Extraction and Characterization of lycopene from Dovyalis Abyssinica Warb (Koshim) Using UV-Vis Spectrophotometry

By

Degu Daba Ejo

A Thesis Submitted to

The Department of Physics

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Physics

(Specialization in Laser Spectroscopy)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

September17, 2017

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by **Degu Daba Ejo** has read and evaluated his/her thesis entitled "**Extraction and Characterization of lycopene from Dovyalis Abyssinica Warb (Koshim) Using UV-Vis Spectrophotometry**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in physics (Laser Spectroscopy)

_____	_____	_____
Advisor	Signature	Date
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I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: _____

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This MSc Thesis has been submitted for examination with my approval as thesis advisor.

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Date of submission: _____

Advisor's approval sheet

To: Physics department

Subject: Thesis Submission

This is to certify that the thesis entitled "**Extraction and Characterization of lycopene from Dovyalis Abyssinica Warb (Koshim) Using UV-Vis Spectrophotometry"** submitted in partial fulfillment of the requirements for the degree of Master's in Physics (Laser Spectroscopy), the Graduate program of the department of Physics, and has been carried out by **Degu Daba Ejo** Id. No GSS/0385/05, under my supervision. Therefore, I/we recommend that the student has fulfilled the requirements and hence hereby he/she can submit the thesis to the department.

_____ _____ _____

Name of major Advisor Signature Date

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Contents

List of figures

List of Tables

Nomenclature

Lyc	Lycopene concentration
A_λ	Absorbance at wavelength λ
α_λ	Molar extinction coefficient
I_0	The intensity of incident light
I_t	The intensity of transmitted light
ν	frequency
λ	wavelength
$^{L}/_{mol \times cm}$	Unit of Molar extinction coefficient
h	Plank's constant
μ	micro
d	The thickness of the liquid layer
nm	nanometer
ASTU	Adama Science and Technology University
$^{mol}/_{L}$	Unit of concentration
$C_{40}H_{56}$	Molecular formula of lycopene
UV/VIS	Ultraviolet/Visible Spectroscopy
$\vec{D}$	The electrical displacement
$\vec{E}$	Electric field strength
$\vec{H}$	The magnetic field strength
$\vec{B}$	Magnetic induction
ϵ_0	Permittivity in vacuum
μ_0	Permeability in vacuum
J	Current density
ρ	Electric charge per unit volume
P	Polarization
M	Magnetization
T	Transmittance
$^{mg}/_{100g}$	Lycopene content
a. u	Absorbance unit
A	Absorbance

Abstract

This study was designed to extract and characterize the lycopene extracted from Koshim fruit juice. The optimization of the solvent extraction process for the maximum extract of lycopene from koshim was carried out by selecting the suitable solvent system. For analysis of lycopene we used a fast and simple spectrophotometric method, at 444nm and 450nm for methanol,446nm and 472nm for hexane and 445nm,450nm and 453nm in tri-mixture extracted at 20^0C, 25^0C,50^0C and 80^0C at time of 10,20 ,30 and 60 minutes respectively. The lycopene concentration was expressed mg/100g product. In koshim extraction the lycopene content had the following values: in methanol approximately (0.179-0.182) mg/100g, in hexane approximately (0.023-0.049) mg/100g, in mixture of (hexane: acetone: ethanol) at 50^0C, 80^0C are (0.195-0.230) and (0.107-0.141) mg/100g respectively. The optimization of the solvent extraction was explored amongst three extraction methods; hexane, methanol and tri-mixture to choose for the best extraction of lycopene from koshim fruit juice. Therefore, the objective of the thesis work is spectrophotometric characterization of these Dovyalis Abyssinica fruits(Koshim) in different solvents and based on this, develop reliable, fast, simple and inexpensive methods for determination of lycopene spectroscopic methods.

1. Introduction

Spectroscopy is the study of matter and its interaction with electromagnetic radiation. If matter is exposed to electromagnetic radiation, e.g. infrared light, the radiation can be absorbed, transmitted, reflected or scattered. All matter contains molecules; these molecules have bonds that are continually vibrating and moving around. Since its development, in the 1950's, the UV-Visible Spectrophotometer has evolved into an accurate and reliable analytical tool and it has become one of the most utilized instruments in today's scientific laboratory. UV/VIS is a subclass of spectroscopy which uses visible light and adjacent near ultraviolet (UV) ranges for the determination of the concentrations and the characterization of dissolved substances. Most dissolved molecules absorb light in the visible or UV range at specific wavelengths [1]. UV-Vis absorption spectroscopy is the measurement of the attenuations or absorption of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurement can be done at a single wavelength or over an extended spectra range. Visible and ultraviolet lights are energetic enough to promote outer electrons to higher energy levels.

