EXTRACTION OF ALPHA-TOCOPHEROL FROM CORN

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A report submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

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“I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree.”

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Date	:…….

DEDICATION

Dedicated to all my beloved family especially my parents, friends and my love.

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Firstly, I would like to express my sincere appreciation to my main supervisor Pn Wan Hanisah binti Wan Ibrahim for her encouragement, guidance, trust, critics and idea in finishing my thesis. I also would like to thanks the personnel at FKKSA Chemical Laboratory for their assistance and coorperation in guiding me to handle the equipment to finish this thesis.

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ABSTRACT

Corn (*Zea mays*) is fruit that have high concentration of Alpha-tocopherol (Vitamin E) in their composition. Alpha-tocopheol is an antioxidant that was used in industries especially pharmaceutical product as a stabilizer, plastic industries, technical oil, and greases. Before extract the Alpha-tocopherol, the corn juice must be blend by using blender to liquefy it. The corn must be manually cut from cob and blend it using blender then the juice was being filter. A High-performance liquid chromatographic (HPLC) method was used to determine the Alpha-tocopherol level in corn. The mixture of acetonitrile/methanol/dicloromethane (60:38:2, v/v) was used as mobile phase at a flow rate of 0.2 mL min^{1} at temperature 30^{0}C. alpha-tocopherol must be determine between 2-5 minute peak. For the result, the peaks for standard are between 2-3 minute. As a result, the peak did not get because of there are some problem at the column. This research finally did not obtain the objective of the research.

ABSTRAK

Jagung (Zea mays) ialah buah-buahan yg mengandungi alpha-tocopherol yg tinggi di dalam setiap kompisisnya. Alpha-tocopherol (vitamin E) ialah antioxidant yang telah banayak diaplikasikan di dalam industri seperti plastic, farmasi dan minyak. Sebelum mengekstrak alpha-tocopherol, beberapa langkah perlu di jalankan. Jagung hendaklah dikisar terlebih dahulu untuk menjadikannya cecair. Jagung tersebut mestilah terlebih dahulu dipisahkan dari tongkolnya kemuadi kisar dituruti dengan menapis sample tersebut. Kemuadia sampel tersebut dicampurkan dengan campuran 80% methanol dan 20% air dengan nisbah (1:2), (1:1) dan (1:4) dan di ekstrak dengan tiga mase yang berbeza iaitu 5,15 dan 25 minit. Bagi menganalisa keputusan, kaedah menggunakan HPLC di jalankan keatas sampel. acetonitrile/methanol/dicloromethane (60:38:2, v/v) akan digunakan sebagai fasa bergerak bagi Alpha-tocopherol.dengan halaju 0.2mLmin^{-1}pada suhu 30^{o}C. Alpa-tocofherol sepatutnya di kesan pada masa minit ke 4-5. tetapi didalam graft analisa lengkuk terdapat pada minit 2-3. Sebagai keputusan graf tersebut tidak menepati keputusan yang terdapat pada kajian lepas kerana terdapat masalah berkaitan dengan “column” pada HPLC. Kajian ini tidak dappat menepati okjektif kajian ini.

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CHAPTER 1

INTRODUCTION

1.2 Introduction

Zea mays are scientific name for corn is including in Poaceae. It is growing to 2m at a fast rate. Corn is cash crops plant. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Wind. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid and neutral soils. It cannot grow in the shade. It requires moist soil.

The seed is diuretic and a mild stimulant. It is a good emollient poultice for ulcers, swellings and rheumatic pains, and is widely used in the treatment of cancer, tumours and warts. It contains the cell-proliferant and wound-healing substance allantoin, which is widely used in herbal medicine (especially from the herb comfrey, Symphytum officinale). It also content antioxidant in it which is Vitamin E. Antioxidant is a substance that capable of protecting other substance from oxidation. Some of antioxidant is made by the body to inhibit the destructive actions of chemicals called free radicals. Some of them cannot make by body such as vitamins C and E. vitamin E (tocopherol) is protective because it help reduce oxidation of lipid membrane and unsaturated fatty acid and prevents the breakdown of other nutrients by oxygen. For main function is to modify and stabilize blood fats so that the blood vessels, heart, and entire body are more protected from free-radical-induced injury.

Extraction is one of chemical separation process. Many biological and inorganic substances occur in a mixture of different component in a solid. In order to separate the desires solute and remove undesirable solute, extraction process can be used. The process is called leaching.

1.2 Problem statement

Nowadays, Malaysian people are more concern to healthy and beauty care. Many skin care and supplement based on Vitamin E (tocopherol). It is used in cosmetics and skin product to prevent cell damage by UV light. It also used in pharmaceutical product as a stabilizer. In plastic industries, technical oil and greases contain α-tocopherol used as an antioxidant. The reason I want to do this project because we can get another alternative beside palm oil to get tocopherol for industries use with lower cost and less time need.

1.3 Objective

The objective of the research is to extract alpha-tocopherol from corn.

1.4 Scope

The scope of this study is:

I. To determine the optimum amount of solvent.
II. To determine the optimum of extraction time.

CHAPTER 2

LITEATURE REVIEW

2.1 Corn

Corn (Zea mays) is the most comment fruit that we can find easily in Malaysia. It is a cash crops plant where it will plant in turn with other plant like potato to stabilize the pH of soil. It is growing to 2m at a fast rate. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Wind. The example of corn is shown in Figure 2.1 and the Agroclimatic requirements of corn is shown in Table 2.1

Figure 2.1: Corn

Table 2.1: Agroclimatic requirements of corn

Temperature	30°C - 35°C
Rainfall	500 - 700 mm/year
Soil type	Deep, friable texture with good water holding capacity and drainage.
Soil pH	5.0 - 6.5

2.2 Antioxidant

2.2.1 Antioxidant

Antioxidants are substance that can delay the onset or slow the rate of oxidation of autoxidizable material. Taking antioxidants combats excessive free radical damage. Molecules become free radicals when the oxygen atom loses an electron and starts to "attack" surrounding molecules, seeking a replacement electron. A chain reaction of cell damage results until an antioxidant halts the process by providing a spare electron. (Steven Shackel, JP)

2.2.2 Free Radicals

In chemistry, Radicals (often referred to as free radicals) are atomic or molecular species with unpaired electrons on an otherwise open shell configuration. These unpaired electrons are usually highly reactive, so radicals are likely to take part in chemical reactions. Radicals play an important role in combustion, atmospheric chemistry, polymerization, plasma chemistry, biochemistry, and many other chemical processes, including human physiology. For example, superoxide and nitric oxide regulate many biological process, such as controlling vascular tone. "Radical" and "Free Radical" are frequently used interchangeably, however a radical may be trapped within a *solvent cage* or be otherwise bound. Historically, "Radical" was used to refer to a collection of atoms that remain unchanged over the course of a reaction, however this usage is, today, uncommon. The first organic free radical (the *triphenylmethyl* radical) was identified by Moses Gomberg in 1900.

In chemistry free radicals take part in radical addition and radical substitution as reactive intermediates. Reactions involving free radicals are usually divided into three categories; initiation, propagation, and termination.

- Initiation reactions are those which result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species as in Reaction 1 above or they may involve reactions of free radicals with stable species to form more free radicals.
- Propagation reactions are those reactions involving free radicals in which the total number of free radicals remains the same.
- Termination reactions are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, for example: $2Cl\cdot \rightarrow Cl_2$

The formation of radicals requires covalent bonds to be broken homolytically, a process that requires significant amounts of energy. For example, splitting H_2 into $2H\cdot$ has a $\Delta H°$ of +435 kJ/mol, and Cl_2 into $2Cl\cdot$ has a $\Delta H°$ of +243 kJ/mol. This is known as the homolytic bond dissociation energy, and is usually abbreviated as the symbol $DH°$. The bond energy between two covalently bonded atoms is affected by the structure of the molecule as a whole, not just the identity of the two atoms, and radicals requiring more energy to form are less stable than those requiring less energy. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. In organic chemistry this is often the O-O bond in peroxide species or O-N bonds. The radical derived from Alpha-tocopherol is shown in Figure 2.2.

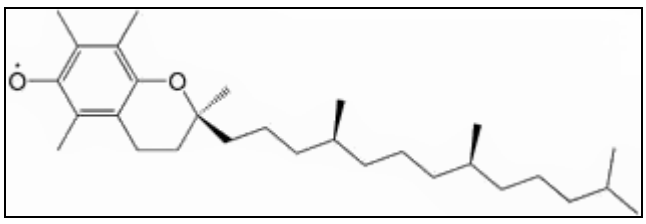

Figure 2.2: The radical derived from *α-tocopherol*

Long lived radicals can be placed into two categories

- **Stable Radicals**

  Radicals can be long lived if they occur in a conjugated π system, such as the radical derived from α-tocopherol (vitamin E)

- **Persistent Radicals**

  Persistent radical compounds are those whose longevity is due to steric crowding around the radical center and makes it physically difficult for the radical to react with another molecule. Examples of these include Gomberg's radical (triphenylmethyl), Fremy's salt (Potassium nitrosodisulfonate, $(KSO_3)_2NO\cdot$)and nitroxides, (general formula $R_2NO\cdot$) such as TEMPO. The longest-lived free radical is melanin, which may persist for millions of years.

