11.2576
3	2.547	VV	0.1448	687.36346	75.07945	0.6578
4	3.163	VV	0.3405	4324.60596	159.42538	4.1383
5	3.412	VV	0.2332	2578.93701	157.33406	2.4678
6	3.693	VV	0.2888	4996.44189	218.50461	4.7812
7	4.093	VV	0.1535	1716.50049	152.33792	1.6426
8	4.264	VV	0.3299	4028.19946	156.68057	3.8547
9	4.773	VV	0.4685	5075.90869	133.82822	4.8573
10	6.239	VV	0.8247	9621.26758	145.26015	9.2068
11	7.329	VV	0.7032	2.29689e4	461.07715	21.9795
12	8.385	VBA	0.7761	3.61282e4	630.88867	34.5720
Totals :				1.04502e5	2485.11486	

Appendix K

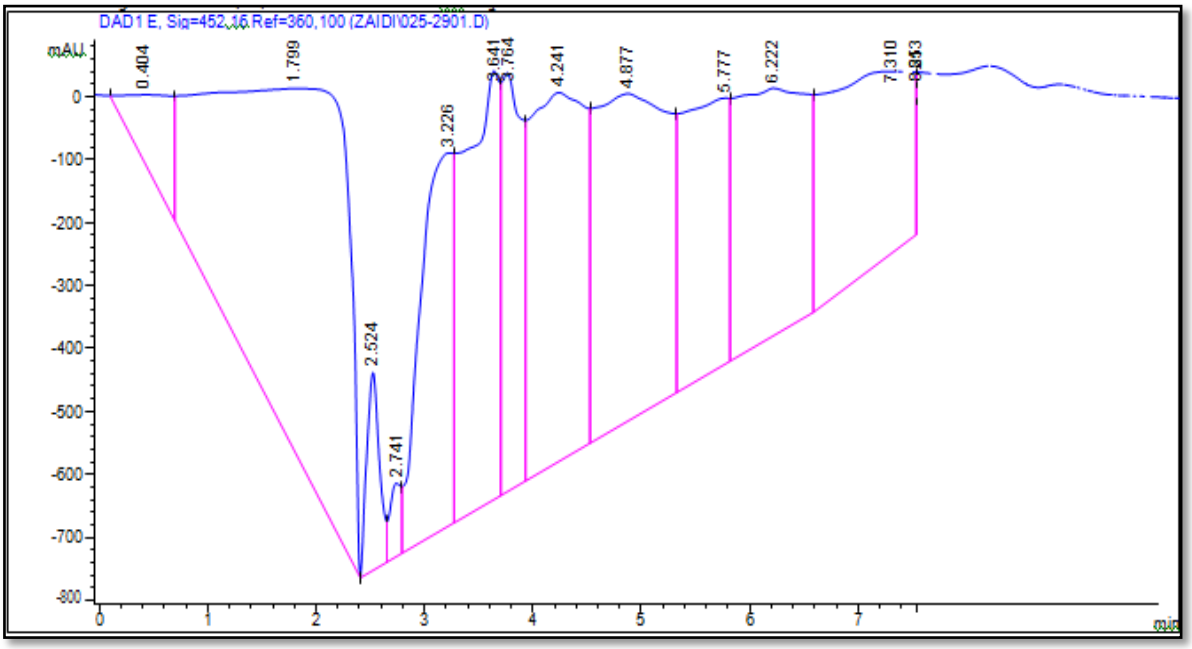
HPLC analysis: Ethanol (6 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.899	BV	0.9112	3.36500e4	514.37952	18.1917
2	2.514	VV	0.1490	3469.37695	378.27710	1.8756
3	3.216	VV	0.4437	1.80686e4	595.95081	9.7682
4	3.637	VV	0.2945	1.51100e4	656.46155	8.1687
5	3.749	VV	0.1784	7968.44189	626.40173	4.3079
6	4.219	VV	0.4206	1.86470e4	556.90814	10.0808
7	4.865	VV	0.5563	2.21672e4	493.29239	11.9839
8	5.736	VV	0.4044	1.26722e4	407.98227	6.8508
9	6.200	VV	0.6255	1.87909e4	374.41190	10.1586
10	7.292	VV	0.9310	2.07775e4	293.82382	11.2326
11	8.309	VV	0.6296	8809.91699	198.58960	4.7628
12	8.915	VBA	0.6004	4843.59473	112.93327	2.6185
Totals :				1.84975e5	5209.41209	