The concentration sample solution can be determined by measuring the absorbance at some wavelength. All molecular materials were first dissolved in their appropriate solvents in well-defined concentrations, filled in a cuvette and then submitted to absorption spectroscopy inside a photo spectrometer [2].Optical spectroscopy is a fast and nondestructive analytical tool. It does not alter or destroy the constituents of the products. Therefore, it can be used for rapid sensing of food constituents online in a food processing plant or in the field. The wavelength range of radiation covered in optical spectroscopy can be divided into Ultraviolet (UV), 200 to 400 nm; and the visible, 400 to 760 nm [3].When photons from UV and visible radiation interact with the electrons in molecular orbital of a matter, electrons from a low energy state in an orbital are promoted to a higher energy state (excited state). The shifting of electrons in discrete energy levels is called electronic transition. In a molecule, the

atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. Because of the superposition of rotational and vibrational transitions on the electronic transitions, the UV/Visible spectra of a molecule have broad absorption bands. Therefore, absorption bands of lycopene have smooth and broad absorbance bands. The molecular spectroscopy which works in the wavelength regions of UV-Vis has various applications. For quantitative determination of one or more species in mixtures by Beer-Lambert's law, for determination of the presence or absence of a particular species in a mixture. In this research, it is intended to carry out quantitative characterization and analysis of Koshim using experimental methods [4]

1.1 Back ground of the study

Lycopene's have recently been used in order to protect human health and to prevent diseases such as various cancers, heart disease, macular degeneration and cataracts. Lycopene's have also been successfully used for many years in the treatment of individuals suffering from photosensitivity disease. Numerous studies have shown that increased intake of lycopene-rich foods, fruits and vegetables has a protective effect against several human chronic diseases. They are also powerful agents for growth inhibition in several human tumor cell lines but not in normal cells. Since many carcinogens inhibit gap junction communications, protection of this activity by dietary substances could be important for protection against cancer, β-carotene may play an important role in facilitating normal cell to cell communication through gap junction. Lycopene plays an important role in the human body's antioxidant defense system and is necessary for human health. Intake of lycopene-rich fruits and vegetables has been linked to reduced risk of cancers and cardiovascular diseases, most likely due to the antioxidant activity of lycopene [5].Lycopene is a natural pigment which protects the body by neutralizing the negative effects of oxidants. In the synthesis of vitamin, A, lycopene plays an important role as an intermediate and carotenoid like β-carotene and β cryptoxent in, influences its development. Therefore, lycopene also reduces low-density lipoprotein cholesterol and a carotenoid and gives color to vegetables and fruits [6], [7].

A great variety of food products of plant and animal origin, contain a wide range of lycopene. In recent years, there has been particular interest on the analysis and extraction of lycopene in foods for various health and nutrition activities. However, the analysis of lycopene is complicated due to several reasons. Diversity and the presence of cis-trans isomeric forms may affect their biochemistry. Highly unsaturated lycopene is subjected to isomerization and oxidation which produces problems associated with work and manipulation on lycopene's. Isomerization of usual configuration of lycopene (trans forms) to the cis forms can occur due to heat, light and acids. This isomerization leads to some loss of color and provitamin A activity. Oxidative degradation is the main reason for loss of lycopene. This degradation depends on the presence of oxygen and is enhanced by light, metals and enzymes. Lycopene has different susceptibilities to oxidation. Subsequent fragmentations produce a series of low-molecular-weight compounds. The last consequences are total loss of color and of vitamin A and other biological activities. Conditions necessary for isomerization and oxidation of lycopene exist during preparation, processing, storage and analysis of food. For this reason, several precautions are necessary when handling lycopene [8].

In recent years, there has been particular interest on the analysis and extraction of lycopene in foods for various health and nutrition activities. Lycopene are synthesized by all plants and many microorganisms (bacteria and fungi), but not by animals. Humans, therefore, rely on dietary intake of Lycopene. Lycopene are one of the most important groups of natural pigments due to their wide distribution, structural diversity and numerous functions. Lycopene have attracted the interest of researchers of different fields including biochemistry, biology, nutrition and food science, medicine and pharmacy for more than a century. They are responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates and birds. For example, β-carotene and lutein are responsible for the orange and yellow colors, respectively [9].