2.3 vitamin E (tocopherol)

Vitamin E was discovered in 1922 when Evans HM et al. (Science 1922, 56, 650) described a "substance X" that was essential to maintain rat fertility. After obtaining similar results, Sure B called the substance "vitamin E" because vitamins A, B, C, and D were already known (Sure B, J Biol Chem 1924, 58, 693). Alphatocopherol is the most common and the most active of the seven currently described forms—alpha, beta, gamma, delta, epsilon, and zeta. Specifically, d-alpha tocopherol is the most potent form, more active than the synthetic dl-alpha tocopherol.

Alpha-tocopherol is basically stable in heat and in acids, other forms are lost in heat, with storage or freezing, or when oxidized by exposure to the air. All vitamin E's are slightly unstable in alkali and are readily used up when in contact with polyunsaturated oils or rancid fats and oils, which are protected from oxidative

destruction by vitamin E. Frying oils, the processing and milling of foods, the bleaching of flours, and cooking remove much of the vitamin E content of whole foods. During the refinement and purification of vegetable oils, vitamin E is lost; the vitamin E-rich by-products of this process are used to make some of the E used in supplements.
(Elson M. Haas M.D)

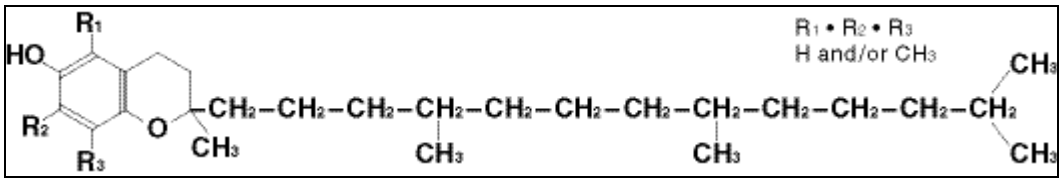

Figure 2.3: Chemical structure of α-tocopherol**.**

Table 2.2: Natural tocopherol homologues

Homologues	R1	R2	R3
Alpha- TOCOPHEROL	CH3	CH3	CH3
Beta- TOCOPHEROL	CH3	H	CH3
Gamma- TOCOPHEROL	H	CH3	CH3
Delta- TOCOPHEROL	H	H	CH3

(Source: rike-vita.co.jp)

Natural TOCOPHEROL exists as a mixture of 4 homologues, Alpha, Beta, Gamma, and Delta is shown in Table 2.2. Available as a mixed product known as MIXED TOCOPHEROL - which is also known as vitamin E. Riken Vitamin Co., Ltd. supplies TOCOPHEROL products and specialties according to requirements by separating or combining the homologues using unique technology. TOCOPHEROLS are transparent, viscous, oily liquids with slightly characteristic odor, and have colors ranging between light yellow to reddish brown. They are insoluble in water but soluble in organic solvents such as ethanol, chloroform, and hexane. The color changes gradually into dark brown after contact with air or light though it dose not alter the antioxidant activity.

2.3.1 Uses of tocopherol

Alpha-Tocopherol has strong vitamin E activity, while Beta, Gamma, and Delta-Tocopherol have strong antioxidant activity outside the body (in foodstuffs etc.). Therefore, Tocopherol preparations which have a high Alpha-Tocopherol content are suitable for health foods and vitamin E enrichment.

There is quite an extensive list of uses for this popular nutrient, most commonly in the middle-aged and older populations. And there are many positive effects. Some of these claims are backed by good research, and more investigation is being done on vitamin E by medical and nutritional scientists. There is hope that the results of this research will enable us to better understand its mechanisms and apply those most effectively to prevent and treat our industrial-age medical conditions.

The antioxidant function that we have discussed gives vitamin E a variety of uses. The protection of cells and tissues against oxidation and injury from unstable molecules, pollution, and fats may also be the basis for the prevention of aging and many chronic diseases. Claims about vitamin E's role in preventing premature aging and promoting longevity are big areas of investigation for vitamin E researchers. These claims are often made and with some good reason. Aging, tissue degeneration, and skin changes may be brought about by the damage that free radicals cause to cells unprotected by antioxidant nutrients in the body.

Cancer and heart and vascular disease may also be created in this way, and vitamin E therapy may help reduce the risks of these major illnesses. Decreased blood clotting and increased tissue oxygenation may also help reduce symptoms of heart and vascular limitations, such as angina pectoris, intermittent claudication (leg pain with walking due to insufficiency of blood and oxygen, for which vitamin E has clearly been helpful), and problems of arterial spasm. In both congenital and rheumatic heart diseases, vitamin E may help reduce symptoms caused by impaired tissue oxygenation.

Vitamin E may be of help in the prevention of atherosclerosis. Its antioxidant effect reduces thrombin formation and thus helps decrease blood clotting, and it also appears to minimize platelet (blood-clotting component) aggregation and stickiness, aspects that either generate or perpetuate the atherosclerotic process. Vitamin E was thought to raise HDL ("good") cholesterol levels, especially when they were low; however, recent research suggests it has a very mild, if any, effect in this regard. Vitamin A and E together can help to decrease cholesterol and general fat accumulation. To assist in healing and to minimize clotting, tocopherol is a useful nutrient before and after surgery, but is limited to dosages of 200–300 IUs per day (higher amounts may actually suppress the healing process).

Also, pre- and postsurgery, vitamin E neutralizes free radical formation and thus reduces possible problems from that. Recently, this antioxidant effect of vitamin E was shown in cardiopulmonary bypass surgery. In regard to its healing powers, vitamin E is used most commonly both internally and externally to assist in the repair of skin lesions, ulcers, burns, abrasions, and dry skin and to heal and/or diminish the scars caused from injury or surgery. (Vitamin A also appears to work in this regard, possibly even better than E in some instances where skin and tissue healing are needed.) Decreasing scars internally may be important in resolving damage from inflammation of blood vessels and may reduce the potential for clotting and thrombophlebitis. Vitamin E, with the help of vitamins C and P (bioflavonoids), may be useful in preventing progression of varicose veins, more so than treating them once they have occurred.

Vitamin E may be very helpful to women. Research shows relief from menstrual pains, as well as general relief from various menstrual disorders. Many problems of menopause, such as headaches, hot flashes, or vaginal itching due to dryness, may be reduced with the use of supplemental vitamin E. When birth control pills are used, the tocopherols may help protect the body from their possible side effects. Estrogen may decrease the effect of vitamin E, so more is needed when estrogen therapy is used.

Vitamin E has been used both topically and orally with some success in the treatment of fibrocystic breast disease, or cystic mastitis, likely due to its protective mechanisms against estrogen, which seems to potentiate this disease.

Vitamin E's antioxidant functions help to protect our cell membranes and lung tissue from pollution, particularly from ozone (O_3) and nitrogen dioxide (NO_2) in the air. Research in rats clearly showed their ability to tolerate increased ozone levels and to survive much longer with vitamin E. There is also some cardiac protection from smoke and alcohol with vitamin E, and it protects against the cardio-toxic effects of adriamycin, an anticancer drug.

Vitamin E has also been used to enhance immunity in the treatment of viral illness and to reduce the neurologic pain from shingles, a viral infection of the nerves and skin. It is also helpful in preventing eye problems, such as poor vision or cataracts, that may be due to oxidation of fatty tissues and free radical formation leading to areas of inflammatory damage. Headaches may sometimes be helped with tocopherol treatment, depending on the cause. Various kidney and liver diseases and muscular dystrophy have all been treated with vitamin E, though more immediate inflammatory problems, as in bursitis, gout, and arthritis seem to benefit more. Leg cramps and circulatory problems associated with diabetes may be helped with vitamin E treatment. For various skin rashes, including those of lupus erythematosus, vitamin E, usually along with vitamin A, may be of some help. (Elson M. Haas M.D)

2.4 Extraction of Antioxidant

2.4.1 Definition of Extraction

Many biological and inorganic substances occur in a mixture of different component in a solid. In order to separate the desires solute and remove undesirable solute, extraction is one type of separation process. The process is also called leaching.

Liquid-liquid extraction is based on the transfer of a solute from one liquid phase into another liquid phase. Extraction becomes a very useful tool if you choose a suitable extraction solvent. You can use extraction to separate a substance selectively from a mixture, or to remove unwanted impurities from a solution.

In the practical use, usually one phase is a water or water-based (aqueous) solution and the other an organic solvent (i.e. vegoil) which is immiscible with water. Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes and other industries. It is interesting to note that liquid-liquid extraction is possible in non aqueous systems, for instance in a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam which modifies the electrochemistry greatly. For example it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinium the sodium cations will not be reduced, instead water is reduced to hydrogen).

