DEVELOPMENT OF HPLC ANALYSIS FOR DETECTION OF LYCOPENE IN TOMATO AND CRUDE PALM OIL

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A thesis submitted in fulfillment of the Requirements for the award of the degree of Chemical Engineering & Natural Resources

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NOVEMBER 2006

To my beloved mother and father.

ACKNOWLEDGEMENT

First of all, I would like to express my appreciation especially to my loving , caring father and mother respectively, Mr Abdul Aziz Bin Mohd Diah and Madam Salihah Binti Hj Jaafar and rest of my family members who are very supportive morally to whatever good thing that I have involved and done all these years.

Secondly, I would like to thank to my final year project supervisor Professor Dr. Mashitah Binti Mohd Yusuf for her understanding, guidance ideas unforgettable advice and moral support in making and finishing this project reality. I also would like to thank to the lectures at Faculty of Chemical and Natural Resources Engineering and all the Teaching Engineers have been teaching me all this while.

Lastly, not forgotten to my partners friends Ms Norsyahila Binti Mohd Seth, Mr Mohd Arif Bin Mokhtarudin, Mr Saifuz Zaman Bin Jamaludin and Mr Rahmat Bin Nor Yatim and also to my others fellow undergraduate who are helpful especially ideas, valuable opinions and advice during the progress of this project. My sincere appreciation also extend to all my colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Thank you.

ABSTRACT

In this study the effect of type and ratio of solvents liquid-liquid extraction from Tomato is considered in this research. Then the method will be done to the Crude Palm Oil (CPO). Extraction is a process to obtaining something from mixture or compound by chemical or physical methods. Liquid-liquid extraction is used to extract and purify carotenoids from CPO and then this sample will be analysis in High Performance Liquid Chromatographer (HPLC). Natural antioxidants occur in carotenoids are lycopene, α-carotene and β-carotene. Natural antioxidants can be founds in almost all plant, microorganism, fungi and animal tissue. Lycopene is antioxidant that uses to avoid some kind of cancer like prostate cancer. Lycopene is mainly found in the red fruid like tomato and watermelon. This is because lycopene is the red pigment that responsible to the red colour of the fruits. Nowadays, major source of lycone are from tomato although the high content of lycopene are found in watermelon.

ABSTRAK

Dalam kajian ini, elemen penting ialah kesan jenis dan kadaran pelarut di dalam pengekstrakan tomato ditentukan semasa eksperimen ini. Kemudiannya metadologi ini akan digunakan pula ke atas sample tomato. Proses pengekstrakan adalah proses untuk mendapatkan sesuatu daripada campuran atau sebatian menggunakan kaedah kimia atau fizikal. Pengekstrakan cecair ke cecair digunakan untuk memisahkan dan menulenkan karotenoid daripada minyak sawit mentah. Kemudiannya sample ini akan dianalisis menggunakan High Performance Liquid Chromatographer (HPLC). Antioksida semulajadi ditemui di dalam karotenoid adalah lycopene, α-karotene dan β-karotene. Antioksida semulajadi boleh didapati pada semua tumbuhan, mikroorganisma, kulat dan tisu haiwan. Lycopene merupakan antioksida yang digunakan untuk merawat beberapa kanser seperti kanser prostat. Lycopene secara umumnya boleh didapati pada buah-buahan berwarna merah seperti tomato dan tembikai. Ini kerana lycopene merupakan pigmen merh yang bertanggungjawab memberikan warna merah kepada buah-buahan tersebut. Pada masa sekarang, sumber utama lycopene adalah daripada tomato walaupun kajian mendapati bahawa kandungan lycopene adalah paling tinggi di dalam tembikai.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE NO **TITLE**

LIST OF SIMBOLS

v = volume

nm = nanometer

µm = micrometer

min = minute

mm = millimeter

ml = milliliter

% = percent

µl = micro liter

lb = pound

psi = pound per square inch

in = inch

CHAPTER 1

INTRODUCTION

1.1 Lycopene

Lycopene (molecular formula: $C_{40}H_{56}$) is a bright red carotenoid pigment, a phytochemical found in tomatoes and other red fruits. Lycopene is the most common carotenoid in the human body and is one of the most potent carotenoid antioxidants. Its name is derived from the tomato's species classification, *Solanum lycopersicum*. Ironically, the highest natural concentrations of lycopene in food are found not in tomatoes, but in watermelon. Almost all dietary lycopene comes from tomato products, however. There is evidence that frequent intake of such products is associated with reduced prostate cancer risk.