In this study, we focus on the extraction and characterization of lycopene from Koshim fruit juice that cultivated in ASTU campus. As a consequence of its wide uses, the request for high-purity, well-characterized, low-cost lycopene is growing continuously. Unfortunately, all the synthetic pathways proposed up to now are not

convenient economically, and at present, the simplest way for obtaining it is the extraction from natural sources [10] .

To understand the extraction of lycopene from Koshim, we need to know the natural sources of lycopene and its intake in human body. As we mentioned, the richest source of lycopene is vegetables and fruits. The content of several carotenoids in the fruits and vegetables, including lycopene, increases with advancing maturity of the fruit. Moreover, the lycopene from the colored fruits and vegetables has a vital significance for economic feasibility for one country, such that used in food products as a value-added and for health benefits, in reducing the risk of cardiovascular diseases, for inhabiting various types of cancers, and reducing many other chronic diseases. So, the extraction of lycopene from the matured Dovyalis Abyssinica(koshim)fruits juices may give option or an alternate bio remedy for healthy benefits, value-added food products and economic development for our country, because it is also part of a yellow-orange colored fruits found in Ethiopia and is edible by human being and wild animals [11].

In this regard, the optimization methods for the extraction and characterization has the way in creating new opportunities for the development of lycopene extraction based on UV-Vis spectroscopy. Thus, the extraction and characterization of lycopene from Koshim fruits is the high interest of this study. Great progress has been made in extraction methods however; there are significant challenges that have to overcome so as to produce efficient lycopene by UV-Vis spectroscopy. The performance of extraction process is influenced by several factors such as extraction time, temperature, stirring and nature of solvent. The most important factors affecting solvent extraction for the determination of total lycopene include extraction duration, solvent-solid ratio and extraction temperature [12].

Thus, the study of extraction and characterization of lycopene by UV-Vis spectroscopy is also an important work of this study. It is widely recognized that the use of lycopene, optimized extraction method and characterization techniques have developed in this study. Therefore, the present work is motivated by the above and aimed at obtaining optimized extraction methods of lycopene from koshim fruit juice by UV-Vis spectroscopy method [13].

1.2 The Objective of the Study

The general objective of this research is to extract and characterize lycopene's from the pulp juice extracted from matured Dovyalis Abyssinica (koshim) fruit using UV-Vis Spectroscopy. The specific objectives of this study are:

- To extract lycopene from matured koshim fruit juice by solvents
- To characterize the extracted lycopene of flesh koshim by UV-Vis spectroscopy.
- To study the effect of time and temperature on extraction of lycopene.

1.3 The Organization of the Study

The thesis is organized as follows. The second chapter is a literature review with details about Physiology and Ecology of Dovyalis Abyssinica (Koshim), solvents, lycopene structure and its properties. The third chapter is the electromagnetic wave theory, Maxwell's equations and the Beer–Lambert law are presented. The fourth chapter describes the materials and methods used in this research, including extraction and analysis methods. In the fifth chapter, the results and discussion of the thesis are presented. The sixth chapter describes the conclusions and future studies.

2. Literature Review

2.1 Physiology and Ecology of Dovyalis Abyssinica (Koshim)

Dovyalis is a genus of shrubs and small trees. Recent genetic evidence has shown the genus to belong to the family Salicaceae. The 15 species are native to Africa (Ethiopia south to South Africa) and southern Asia (India, Sri Lanka). Some are cultivated for their fruit. They are dense, thorny plants growing to 3–6 m tall, with sharp, 3–6 cm long stem spines in the leaf axils. Buds at the base of the spine produce clusters of alternately arranged simple ovate leaves 3–10 cm long. The flowers are inconspicuous, solitary or clustered, with no petals. They are dioecious, with male and female flowers on separate plants. The fruit is an edible, yellow to purple globose berry 2–4 cm diameter, containing several small seeds. They are very juicy and with an acidic flavor. Several species are grown for their fruit; Dovyalis caffra is popular in southern Africa, and *Dovyalis hebecarpa* in India and Sri Lanka. Some, notably *Dovyalis Abyssinica*, are also grown as ornamental plants and as hedges, where the spines are valued for deterring intrusion by livestock or burglars. The tropical apricot is a hybrid between *Dovyalis hebecarpa* and *Dovyalis Abyssinica* that was developed in Florida in 1953 and is cultivated for its fruit [14].