2.4.2 Solvent extraction

A solvent is a liquid that dissolves a solid, liquid, or gaseous solute, resulting in a solution. The most common solvent in everyday life is water. The term organic solvent

refers to most other solvents that are organic compounds and contain carbon atoms. Solvents usually have a low boiling point and evaporate easily or can be removed by distillation, thereby leaving the dissolved substance behind. Solvents should therefore not react chemically with the dissolved compounds, they have to be inert. Solvents can also be used to extract soluble compounds from a mixture, the most common example is the brewing of coffee or tea with hot water. Solvents are usually clear and colorless liquids and most of them have a characteristic odor. The concentration of a solution is the amount of compound that is dissolved in a certain volume of solvent. The solubility is the maximal amount of compound that is soluble in a certain volume of solvent at a specified temperature.

The solvents are grouped into non-polar, polar aprotic, and polar protic solvents and ordered by increasing polarity. The polarity is given as the dielectric constant. The density of nonpolar solvents that are heavier than water is bolded. The boiling point, polarity and density of solvent is shown in table 2.3.

Table 2.3: Solvent chemical Formula,boiling point, polarity and density**.**

Solvent	Chemical Formula	Boiling point	Polarity	Density
Non-Polar Solvents				
Hexane	CH_3-CH_2-CH_2-CH_2-CH_2-CH_3	69 °C	2.0	0.655 g/ml
Benzene	C_6H_6	80 °C	2.3	0.879 g/ml
Toluene	C_6H_5-CH_3	111 °C	2.4	0.867 g/ml
Diethyl ether	CH_3CH_2-O-CH_2-CH_3	35 °C	4.3	0.713 g/ml
Chloroform	$CHCl_3$	61 °C	4.8	**1.498 g/ml**
Ethyl acetate	CH_3-C(=O)-O-CH_2-CH_3	77 °C	6.0	0.894 g/ml

Dichloromethane	CH_2Cl_2	40 °C	9.1	**1.326 g/ml**
Polar Aprotic Solvents				
1,4-Dioxane	/-CH_2-CH_2-O-CH_2-CH_2-O-\	101 °C	2.3	1.033 g/ml
Tetrahydrofuran (THF)	/-CH_2-CH_2-O-CH_2-CH_2-\	66 °C	7.5	0.886 g/ml
Acetone	CH_3-C(=O)-CH_3	56 °C	21	0.786 g/ml
Acetonitrile (MeCN)	CH_3-C≡N	82 °C	37	0.786 g/ml
Dimethylformamide (DMF)	H-C(=O)N(CH_3)$_2$	153 °C	38	0.944 g/ml
Dimethyl sulfoxide (DMSO)	CH_3-S(=O)-CH_3	189 °C	47	1.092 g/ml
Polar Protic Solvents				
Acetic acid	CH_3-C(=O)OH	118 °C	6.2	1.049 g/ml
n-Butanol	CH_3-CH_2-CH_2-CH_2-OH	118 °C	18	0.810 g/ml
Isopropanol	CH_3-CH(-OH)-CH_3	82 °C	18	0.785 g/ml
n-Propanol	CH_3-CH_2-CH_2-OH	97 °C	20	0.803 g/ml
Ethanol	CH_3-CH_2-OH	79 °C	24	0.789 g/ml
Methanol	CH_3-OH	65 °C	33	0.791 g/ml
Formic acid	H-C(=O)OH	100 °C	58	1.21 g/ml
Water	H-O-H	100 °C	80	0.998 g/ml

(From: www.wikipedia.org)

2.4.4 Methanol As Solvent In Extraction

Methanol, also known as methyl alcohol or wood alcohol, is a chemical compound with chemical formula CH_3OH. It is the simplest alcohol, and is a light, volatile, colourless, flammable, poisonous liquid with a very faint odor. It is used as an antifreeze, solvent, fuel, and as a denaturant for ethyl alcohol. The formula of methanol is shown in Figure 2.4

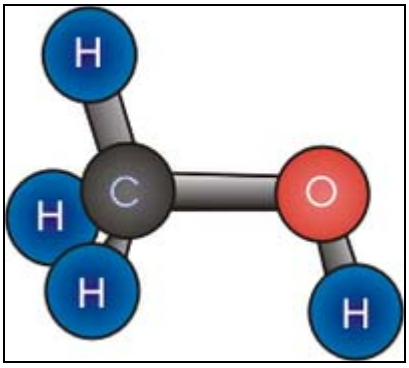

Figure 2.4: Methanol atom structure

Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria. As a result, there is a small fraction of methanol vapor in the atmosphere. Over the course of several days, atmospheric methanol is oxidized by oxygen with the help of sunlight to carbon dioxide and water.

Methanol burns in air forming carbon dioxide and water:

$$2\ CH_3OH + 3\ O_2 \rightarrow 2\ CO_2 + 4\ H_2O \text{----------[1]}$$

A methanol flame is almost colorless. Care should be exercised around burning methanol to avoid burning oneself on the almost invisible fire.

Because of its poisonous properties, methanol is also used as a denaturant for ethanol. Methanol is often called wood alcohol because it was once produced chiefly as

a byproduct of the destructive distillation of wood. It is now produced synthetically by the direct combination of hydrogen and carbon monoxide gases, heated under pressure in the presence of a catalyst. General information, properties and structure of methanol is shown in Table 2.4. (From Wikipedia, the free encyclopedia)

Table 2.4: Methanol general information, properties and structure

General	
Systematic name	methanol
Other names	hydroxymethane, methyl alcohol, wood alcohol carbinol
Molecular formula	CH_3OH
SMILES	CO
Molar mass	32.04 g/mol
Appearance	colourless liquid
CAS number	[67-56-1]
Properties	
Density and phase	0.7918 g/cm^3, liquid
Solubility in water	Fully miscible
Melting point	-97 °C (176 K)
Boiling point	64.7 °C (337.8 K)
Acidity (pK_a)	~ 15.5
Viscosity	0.59 mPa·s at 20 °C
Structure	
Molecular shape	Tetrahedral and Bent
Dipole moment	1.69 D (gas)

2.4.4.1 uses of methanol

Methanol is used on a limited basis to fuel internal combustion engines, mainly by virtue of the fact that it is not nearly as flammable as gasoline. Methanol blends are the fuel of choice in open wheel racing circuits like Champcars, as well as in radio

controlled model airplanes (required in the "glow-plug" engines that primarily power them), cars and trucks. Dirt circle track racecars such as Sprint cars, Late Models, and Modifieds use methanol to fuel their engines. Drag racers and mud racers also use methanol as their primary fuel source. Methanol is required with a supercharged engine in a Top Alcohol Dragster and, until the end of the 2005 season, all vehicles in the Indianapolis 500 have to run methanol. Mud racers have mixed methanol with gasoline and nitrous oxide to produce more power than gasoline and nitrous oxide alone.

One of the drawbacks of methanol as a fuel is its corrosivity to some metals, including aluminium. Methanol, although only a weak acid, attacks the oxide coating that normally protects the aluminium from corrosion:

$$6CH_3OH + 2Al \rightarrow 2Al^{3+} + 6CH_3O^- + 3H_2 \text{----------[2]}$$

The resulting methoxide salts are soluble in methanol, so the corrosion continues until the metal is eaten away.

When produced from wood or other organic materials, the resulting organic methanol (bioalcohol) has been suggested as renewable alternative to petroleum-based hydrocarbons. However, one cannot use BA100 (100% bioalcohol) in modern petroleum cars without modification. Methanol is also used as a solvent and as an antifreeze in pipelines. The largest use of methanol by far, however, is in making other chemicals. About 40% of methanol is converted to formaldehyde, and from there into products as diverse as plastics, plywood, paints, explosives, and permanent press textiles.

In some wastewater treatment plants, a small amount of methanol is added to wastewater to provide a food source of carbon for the denitrification bacteria, which convert nitrates to nitrogen.

In the 1990s, large amounts of methanol were used in the United States to produce the gasoline additive methyl tert-butyl ether (MTBE). The 1990 Clean Air Act

required certain major cities to use MTBE in their gasoline to reduce photochemical smog. However, by the late 1990s, it was found that MTBE had leaked out of gasoline storage tanks and into the groundwater in sufficient amounts to affect the taste of municipal drinking water in many areas. Moreover, MTBE was found to be a carcinogen in animal studies. In the resulting backlash, several states banned the use of MTBE, and its future production remains uncertain.

Direct-methanol fuel cells are unique in their low temperature, atmospheric pressure operation, allowing them to be miniaturized to an unprecedented degree. This, combined with the relatively easy and safe storage and handling of methanol may open the possibility of fuel cell-powered consumer electronics.

Other chemical derivatives of methanol include dimethyl ether, which has replaced chlorofluorocarbons as an aerosol spray propellant, and acetic acid.