Appendix L

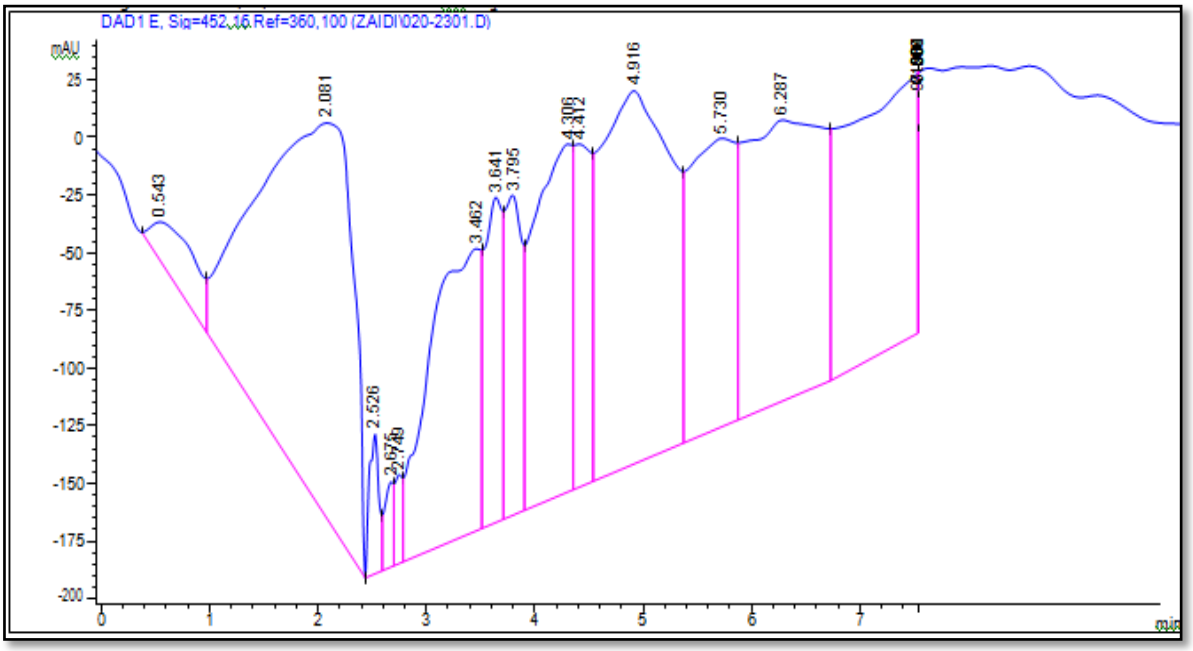
HPLC analysis: ethanol (8 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.404	BV	0.5116	3569.43311	103.18842	1.7444
2	1.799	VV	1.0414	4.68832e4	574.88092	22.9119
3	2.524	VV	0.1328	2553.26367	314.31064	1.2478
4	2.741	VV	0.1058	835.84717	116.31948	0.4085
5	3.226	VV	0.3180	1.23839e4	592.21460	6.0520
6	3.641	VV	0.2927	1.56682e4	680.41174	7.6571
7	3.764	VV	0.1714	8513.03516	664.79767	4.1603
8	4.241	VV	0.4493	2.03385e4	585.48193	9.9395
9	4.877	VV	0.5991	2.39212e4	519.65173	11.6903
10	5.777	VV	0.3957	1.27162e4	421.83902	6.2144
11	6.222	VV	0.5701	1.78486e4	392.83075	8.7227
12	7.310	VV	0.9732	2.26708e4	309.99963	11.0793
13	8.213	VV	0.6779	1.08841e4	227.60696	5.3191
14	8.853	VBA	0.6088	5837.19922	133.85307	2.8527