Lycopene is a powerful antioxidant.. As an antioxidant, it is about twice as powerful as beta-carotene. There is some evidence that lycopene may help prevent cancer and other diseases, but conclusive proof is lacking. Thus far, the best evidence for lycopene has to do with preventing complications during pregnancy.

1.2 Oil Palm

Local name is Kelapa Sawit. Scientific name is (*Elaeis guineensis* Jacq.). The magic tree from which the oil palm fruits, and subsequently the palm oil and its by-products, were produced is not native to Malaysia. The Oil Palm trees being grown in Malaysia is from the E. Guineensis family.

Oil Palm is not native to Malaysia. In fact, it originated in Africa. Crossing the oceans to land on our shores over 100 years ago, it has since found an ideal new home on our soil. Today, it thrives as our most important national crop. The Tenera is the most common types of oil palm species grown in Malaysia. It is a cross between the Dura and Pisifera species.

The oil palm was introduced to Malaysia in 1870 as an ornamental plant. Its use as a crop was not developed until 1917, when it was grown commercially. The modem expansion of the industry can be traced back to the 1960s when the Malaysian Government embarked on a massive programme of agricultural diversification. Today oil palm is the leading agricultural crop in Malaysia, covering about two million hectares or a third of the total cultivated area.

It's mind-boggling to think that we have over 500 million oil palm trees in Malaysia. It's even more astonishing when you consider all the love and care that goes into caring for each one of them. Everyday, research and experiments are being conducted to produce seeds that yield more and better quality oil.Only the finest seeds are germinated under controlled conditions. The germinated seeds are planted into polybags and kept in nurseries to grow for 12 to 15 months. When they are ready, the young palm seedlings are transferred into a well-irrigated field. The seedlings are left to grow for about 32 months or nearly 3 years. Fertilisers are added from time to time to ensure that the growing trees have sufficient nutrients. Mature trees will sprout oil palm fruits which are called Fresh Fruit Bunches (FFB). Each tree will continue to produce about 12 FFB each year for 20 to 30 years.

Oil palm fruits turn yellowish-red in colour when ripe. They must be harvested at just the right time when the amount and quality of oil is best. Considering how one FFB can weigh between 20 and 30kg, harvesting is not easy. Care must be taken to prevent the fruits from getting bruised or spoilt. Rough handling can reduce the quality of the oil obtained eventually. Harvested FFB are loaded onto a lorry or rail cage to be transported to the mills for processing as soon as possible. Most mills are usually located in or near the oil palm plantations.

Cultivation of the oil palm has expanded tremendously in recent years such that it is now second only to soybean as a major source of the world supply of oils and fats. Presently, Southeast Asia is the dominant region of production with Malaysia being the leading producer and exporter of palm oil. This paper reviews the various factors that have led to oil palm occupying its present position, including biological, technical, managerial, environmental, and socio-political aspects.

Biological features recognised as critical to the high productivity of the crop are examined. These include its perennial and evergreen nature (giving a continuous year-round canopy cover intercepting a high proportion of incoming radiation), the year-round production of fruit bunches and the high partition of total assimilates into harvested product.

Scientific and managerial aspects contributing to the success of the crop include the significant genetic improvements and production of high quality planting materials, the development and application of finely-tuned agronomic practices, the appropriate scale and efficient organisation of oil palm plantations and the continuous R&D and good infra-structural support provided in the main producing countries.

The programmes of crop improvement through the utilisation of traditional breeding and selection methods, the development and benefits of vegetative propagation techniques using tissue culture and ongoing efforts to apply molecular and genetic engineering techniques to improve and modify oil composition, are reviewed. Finally, the nutritional qualities of palm oil as a healthy component of diet are briefly described.

1.3 Objectives Of This Study

- To determine that oil palm waste (CPO) has an amount of lycopene.
- To study the effect of lycopene from oil palm waste as antioxidant.

1.4 Scopes Of This Study

- To study the effect of solvents type in the extraction process to the sample.
- To determine the most effective solvents ratio that produces high yield of Lycopene in the extraction process.

1.5 Problem Statement

- The demand on lycopene has increased significantly, with increased of consumer awareness about cancer.
- The raw materials (CPO) are easily finded in our country.
- Lycopene from oil palm has a lower cost than the other sources like tomato (main sources nowaday).