2.5 High Performance Liquid Chromatography (HPLC)

2.5.1 Introduction

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flowthrough time, thus reducing purification times of compounds being isolated by column chromatogaphy. However, flow rates were inconsistant, and the question of whether it was better to have constant flow rate or constant pressure was debated. (Analytical Chem. 1990).

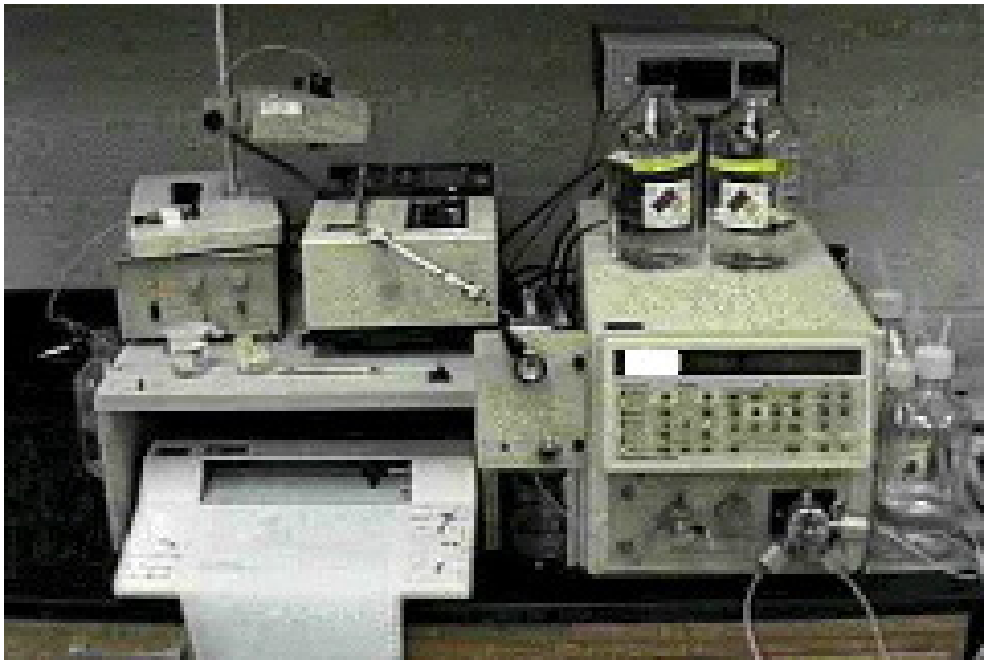

Figure 2.4: HPLC

2.5.2 HPLC Working Principles

High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds.

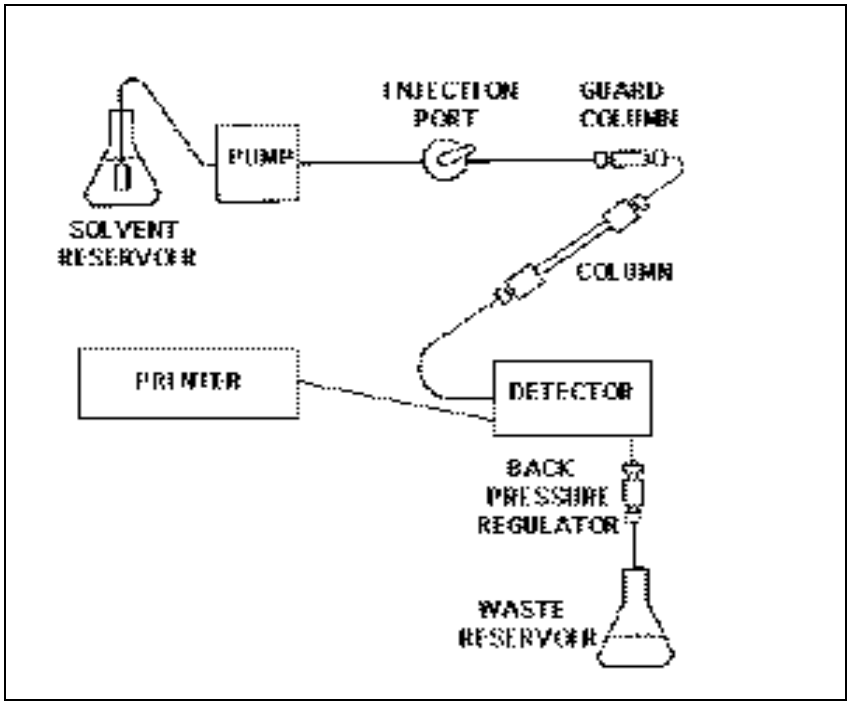

Figure 2.5: HPLC working principle
(From: kerouac.pharm.uky.edu)

By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification, and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge.

The past decade has seen a vast undertaking in the development of the micro-columns, and other specialized columns. The dimensions of the typical HPLC column are with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 μm to 200 μm. Fast HPLC utilizes a column that is shorter than the typical column, with a length of about 3 mm long, and they are packed with smaller particles.

Although HPLC is widely considered to be a technique mainly for biotechnological, biomedical, and biochemical research as well as for the pharmaceutical industry, these fields corrently comprise only about 50% of HPLC users.(Analytical Chem. 1990). Currently HPLC is used by a variety of fields including cosmetics, energy, food, and environmental industries

2.5.2.1 Detector

The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled (in most cases). There are many types of detectors that can be used with HPLC. Some of the more common detectors include: Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infra Red (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).

Mass Spectroscopy (MS) Detectors- The sample compound or molecule is ionized, it is passed through a mass analyzer, and the ion current is detected. There are various methods for ionization:

A)	**Electron Impact (EI)**- An electron current or beam created under high electric potential is used to ionize the sample migrating off the column.
B)	**Chemical Ionization**- A less aggresive method which utilizes ionized gas to remove electrons from the compounds eluting from the column.
C)	**Fast Atom Bombarbment (FAB)** - Xenon atoms are propelled at high speed in order to ionize the eluents from the column.

Has detection limit of 10^{-8} to 10^{-10} gm/ml

2.6 Literature Review from Previous Study

From previous study, they covered vitamin E level in corn. Maize kernels have been studied for their different levels and isoforms of tocopherols. Early studies focused on three isoforms, α-tocopherol, γ-tocopherol and δ-tocopherol with γ-tocopherol generally regarded as being in the highest concentration . The corn kernel consists of two parts, the endosperm which is mostly starch and some protein, and the germ. The germ of the kernel is a combination of the reproductive organ or embryo and the scutellum. The scutellum is the non-germinating tissue surrounding the embryo where most of the oil is stored. The distribution of the tocopherols in the kernels has been evaluated by hand dissection of the kernel in different corn lines. The germ of the kernel contains 70% to 86% of the tocopherols, with the endosperm having 11% to 27%, although the levels of tocopherol storage is genotype dependent. For individual tocopherols, 94% to 96% of α-tocopherol and 93% to 96% of γ-tocopherol are found within the germ; for δ-tocopherol it is only found in the germ. Considerable variation is present among different inbreds for tocopherol levels, as well as different ratios of α-tocopherol to γ-tocopherol. The nature of inheritance, as calculated by heritability values for α- and γ-tocopherol as well as total tocopherols, indicate effective selection for levels of tocopherols should be attainable.

Measuring tocopherols in corn grain requires a series of steps and procedures that are very labor intensive, time consuming and require expensive equipment and some expertise. The grain is first ground, followed by a series of extractions, and finally the extracted samples are run on a high performance liquid chromatographer (HPLC). The extraction process requires the most time, with 16 to 24 samples extracted by one person in about three hours depending on the experience of the person. This is followed by the running of the sample on the HPLC which requires six minutes per sample. In total, 16

to 24 samples can be analyzed by one person in about 4.5 to 5.5 hours. Typical commercial corn breeding operations, which produce the hybrids that are planted on approximately 99% of the corn acreage in the US, do not have the time to perform these expensive assays. The major reason they do not have the time is that presently there is not enough of an economic incentive. **(**Torbert R. Rocheford et al)

oncentration of alpha-Tocopherol (*x*) versus the HPLC alpha-Tocopherol peak area (*y*) graph was used as calibration. Alpha-tocopherol peak area will calculate and refer to the standard calibration.The analysis of the total alpha-tocopherol using HPLC method was resulting as the figure 1 below (*Torbert R. Rocheford,2002*):