Appendix M

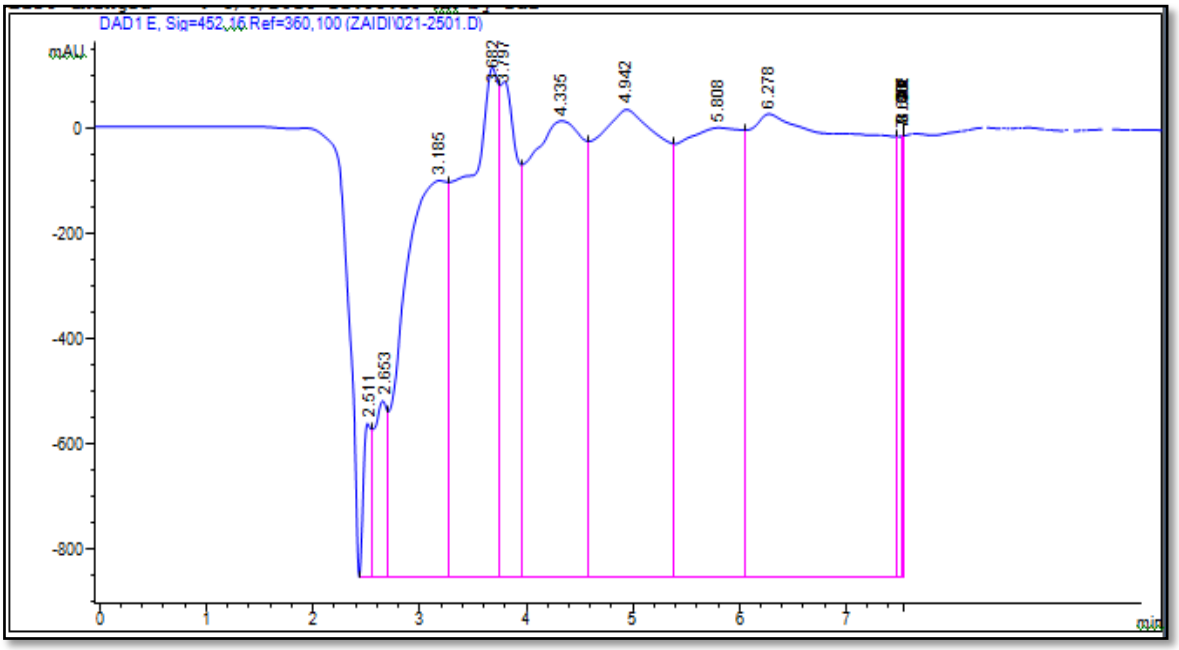
HPLC analysis: 2-propanol (2 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.543	BV	0.5278	664.07471	16.70418	1.1313
2	2.081	VV	0.7617	9836.62793	171.37337	16.7572
3	2.526	VV	0.0838	372.73328	60.53062	0.6350
4	2.675	VV	0.0841	208.53403	36.78984	0.3552
5	2.749	VV	0.0681	185.04291	38.46238	0.3152
6	3.462	VV	0.4247	4094.28027	122.26511	6.9748
7	3.641	VV	0.1548	1580.44067	140.94336	2.6924
8	3.795	VV	0.1543	1526.16699	138.77032	2.5999
9	4.306	VV	0.3356	3689.80933	150.71292	6.2858
10	4.412	VV	0.1543	1609.30408	148.61534	2.7415
11	4.916	VV	0.5918	7233.97168	161.74110	12.3234
12	5.730	VV	0.3940	3684.50977	124.95429	6.2767
13	6.287	VV	0.6197	6008.46338	121.77051	10.2357
14	7.638	VV	0.7467	6910.52002	117.40870	11.7724
15	8.209	VV	0.4872	4045.79932	106.98836	6.8922