In early this century, scientist found that some cancers can be avoid by Lycopene. The major sources of lycopene came from tomato. But lycopene also can be find in other red fruits such as watermelon and carrot. Crude palm oil maybe containing an amount of lycopene because it is red in colour.

Every single day, we can hear many peoples dies cause of cancers. The increasing of human awareness make the demand on lycopene increase significantly with it. So, many company are trying to get more sources to get this antioxidant. Nowadays, Malaysia is among the larger country that produced the CPO.

CHAPTER 2

LITERTURE REVIEW

2.1 Introduction

Nowadays the major interest of carotenoids, which are found in plants, is not only due to their provitaminb A activity but also to their antioxidant action by scavenging oxygen radicals and reducing oxidative stress in the organism. There are many studies showing strong correlations between carotenoid intake and a reduced risk of some diseases, as cancer, aterogenesis, bone calcification, eye degeneration and neuronal damages.

Lycopene is the compound responsible for red colour in tomato, watermelon and other fruits, and it is also used as a colour ingredient in many food formulations. A great interest has recently been focused on lycopene due to its preventive activity against several pathologies, such as cardiovascular disease, hepatic fibrogenesis , solar light induced erythema, human papillomavirus persistence and some cancer types, such as prostate, gastrointestinal and epithelial. Lycopene has also been recently reported to play a role in lung function as well as in foetal growth. Finally, it is also important to consider the synergic action of carotenoids with other bioactive compounds present in fruits and vegetables.

Carotenoid analysis in food products may be carried out by different methods: HPLC, or colour evaluation. Although spectrophotometry or colorimetry can be used to rapidly assess the lycopene content of products derived from tomatoes, a highly versatile, sensitive and selective method such as HPLC is needed for reliable analysis of food samples. HPLC analysis of carotenoids is usually done with C18 or C30 RP-columns, operated with isocratic or gradient elution with a wide variety of mixtures of different organic solvents as mobile phases, using UV–vis (k _ 450 nm) or photodiode array or MS detection. Heating the column is sometimes used to improve pigment separation as well as to standardize the separation conditions. For the extraction of carotenoids from the samples, different systems can be used, like liquid–liquid extraction, solid phase extraction or supercritical fluid extraction.

AOAC (1993) recommends methanol/tetrahydrofuran (THF) (50:50 v/v) for extracting the carotenoids, while other sources use ethyl acetate (100%) or different mixtures of ethanol/hexane, acetone/ethanol/hexane, ethyl acetate/hexane or acetone/hexane.

The instability of lycopene during processes of extraction, handling, and elimination of organic solvents makes the preparation of a sample for analysis an extremely delicate task, often requiring successive and complex procedures to ensure that all the carotenoids are extracted. Besides, not all the analytical methods available for carotenoid analysis in food products are suitable for lycopene-rich foods due to the low solubility of lycopene in some of the solvents employed – as in the case of methanol – and due to the fact that the use of other solvents may interfere with the mobile phases applied for carotenoid separation. There are many HPLC methods that can be applied to the determination of carotenoids. However, this kind of compounds needs a very careful and tedious manipulation due to their chemical lability. Therefore the development of new methodologies of extraction/separation is of relevance and the necessity for a reliable and rapid analysis method for lycopene in vegetable products has been recognized. This work is aimed at solving the problems above mentioned with a quite simple preparation of the samples, the selection of extraction solvent mixtures more compatible with mobile phase, and short run times, by developing a suitable, reliable, rapid and simple HPLC method for lycopene analysis on CPO and its compare it with a reference spectrophotometric method.

2.2 Oil Palm

Figure 2.1 : The Oil Palm

From its home in West Africa, the oil palm (*Elaeis guineensis* Jacq.) has spread throughout the tropics and is now grown in 16 or more countries. However, the major centre of production is in South East Asia (SEA) with Malaysia and Indonesia together accounting for around 83 % of world palm oil production in 2001. The recent changes in world mature areas are shown in Table 1. Malaysia is presently the world's leading exporter of palm oil having a 60 % market share and palm oil is second only to soybean as the major source of vegetable oil.

Oil palm production in Malaysia presently occupies around 3.7 million hectares of which over two million are in Peninsular Malaysia and the rest in the East Malaysian states of Sabah and Sarawak. Production is divided between large estates managed by publicly listed companies, smaller independent estates, independent smallholders and government smallholder settler schemes.