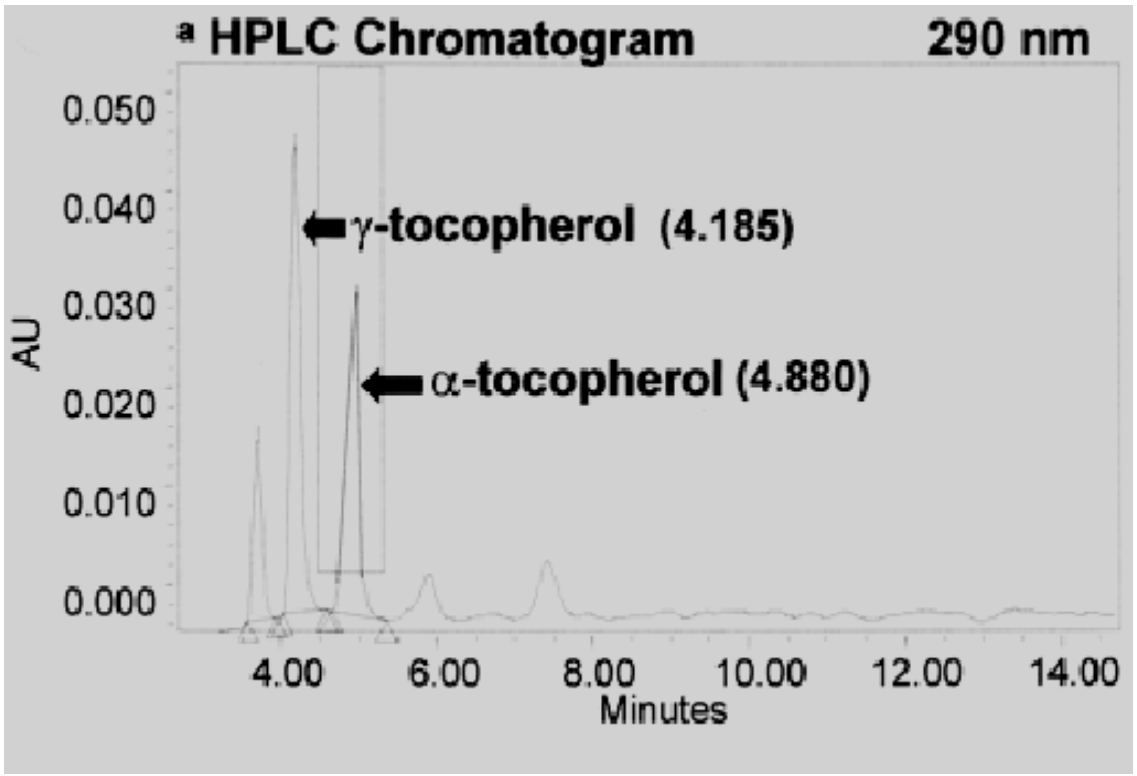

Figure 2.6: Tocopherol concentrations are determined by HPLC.(a) Chromatogram shows γ-tocopherol and α-tocopherol and their respective retention times of 4.185 and 4.880.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

A process to extract alpha-tocopherol from corn, there are few steps that need to be done before extracting process. The samples then will be analyzed by using High Performance Liquid Chromatography (HPLC) to detect that the alpha-tocopherol are attend in the sample. The extracting process will be run in same range of temperature but in different range of amount of solvent to find the optimum condition to extract the alpha-tocopherol and the optimum time to extract.

3.2 Methods to extract Alpha-tocopherol

A few steps must be following to run this experiment. Below is experimental flow chart:

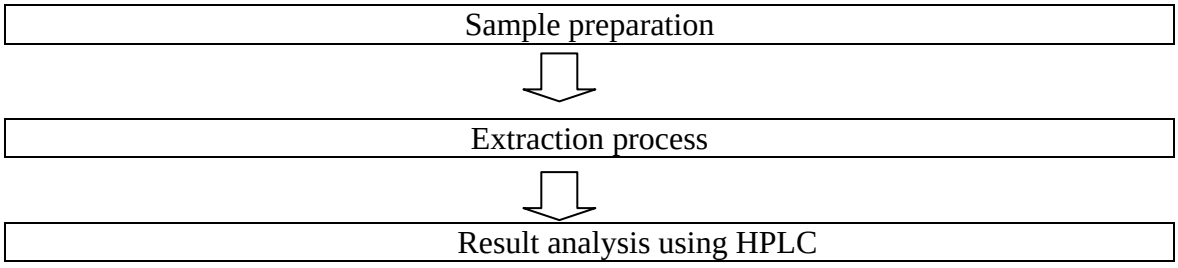

Figure 3.1: experiment flow chart.

3.2.1 Sample preparation

For fresh corn, 10 unhusked ears of corn were randomly selected *(Corey E. Scott July 2003).* Kernels from fresh corn samples were manually cut from the cob and blend it using blender at 30Hz in 3minutes with H_2O (2:1).

3.2.2 Extraction process

Corn was extract with methanol / H_2O (80:20 v/v) with ratio corn / solvent (1:2 v/v) at ambient temperature. The sample also been prepared with corn / solvent, (1:1 v/v) and (1:4 v/v) during extraction process. (Li Peiwu, 1999). Then shake it with shaker at ambient temperature in 5, 15, and 25minutes. Methanol solution will extract alpha-tocopherol from the sample. Table 3.1 below shown the experiment progress properly.

Table 3.1: Experimental summary to determine Alpha-Tocopherol

(1:2v/v) corn / solvent extraction	5 minute extraction time
	15 minute extraction time
	25 minute extraction time
(1:1v/v) corn / solvent extraction	5 minute extraction time
	15 minute extraction time
	25 minute extraction time
(1:4v/v) corn / solvent extraction	5 minute extraction time
	15 minute extraction time
	25 minute extraction time

3.2.3 Result analysis

The samples were analysis using HPLC, *Agilent*. Alpha-Tocopherol will detect using acetonitrile/methanol/dicloromethane (60:38:2, v/v) *(Sergi Munné-Bosch ,1999)* as mobile phase at a flow rate of 1.1 mL min^{-1} at 30oC. 5 micro liters of sample was injected, and duplicates were run for each extract. Alpha-Tocopherol standard solution was used for calibration.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The research entitled “Extraction of alpha-tocopherol from corn” had been completely done. The experiment started with sample preparation then extracts the sample. After that analysis the sample using HPLC to detect alpha- tocopherol. The procedure of doing this experiment is carefully followed to ensure the optimum data obtained from each experiment run.

4.2 Analysis using HPLC

Before analysis the sample that have been extract, standard solution have been prepared and analyzed at 100ppm, 50ppm and 10ppm using HPLC system. The grafts that have been getting from analysis are shown in Figure 4.1, 4.2 and 4.3.

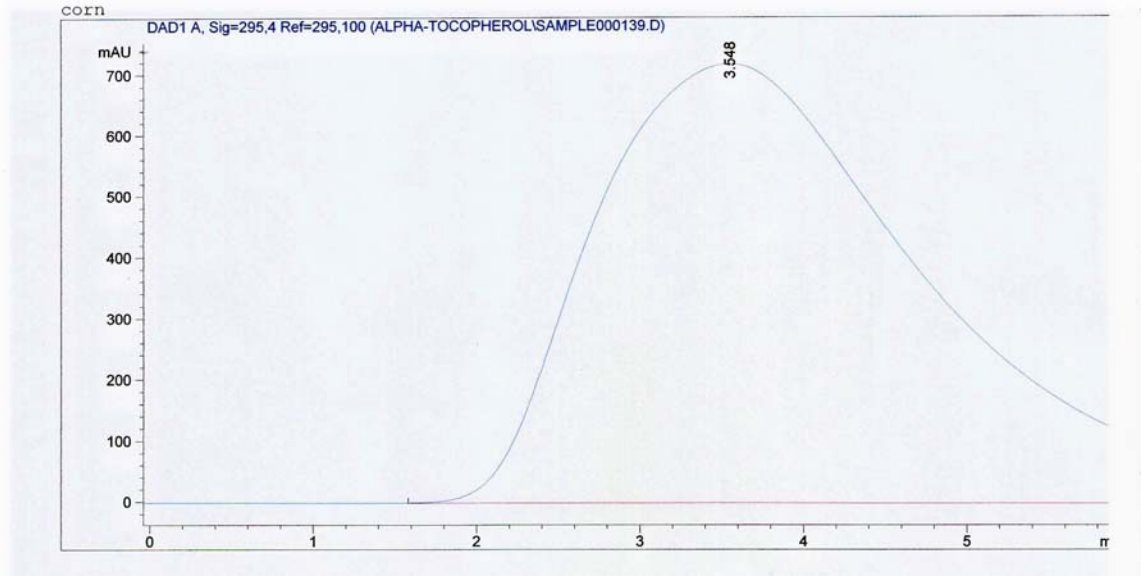

Figure 4.1: Standard solution 10ppm

From the figure 4.1 shown above, the peak was at 3.548 minute. For standard curve, the peak that should get was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