Appendix N

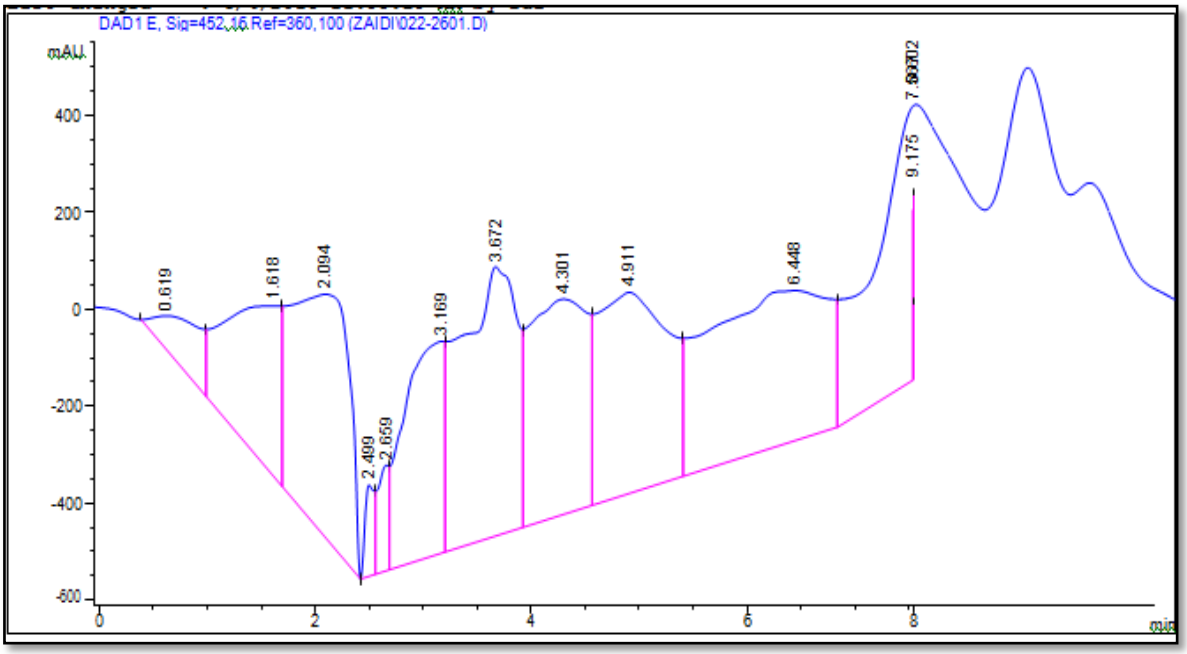
HPLC analysis: 2-propanol (4 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.511	BV	0.0765	1462.65686	291.55646	0.4006
2	2.653	VV	0.1206	2884.36426	334.55951	0.7900
3	3.185	VV	0.4539	2.14173e4	752.93304	5.8660
4	3.682	VV	0.3148	2.36197e4	967.61133	6.4692
5	3.797	VV	0.1562	1.04973e4	940.67352	2.8751
6	4.335	VV	0.5095	3.15743e4	865.82422	8.6479
7	4.942	VV	0.5889	4.11600e4	887.70343	11.2733
8	5.808	VV	0.5249	3.35658e4	853.26056	9.1934
9	6.278	VV	1.0170	7.28635e4	879.09723	19.9567
10	7.659	VV	0.2761	1.66081e4	841.91217	4.5488
11	8.302	VV	0.5464	3.55816e4	853.42676	9.7455
12	8.701	VV	0.4582	2.92785e4	853.43872	8.0191
13	9.468	VV	0.5161	3.22246e4	850.34570	8.8260
14	9.863	VBA	0.2179	1.23712e4	849.49097	3.3884

Appendix O

HPLC analysis: 2-propanol (6 hours of extraction time)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.619	BV	0.5881	2832.57422	69.40517	1.4495
2	1.618	VV	0.3908	1.11564e4	352.88290	5.7090
3	2.094	VV	0.5128	1.84708e4	502.56232	9.4520
4	2.499	VV	0.0870	1116.41528	188.76221	0.5713
5	2.659	VV	0.1026	1539.63989	217.44257	0.7879
6	3.169	VV	0.3676	1.14492e4	437.08200	5.8588
7	3.672	VV	0.4541	2.01994e4	555.60620	10.3365
8	4.301	VV	0.5134	1.63414e4	444.00607	8.3623
9	4.911	VV	0.6106	1.86619e4	415.01321	9.5498
10	6.448	VV	1.0117	2.51807e4	309.34924	12.8856
11	7.567	VV	0.7652	3.48140e4	614.26031	17.8152
12	8.602	VV	0.5253	2.22709e4	616.78943	11.3966
13	9.175	VBA	0.4865	1.13840e4	338.38022	5.8255
Totals :				1.95417e5	5061.54186	

Appendix P

Technical report

EXTRACTION OF BETA-CAROTENES UNDER DIFFERENT CONDITONS

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Abstract

Extraction of carotene from carrot (Daucus Carota.L) using Soxhlet extraction method is done. Beta-carotene is the highest constituents in carotene. Fresh carrots are used as sample in this study. The effect of different conditions, 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. It is expected 2-propanol (solvent) and 2-4hours (duration of extraction) will have the highest yield of carotene.