With good quality planting materials and agronomic practices, oil palm begins producing the oil-bearing fruit bunches as early as two and a half years after planting. While the lifespan of oil palm, as demonstrated by specimens planted in the Bogor Botanic Garden, Indonesia, is at least 120 years, the crop is generally grown for 25-30 years before being replanted. This is mainly because old palm becomes too tall to harvest economically.

Both the public and private sectors carry out oil palm research and development (R&D). In Malaysia, the Palm Oil Research Institute of Malaysia (PORIM) was set up in 1974 and was merged in 2000 with the Palm Oil Licensing Authority (PORLA) to form the Malaysian Palm Oil Board (MPOB). MPOB now deals with all aspects of oil palm and palm oil development and provides regulatory, training and technical advisory services to all sectors of the industry. Other research organizations that conduct research on oil palm and palm oil include the Indonesian Oil Palm Research Institute (IOPRI), Nigerian Oil Palm Research Institute (NIFOR), CENIPALMA in Columbia, CIRAD in France and Bah Lias Research Station in Indonesia. In Malaysia there are also many local plantation companies with R&D facilities such as FELDA, Golden Hope, United Plantations and Applied Agricultural Research.

The intensive research on oil palm and palm oil globally accounts for its significant contribution and status in the oils and fat market. In Malaysia, the success of the oil palm is attributed to many factors, which include favourable climatic conditions, well-established infrastructure, management skills and technology for oil palm cultivation and a land ownership structure which favours estate type of agriculture. Nevertheless, to stay competitive and to ensure agricultural sustainability (that is economic, social and environmental), appropriate R&D in various disciplines such as crop physiology, agronomy, genetics, tissue culture and biotechnology, must be strategically planned and implemented. The paper aims to provide a comprehensive overview on achievements in the areas mentioned and examine the potential of oil palm as a sustainable crop in the future.

Countries	1980	1990	2000	Annual growth rate (%) 1990-2000*
Indonesia	230	617	2014	12.6
Thailand	15	94	199	7.8
Malaysia	805	1746	2941	5.5
Colombia	27	81	134	5.2
Others	151	527	731	3.3
Nigeria	220	270	360	2.9
Ivory Coast	100	128	139	0.8
Total	1756	3463	6563	6.6

Figure 2.2 : The Countries that grow palm oil

2.3 Palm Oil

Palm oil is derived from the flesh of the fruit of the oil palm species E. Guineensis. In its virgin form, the oil is bright orange-red due to the high content of carotene. Palm oil is semi-solid at room temperature; a characteristic brought about by its approx. 50 percent saturation level. Palm oil (and its products) has good resistance to oxidation and heat at prolonged elevated temperatures; hence, making palm oil an ideal ingredient in frying oil blends. Manufacturers and end-users around the world incorporate high percentages of palm oil in their frying oil blends for both performance and economic reasons. In fact, in many instances, palm oil has been used as 100 percent replacement for traditional hydrogenated seed oils such as soybean oil and canola. Products fried in palm oil include potato chips, french fries, doughnuts, ramen noodles and nuts.

Another positive attribute of palm oil as a frying oil is that it imparts longer shelf life to the fried products; generally attributed to comfortable level of unsaturation, absence of linolenic acid and presence of natural antioxidants in the oil. See palm olein below for more information.

Palm oil, because of its natural solid-liquid content, is suited to be used in high percentages in vegetable oil shortenings, biscuit fats and bakery fats. In margarine production, palm oil is highly suited as a component as it imparts the desirable beta prime crystalline tendency in the fat blend. Palm oil's natural semi-solid consistency means need for no or little hydrogenation.

The concepts of antioxidants, free radicals, and singlet O2 species are terms that have been topics of research for decades. However, not until recently has the public and scientific recognition of their relationship to overall health come into its own. Crude palm oil has other essential carotenoids. One of the most important up-and-coming carotenoids is lycopene. Although it does not have any provitamin A activity, lycopene is the most efficient biological singlet oxygen quencher of the carotenoids yet discovered, with a quenching efficiency more than two times greater than beta carotene and 100 times greater than vitamin E.