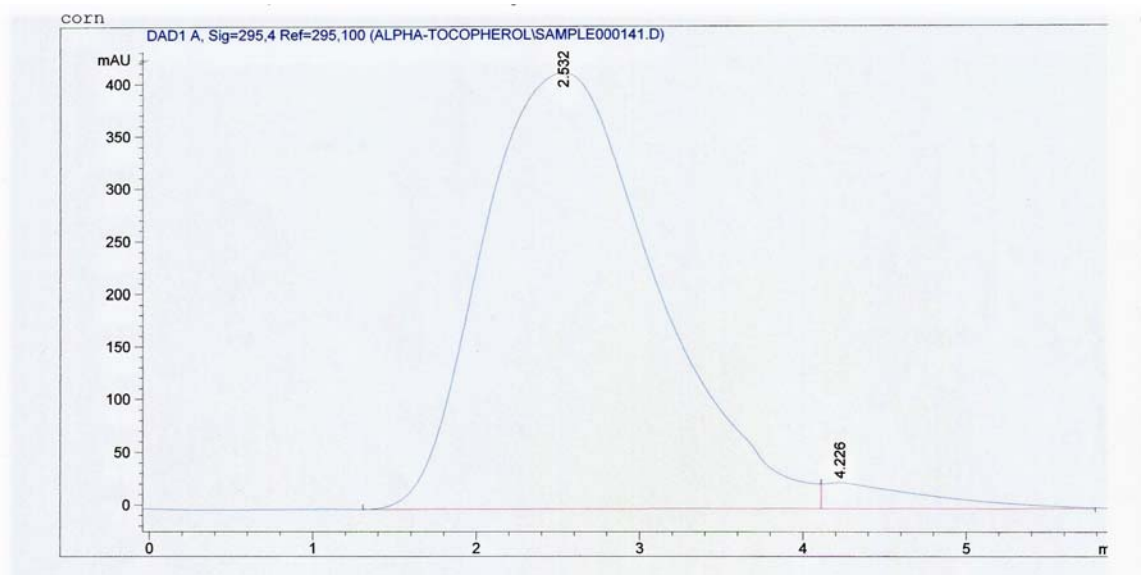

Figure 4.2: Standard solution 50ppm.

From the figure 4.2 shown above, the peak was at 2.532 and 4.226minutes. For standard curve, the peak that should get was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

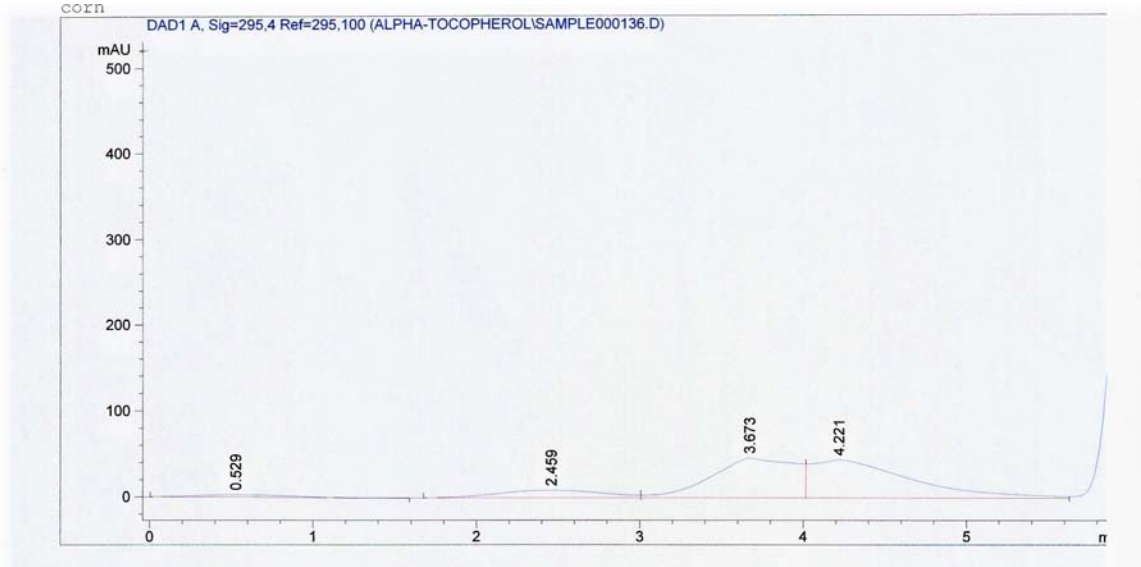

Figure 4.3: Standard solution 100ppm

From the figure 4.3 shown above, the peak was at 0.529, 2.459, 3.673 and 4.221 minutes. For standard curve, the peak that should get was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

After get the standard curve, sample that have been extract were analyze using HPLC. The graft that got from analysis is shown in figure 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11 and 4.12.

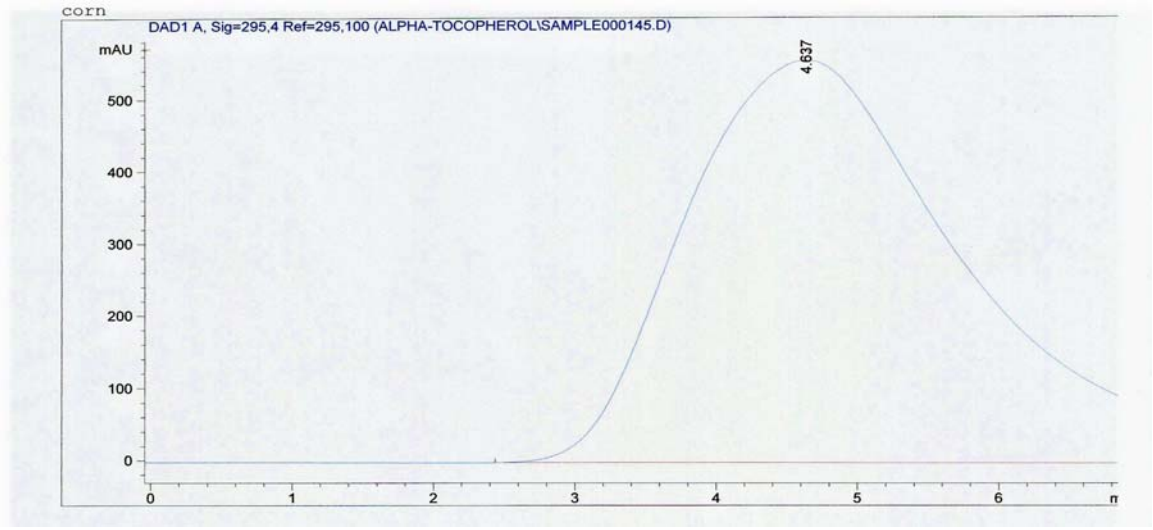

Figure 4.4: Corn 1:2; 5min

From the figure 4.4 shown above, the peak was at 4.637minute. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

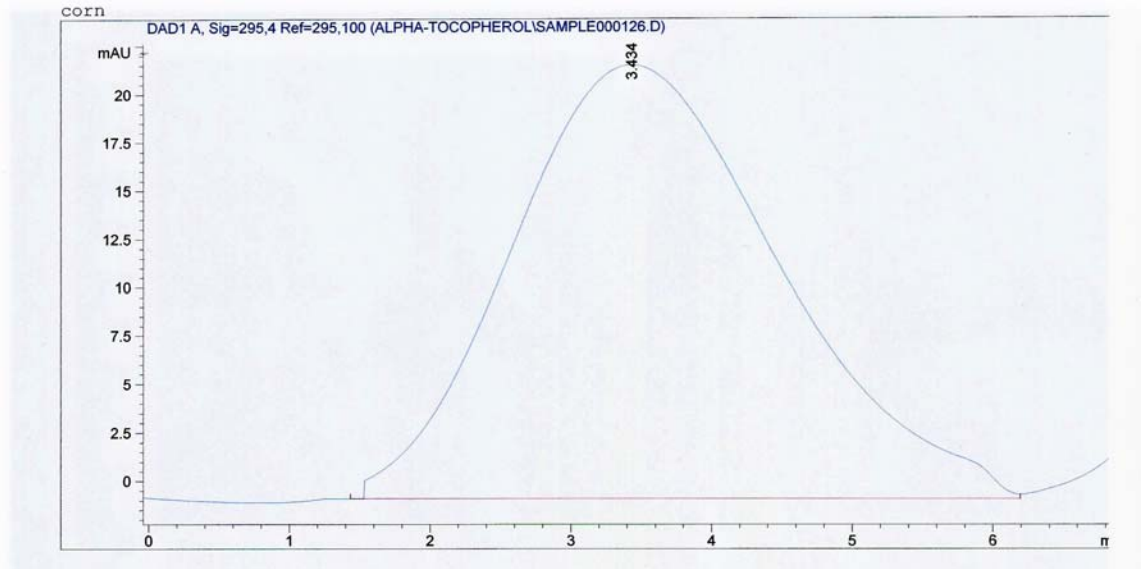

Figure 4.5: Corn 1:2; 15min

From the figure 4.5 shown above, the peak was at 3.434 minute. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