Key words: *Carotenoids, carrot, extraction, solvent, time, HPLC*

1. INTRODUCTION

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.

2. MATERIALS AND METHODS

2.1 Chemicals

Solvents used in this experiment are acetone, 2-propanol, and ethanol. For analyzing the samples, HPLC standard chemicals used are acetonitrile, methanol and dichloromethane.

2.2 Sample preparation

The sample which was carrot needs to be prepared to produce a kind of powder. This was been done by slicing the carrot into smaller size before it was dried in microwave. The temperature of microwave was make up to 60°C. The carrot was dried for 24 hours. Take out the carrot from the microwave and grind it into small particle by using blender. After that, put the carrot powder in the weighing boat and its weight is measured.

2.3 Soxhlet extraction

Extraction was performed using Soxhlet extraction method. 25g of carrot powder was weight by using an electronic balance and was placed in the porous cellulose thimble. The thimble was placed in the extraction chamber, which was suspended above the flask containing the solvent and below the condenser. 150ml of solvent was placed in the round bottom flask which is located below the extraction chamber. Acetone, ethanol, and 2-propanol were used as solvents in this study. The operating temperature was depends on the boiling point of the solvent. The extractions time durations are 2hr, 4hr, 6hr, and 8hr.

2.4 Solvent separation method

After finished the extraction process, the extracted compound should be separated from the solvent used. Firstly, the extracted oil was filtered by using filter paper to removed suspended solid. After that, put the extracted mixture in rotary evaporator. The extracted mixture was placed in the rotary flask. The flask was attached to the rotary evaporator. The temperature used are depends on the boiling point of the solvent used. The oil obtain after separation method was weighted using analytical balance and placed in the sample bottle.

2.5 High performance liquid chromatography (HPLC)

Carotene, which is the extracted compound, will be analyzed by using HPLC system. The identification of beta carotene in the extract samples are performed by referring to (Mendes et al., 1999). HPLC system used is equipped with a Supercosil LC-18 column ($15cm \times 4.6cm \times$

$5\mu m$). Before transferred the extracted samples into HPLC vial the extracted sample are filtered by using nylon filter first. This is to remove any particle in the samples to avoid damage occurring in the HPLC system. Note that, the syringe should be changed if the extracted samples are in different type of solvents. This is to preserve the accuracy of the result. After that, the mobile phase is prepared. The chemicals use for mobile phase in this analysis are dichloromethane, methanol and acetonitrile. According to (Ahamad M.N. et al., 2007), the mobile phase consisted of acetonitirle, dichloromethane and methanol by the ratio 70:20:10. So, 700ml of acetonitrile and 200ml of dichloromethane and 100ml of methanol are filled in 1L Schott bottle. The mobile phase is then degas by using pump. The peak for beta-carotene are monitored by a UV detector at 452nm. The flow rate is 2mL/min.

3.0 RESULTS AND DISCUSSIONS

Solvent	Oil weight (g)	Sample weight (g)	Percentage yield (%)
Acetone	12.905	25	51.62
Ethanol	8.05	25	21.47
2-propanol	5.37	25	32.18

Table 4.1: Percentage yield for oil for each solvent

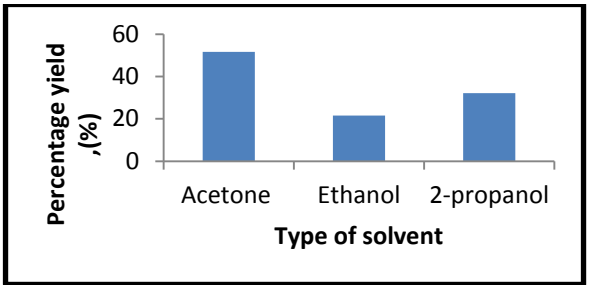

Figure 4.1: Graph of percentage yield versus type of solvent

Table 4.1 and Figure 4.1 show the percentage yield for oil for each solvent. When using acetone, the percentage yield of 51.62% is obtained. This project is also study the effect of extraction time on the yield of beta-carotene. Figure 4.2 shows the graph of percentage yield versus extraction time for each solvent. The extraction duration time that are used for this study are 2hr, 4hr, 6hr, and 8 hr.