While it appears that beta carotene is a very important plasma carotenoid, increased attention should be directed to other cartenoids, such as lycopene, which actually has a higher plasma concentration than beta carotenoids. Lycopenes have also been identified in low-density lipoprotiens, and therefore may function in the prevention of oxidized LDL in causing atherosclerosis. Physiological functions of other carotenoids, as found in crude palm oil, may be highly specialized, as indicated by the presence of zeanthin and its isomer lutein in the macula area of the retina, while beta carotene is virtually absent. It is worthy to note that dark green vegetables are very high in lutien carotenoids. It’s also important to note that crude palm oil cartenoid extract is devoid of any saturated fats, which are normally found in tropical oils. The potential of crude palm oil carotenoid extract as an economical source of richly diversified carotenoids is enormous.

2.3.1 Benefits of palm oil

Red palm oil is particularly healthy because it contains the above-mentioned carotenoids and a special form of vitamin E. Most people are not aware of the fact that many different kinds of vitamin E occur in nature and that some forms of vitamin E are more beneficial than others. Red palm oil contains vitamin E tocotrienols, a powerful form of vitamin E, which acts as a super-antioxidant.

The carotenoids in red palm oil also act as antioxidants and one of these carotenoids, namely lycopene, is associated with a reduced risk of certain types of cancer.

Benefit of palm oil:

- a reduction in blood cholesterol levels.
- a reduction in blood clotting, combined with blood vessel dilation, thus preventing heart attacks and strokes.
- inhibition of the growth of breast cancer cells, which suggests that red palm oil may act as a chemopreventive agent.
- a 45% enhancement of the efficiency of breast cancer drugs such as Tamoxifen.

2.3.2 Vitamins present in Palm Fruit Oil

2.3.2.1 Carotenoids

The striking ruby-red colour of Palm Fruit Oil is due to the exceptionally high levels of caroteinoids (e.g. a and carotenes and lycopene in the product Palm Fruit Oil has on average 13 to 15 times more carotenes than carrots and 40 to 50 times more than tomatoes. These carotenes are non toxic, pigmented precursors of vitamin A. Our bodies convert them into vitamin A as and when required. Vitamin A is important in, amongst others, maintaining good vision and supporting the immune system. Carotenes have strong antioxidant properties - they remove damaging oxygen-free radicals, prevent certain forms of cancer and delay the aging process.

2.3.2.2 Vitamin E: tocopherols and tocotrienols

Palm Fruit Oil also has high levels of tocopherols and tocotrienols, which are two forms of the strong antioxidant, vitamin E. They, too, remove damaging oxygen-free radicals from the body. Among all vegetable oils, Palm Fruit Oil has by far the highest level of naturally occurring tocotrienols.

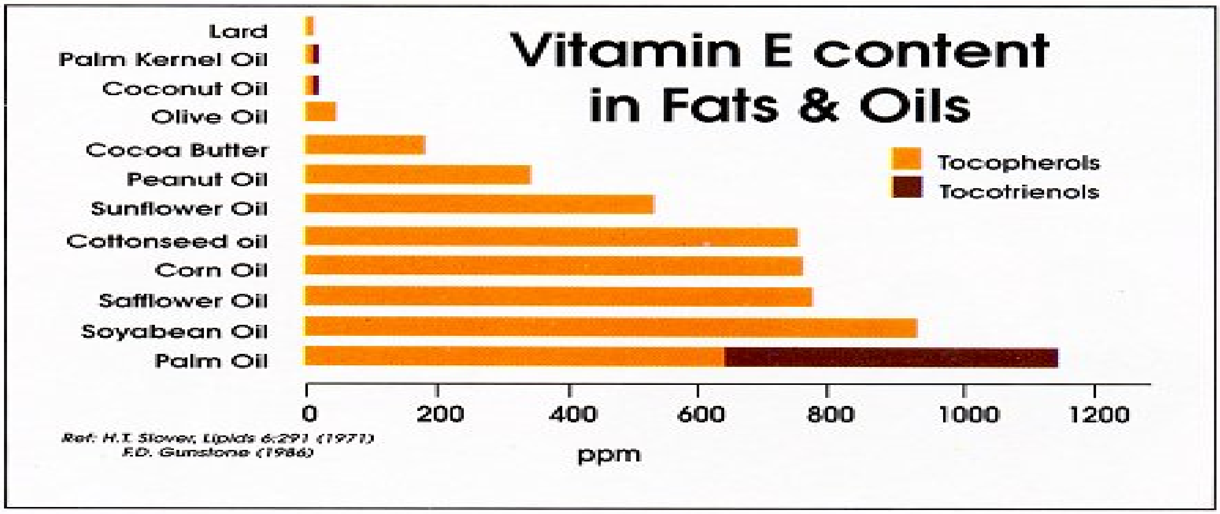


Figure 2.3 : Vitamin E in oil

]2.3.2.3 Fatty acids present in Palm Fruit Oil

Palm Fruit Oil is an efficient source of energy, providing equal amounts of saturated and unsaturated fatty acids. The composition of fatty acids in Palm Fruit Oil is (on average) as follows:

- 40% oleic acid (mono-unsaturated), 10% linoleic acid and 0,4% linolenic acid (both polyunsaturated)
- 44% palmitic acid (monosaturated), 4.6% stearic acid, 1.1% myristic acid and 0.2% lauric acid (all three saturated).

The harmful effects usually associated with saturated fatty acids have not been observed with Palm Fruit Oil. This is apparently due to their structure, their position in the fat molecule and the balanced composition of fatty acids found in it.

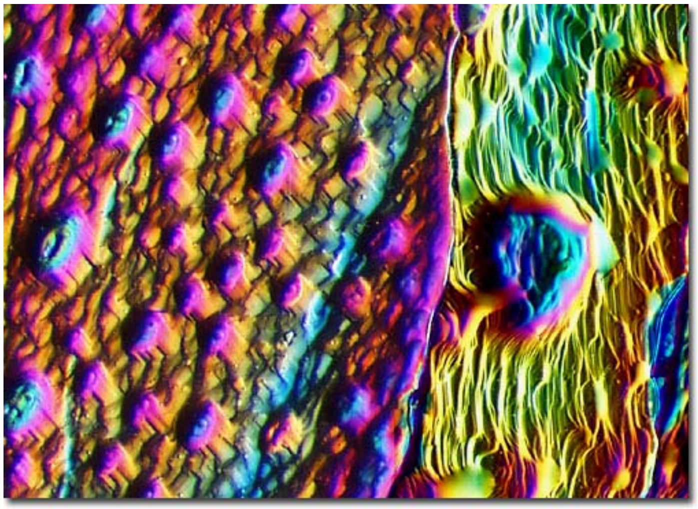
Fatty Acid	Fatty Acid Name	*Wilderness Family Naturals* Palm Oil
C12:0	Lauric Acid	0.4 %
C14:0	Myristic Acid	1.1 %
C16:0	Palmitic Acid	31.5 %
C16:1	Palmitoleic Acid	-
C18:0	Stearic Acid	3.2 %
C18:1	Oleic Acid	49.2 %
C18:2	Linoleic Acid	13.7 %
C18:3	Linolenic Acid	0.3 %
C20:0	Arachidic Acid	0.4 %

Figure 2.4 : Fatty Acids in palm oil

Carotenoids	%	Mg/kg (in palm oil)
α - carotene	36.2	500 - 700
β - carotene	54.4	
Lycopene	3.8	
Xanthophylls	2.2	

Figure 2.5 : Ranges in content for various carotenoids in the unsaponifiable fraction of palm oil

2.4 Lycopene



Lycopene is a member of the carotenoid family of chemical substances. Lycopene, similar to other carotenoids, is a natural fat-soluble pigment (red, in the case of lycopene) found in certain plants and microorganisms, where it serves as an accessory light-gathering pigment and to protect these organisms against the toxic effects of oxygen and light. Lycopene may also protect humans against certain disorders, such as prostate cancer and perhaps some other cancers, and coronary heart disease.

Carotenoids are the principal pigments responsible for the colors of vegetables and fruits (see Beta-Carotene and Lutein and Zeaxanthin). Lycopene is responsible for the red color of red tomatoes. In addition to tomatoes (*Lycopersicon esculentum*) and tomato-based products, such as ketchup, pizza sauce, tomato juice and tomato paste, lycopene is also found in watermelon, papaya, pink grapefruit and pink guava.

Lycopene is an acyclic isomer of beta-carotene. Beta-carotene, which contains beta-ionone rings at each end of the molecule, is formed in plants, including tomatoes, via the action of the enzyme lycopene beta-cyclase. Lycopene is a 40 carbon atom, open chain polyisoprenoid with 11 conjugated double bonds.