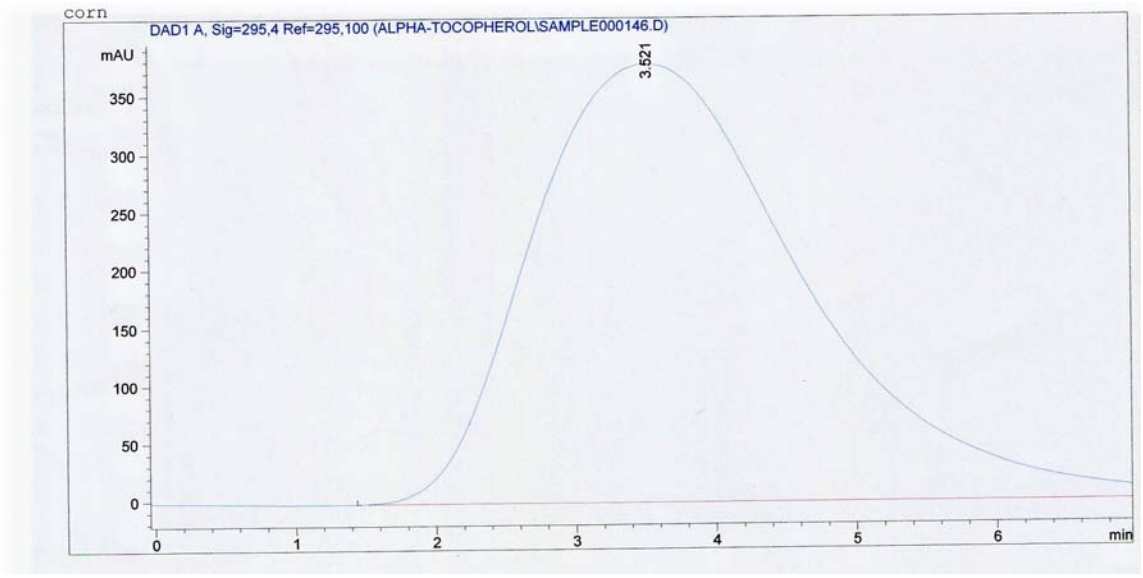

Figure 4.6: Corn 1:2; 25min

From the figure 4.6 shown above, the peak was at 3.521 minute. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

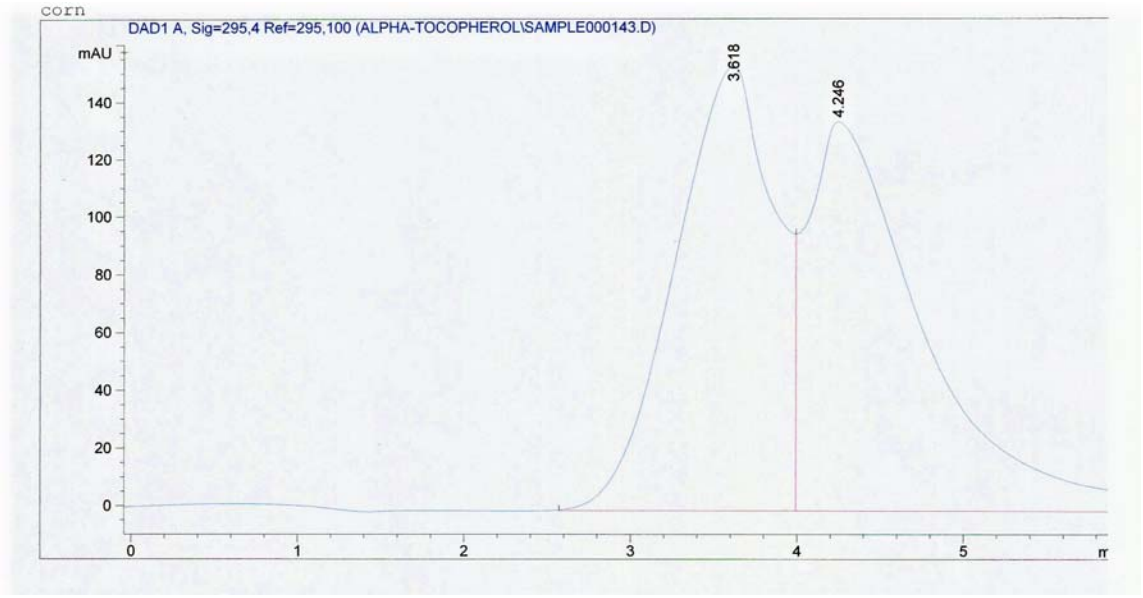

Figure 4.7: Corn 1:1; 5min

From the figure 4.7 shown above, the peak was at 3.618 and 4.246 minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

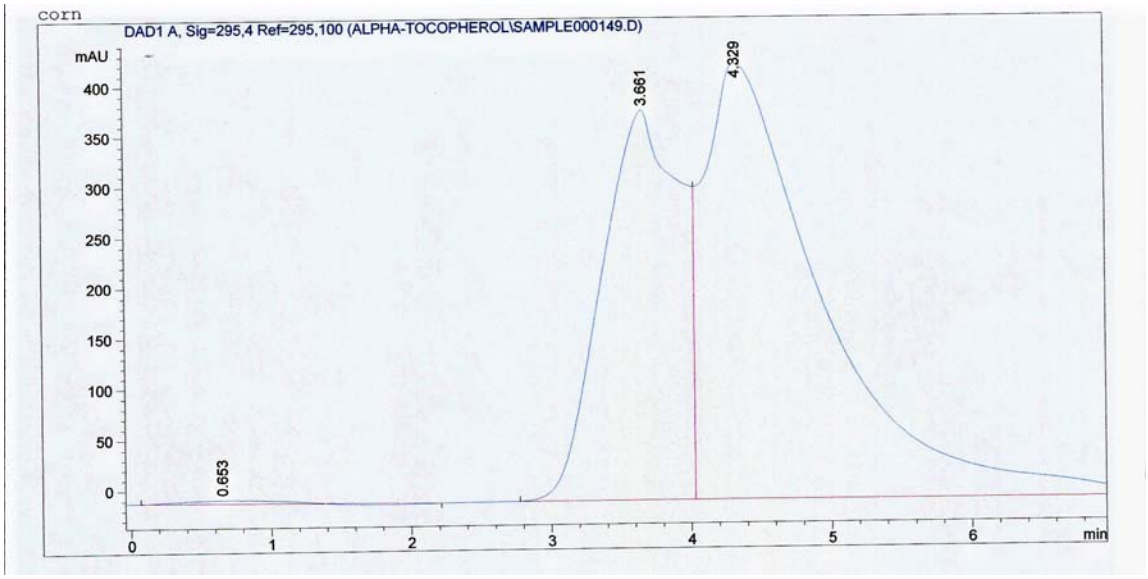

Figure 4.8: Con 1:1; 15min

From the figure 4.8 shown above, the peak was at 3.661 and 4.329 minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

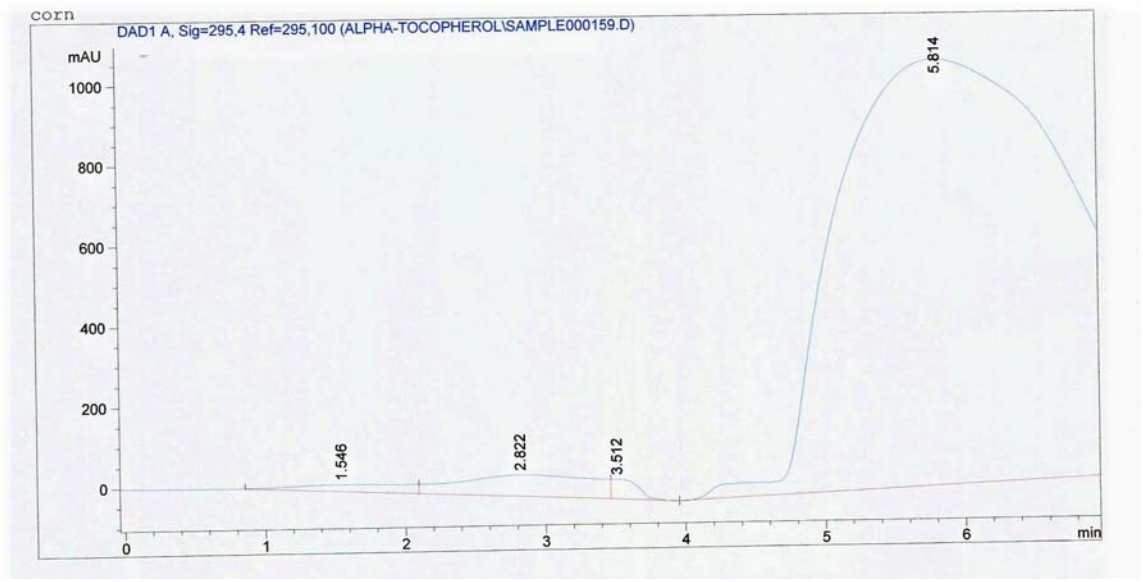

Figure 4.9: Con 1:1; 25min

From the figure 4.9 shown above, the peak was at 1.546, 2.822, 3.512and 5.814 minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