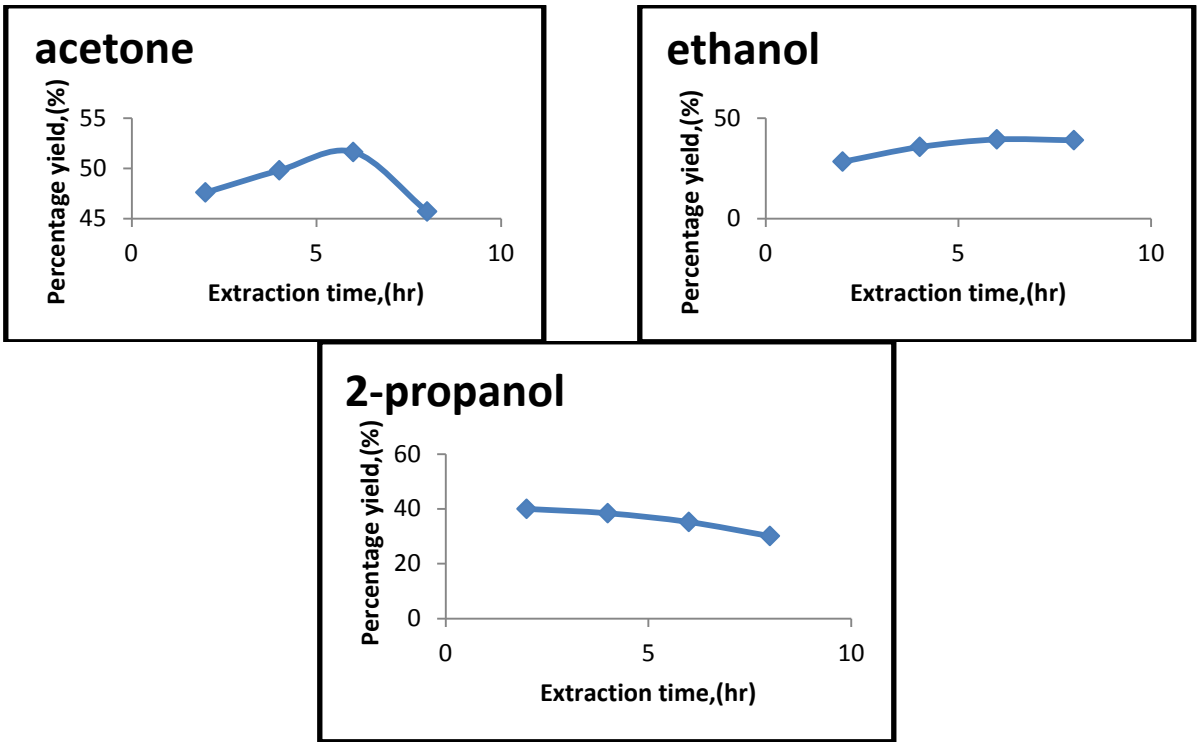

Figure 4.2: Graph of percentage yield versus extraction time for each solvent

The extracted oil is then being analyzed by using HPLC. The beta-carotene yield in the extracted oil is identified by monitoring the peak area at fixed retention time. According to (Ahamad M.N. et al., 2007), the mobile phase consisted of acetonitirle, dichloromethane and methanol by the ratio 70:20:10. So, 700ml of acetonitrile and 200ml of dichloromethane and 100ml of methanol are filled in 1L Schott bottle. The mobile phase is then degas by using pump. The peak for beta-carotene are monitored by a UV detector at 452nm. The flow rate is 2mL/min.