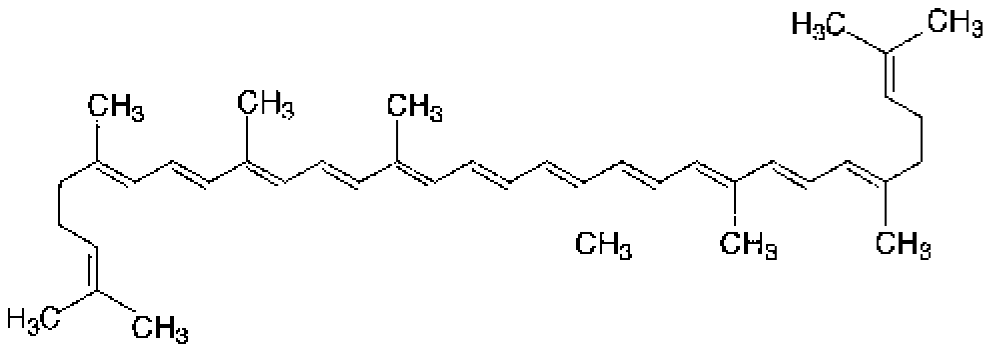


Figure 2.6 : Structure of Lycopene.

All-trans lycopene is the predominant geometric isomer found in plants. *Cis* isomers of lycopene are also found in nature, including 5-*cis*, 9-*cis*, 13-*cis* and 15-*cis* isomers. Lycopene found in human plasma is a mixture of approximately 50% *cis* lycopene and 50% all-*trans* lycopene. Lycopene in processed foods, is mainly in the form of the *cis*-isomer.

Lycopene is a lipophilic compound and is insoluble in water. Lycopene is also known as psi-carotene. Its molecular formula is $C_{40}H_{56}$ and its molecular weight is 536.88 daltons. In contrast to beta-carotene, lycopene has no vitamin A activity and thus is a nonprovitamin A carotenoid.

Tomato	mg/100g
Rambo	4.5
Raf	3.1
Cherry 1	3.4
Cherry 2	7.1
Canario	2.8
Raf	3.7
Watermelon	mg/100g
Dulce Maravilla	7.3
Seedless	6.5
Persimmon	mg/100g
Rojo Brillante 1	0.4
Rojo Brillante 2	0.3
Sharoni	0.2

Figure 2.7 : Lycopene contents in other sources

2.5 Liquid-Liquid Extraction

Liquid-Liquid extractors are often a neglected part of process plants because they are not well understood and generally form only a small part of the overall process scheme. Often, significant savings in operating costs can be achieved by fine-tuning extraction systems.

Liquid-Liquid extraction is a mass transfer operation in which a liquid solution (the feed) is contacted with an immiscible or nearly immiscible liquid (solvent) that exhibits preferential affinity or selectivity towards one or more of the components in the feed. Two streams result from this contact: the extract, which is the solvent rich solution containing the desired extracted solute, and the raffinate, the residual feed solution containing little solute.

The following need to be carefully evaluated when optimizing the design and operation of the extraction processes.

- Solvent selection
- Operating Conditions
- Mode of Operation
- Extractor Type
- Design Criteria

2.5.1 Solvent Selection

Solvents differ in their extraction capabilities depending on their own and the solute's chemical structure. Once the functional group is identified, possible solvents can be screened in the laboratory. The distribution coefficient and selectivity are the most important parameters that govern solvent selection. The distribution coefficient (m) or partition coefficient for a component (A) is defined as the ratio of concentration of a A in extract phase to that in raffinate phase. Selectivity can be defined as the ability of the solvent to pick up the desired component in the feed as compared to other components.

The desired properties of solvents are a high distribution coefficient, good selectivity towards solute and little or no miscibility with feed solution. Also, the solvent should be easily recoverable for recycle. Designing an extractor is usually a fine balance between capital and operating costs. Usually, good solvents also exhibit some miscibility with feed solution (see Table 1). Consequently, while extracting larger quantities of solute, the solvent could also extract significant amount of feed solution.

Solvent	Distribution Coefficient @ 20°C	Miscibility with water wt% @ 20°C
n-Butanol	1.6	>10
Ethyl Acetate	0.9	10
MIBK	0.7	2.0
Toluene	0.06	0.05
n-Hexane	0.01	0.015

Figure 2.8 : Solvents for Acetic Acid Extraction