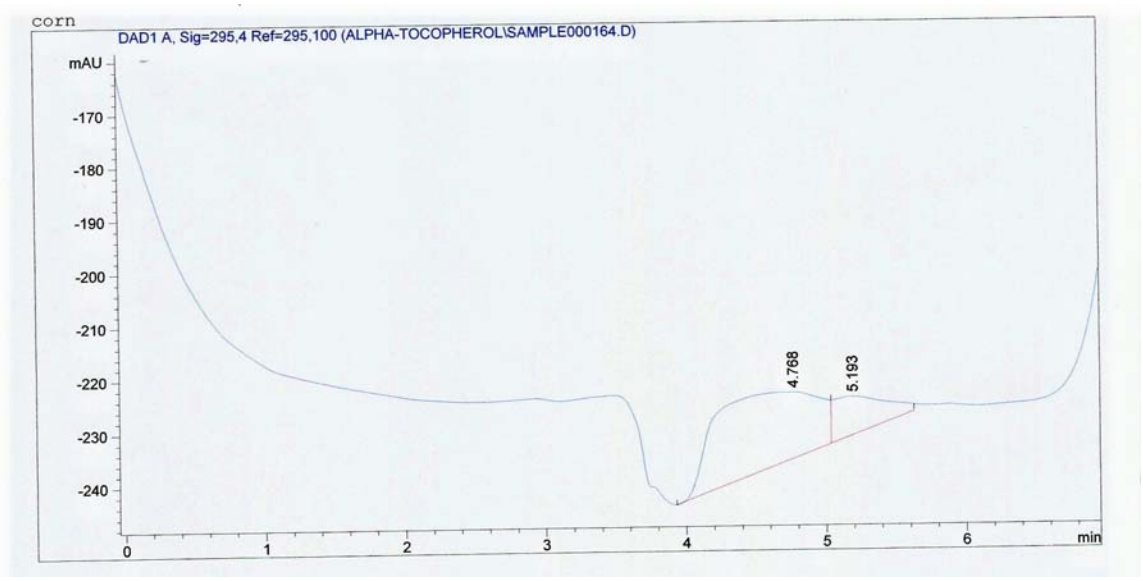

Figure 4.10: Con 1:4; 5min

From the figure 4.10 shown above, the peak was at 4.768 and 5.193 minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

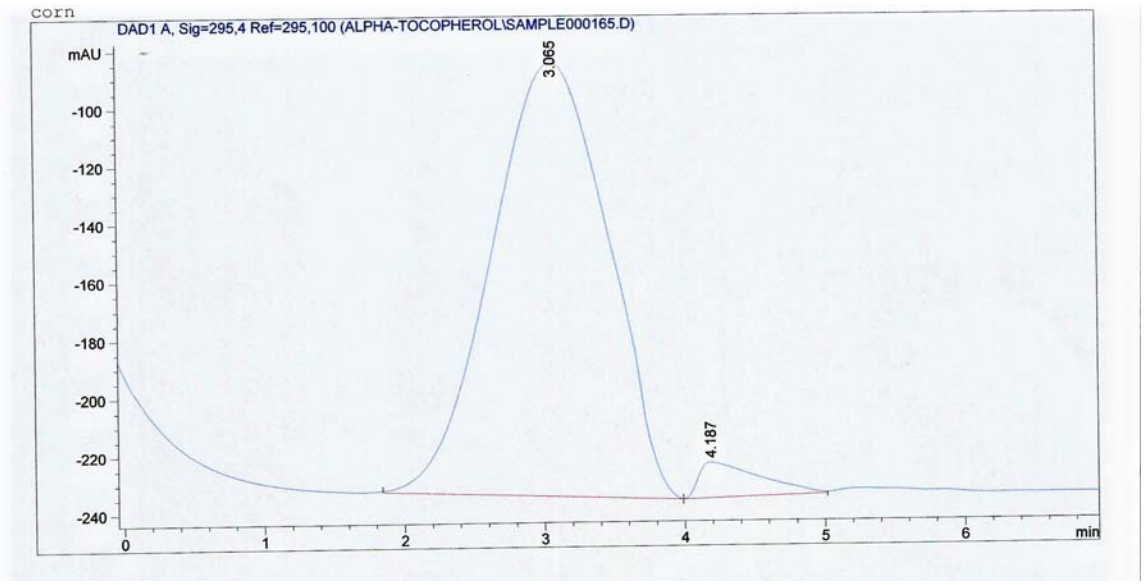

Figure 4.11: Con 1:4; 15min

From the figure 4.11 shown above, the peak was at 3.065 and 4.187minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

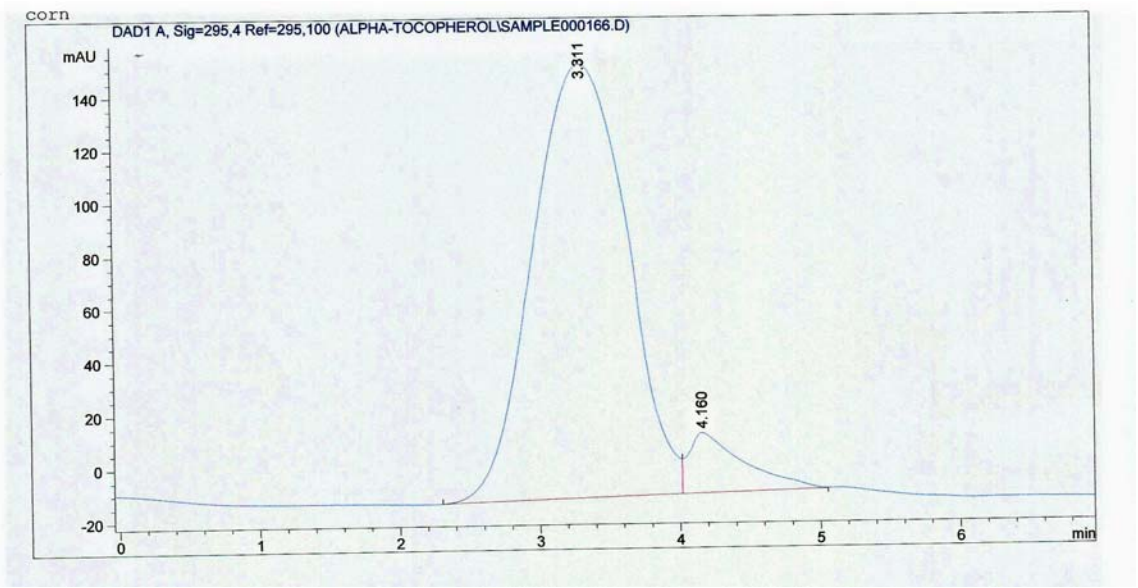

Figure 4.12: Con 1:4; 25min

From the figure 4.12 shown above, the peak was at 3.311 and 4.160 minutes. From previous study, the peak for alpha-tocopherol was at 4.88 minute. The graft shown was not obtaining the graft from previous study.

4.3 Discusion

It is observe that the result did not obtain the previous study have before. Every graft did have pick at retention time between 4 and 5 minute but not exactly at 4.88 retention time. I believe the main reason of this problem is analyze equipment (HPLC). There are a few reasons that possibly can explain why the alpha-tocopherol cannot be detected by using HPLC.

- As we know HPLC is very sensitive equipment. During this time, many students are using HPLC to analysis their sample. Same column are using to analyze many different sample with different structure. As we know, students handle the equipment alone but they did not have skill to handle the equipment. So the column is not clean, it make a reason why the equipment cannot operate properly.

- One of the main reason is the column it self. The column that are suppose to be use to detect alpha-tocopherol is C_{18} reversed-phase column. During the experiment only C_{14} column that water based are provided. So the column cannot detect tocopherol well because tocopherol is oil based.
- Filter the sample is the main factor to prevent the column from stuck. When the sample didn't filter properly, so in hard for the column to detect the component in the sample.

There are some ways to prevent the problem above. I believe that the way that I recommend can reduce the error in this experiment. They are:

- Gets the right column to detect the sample during analysis using HPLC. For alpha-tocopherol C_{18} reverse phase column are suitable.
- Guide from professional advisor to handle the equipment. So the student can learn to handle the equipment properly.
- Another method can be added to analyze the samples that have been extracting. For example using DPPH to detect alpha tocopherol in sample

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study was trough the procedure to extract alpha-tocopherol from corn. After preparing sample, it was extract with solvent extraction then analyzes it using HPLC. The result that was get was not obtaining the expected result. It is not successfully complete because of the problem that gets during analysis using HPLC. The optimum amount of solvent and optimum time of extraction cannot be detected.

5.2 Recommendation

From study that has been performing during this research, there are several important recommendations that should be done in future study to improve the result to analyze the sample. They are:

- Choose another method to extract alpha- tocopherol. Then it can be compare between extract with methanol and other chemicals for example hexane.

- Use ultrasonic separation to get high concentration of sample after extraction process. It cannot be use as method because the equipment is not provided in KUKTEM.
- Choose other raw materials that have potential in alpha tocopherol concentration in their composition. For example rosemary plant.