Acetone		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	15.6849
4	4.897	16.7980
6	**4.897**	**19.7714**
8	4.897	14.4280

Ethanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	5.2255
4	4.897	9.1473
6	**4.897**	**11.7953**
8	4.897	11.5686

2-propanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	**4.897**	**11.9622**
4	4.897	11.0787
6	4.897	9.5225
8	4.897	7.8413

Table 4.2: Peak area of beta-carotene of different extraction time for each solvent

Beta-carotene is a non polar solvent due to (Pavia D.L. et al., 1999). Beta carotene is a hydrocarbon, making it quite non-polar. According to (Belitz et al., 2004), carotenoids are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, petroleum ether because carotenoids are liposoluble. Ethanol is the most polar solvent among all the solvents. Ethanol is the polar protic solvent. Protic refer to a hydrogen atom attached to an electronegative atom. In ethanol, oxygen is an electronegative atom. In other words polar protic solvents are compounds that can be represented by the general formula ROH. The polarity of the polar protic solvents come from the bonding of O-H bond. The large difference in electronegativities of the oxygen and the hydrogen atom, combine with the small size of the hydrogen atom, warrant separating molecules that contain an OH group from those polar compounds that do not. While, on the other hand, acetone with the general formula RCOR is less polar compare to ethanol and 2-propanol. This is because the bonding is weaker than 2-propanol and ethanol. Beta-carotene is substance which is quite non-polar because it is hydrocarbon. That is why acetone has the higher yield of beta-carotene because less polar compound is best to be extracted by the usage of less polar solvent too.

In this study, the extraction time those being examined for beta-carotene yield are 2.0, 4.0, 6.0 and 8.0 hours. In any extraction process has their optimum extraction time. This optimum extraction time indicates the highest yield of desired compound. For example, in this analysis the optimum extraction time for acetone is 6 hours with the yield of 19.7714% beta-carotene. Extraction that exceeds this optimum extraction time which is 6 hours will decrease in the yield of beta carotene. So, it will be useless if the extraction time exceeds the optimum extraction time. That is why it is important to obtain the optimum extraction time. For ethanol, the highest yield of beta-carotene is 11.7953% of peak area. This is at 6 hours of extraction time. So, the optimum extraction time for ethanol is 6 hours. While for 2-propanol the highest yield is 11.9622% peak area with 2 hours of extraction time. So, the optimum extraction time for 2-propanol is 2 hours. Based on the peak area among all three solvents, it can be concluded that acetone is the best solvent with the optimum extraction time 6 hours.

4.0 CONCLUSION

To extract beta-carotene from carrot, one of the method is via Soxhlet extraction. This is one of the oldest and easiest methods being used for the extraction of essential oil commonly. Thus, Soxhlet extraction has found to be economically viable and safe to operate. In this research, the affecting parameters such as type of solvent and extraction time can be identified by analyze the carotene yield.

Among the three solvents used in this study, acetone is the best solvent. The optimum extraction time for acetone is 6 hours.

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APPENDICES

Data collection:

Percentage yield of oil for different type of solvent

Solvent	Oil weight (g)	Sample weight (g)	Percentage yield (%)
Acetone	12.905	25	51.62
Ethanol	8.05	25	21.47
2-propanol	5.37	25	32.18

Percentage yield of oil for different extraction time for acetone

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	11.9	25	47.6
4	12.45	25	49.8
6	12.91	25	51.62
8	11.43	25	45.7

Percentage yield of oil for different extraction time for ethanol

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	7.09	25	28.34
4	8.92	25	35.67
6	9.86	25	39.42
8	9.75	25	39.01

Percentage yield of oil for different extraction time for 2-propanol

Extraction time (h)	Oil weight (g)	Sample weight (g)	Percentage yield (%)
2	10.00	25	40.01
4	9.61	25	38.43
6	8.81	25	35.24
8	7.53	25	30.11

Peak area of beta-carotene for acetone with different extraction time

Acetone		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	15.6849
4	4.897	16.7980
6	**4.897**	**19.7714**
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Peak area of beta-carotene for ethanol with different extraction time

Ethanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	4.897	5.2255
4	4.897	9.1473
6	**4.897**	**11.7953**
8	4.897	11.5686

Peak area of beta-carotene for 2-propanol with different extraction time

2-propanol		
Extraction time (h)	Retention time(min)	Peak area (%)
2	**4.897**	**11.9622**
4	4.897	11.0787
6	4.897	9.5225
8	4.897	7.8413