In Ethiopia this plant, usually found along river courses in humid lower highland forest and Juniperus and Podcarpus forest, of Moist and wet Wayne Degas and Dega Agro climatic zones in most regions, 1,700-3,000m [15]. The fruit is edible (eaten raw), but very acidic. A round berry about 2cm across, surrounded by the calyx, green and hairy at first then smooth orange-yellow melting juicy flesh at maturity, with edible sweet-sour flesh around the seeds. It is used for making jam, and added to porridge as a flavoring. Roots and stem are good for making soup. The roots also have medical properties with alleged effect on gonorrhea, bilharzias, stomach-ache and fever. The leaves provide fodder for livestock, primarily goats and sheep. Flowers attract bees and the plant is used as a live fence. The fruit is edible and is tart (containing a sweet or

savory filling) with aroma and flavor suggesting fresh apricot, but acidic; excellent for jelly [16].

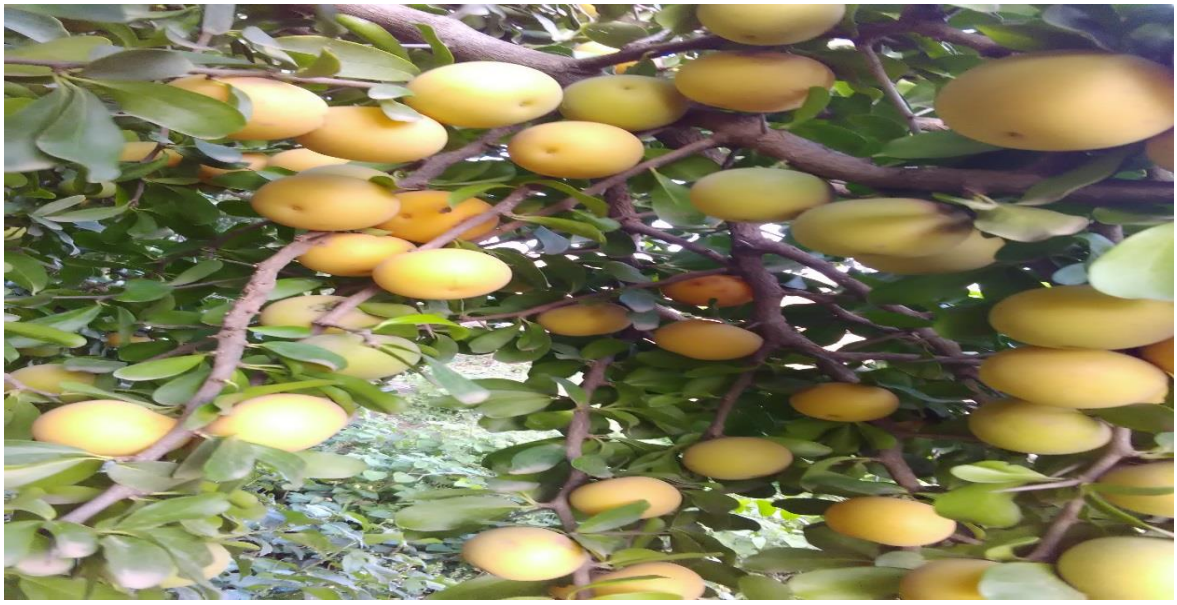

Figure 1: Dovyalis Abyssinica fruits found in ASTU campus

2.2 Lycopene

Lycopene incredible little molecule was first isolated in 1910, and the full molecule structure was discovered in 193. It is a phytonutrient. Phytonutrients are antioxidants found in plant life. These nutrients are not originally created by the human body, but rather produced by plants as a defense against environmental damage, such as pests, toxins and UV damage. Instead of allowing free radicals to run free within the plant, it creates various types of phytonutrients to protect itself [17]. We are subjected to a lot of dangerous environmental chemicals and other things, like prolonged sun exposure, that can cause free radicals to damage cells throughout our entire bodies as well. If you regularly eat plants of all colors, you can ensure you get enough phytonutrients to keep your body healthy . There are more than 25,000 different kinds of phytonutrients found in plant foods, and one of the top five important classes is carotenoids. Carotenoids both help plants absorb lights and protect chlorophyll from UV damage. Of the 600 different types, lycopene makes this top five as well. Lycopene

structure provides very special properties which are the basis of their varied functions in all kinds of living organisms. They are essential for photosynthesis and living in an oxygen atmosphere. The first study on the chemistry of tomato pigments dates about one hundred years ago [18]. Since then, the interest toward carotenoids has been rapidly growing; in the last fifteen years, an exponential increase in the number of scientific papers concerning lycopene pigments has been observed, contemporary with the recognition of the relevant physiological role played by natural antioxidants. Lycopene is one of the most extensively studied fruit carotenoids. One research study on the absorption of this nutrient by scientists at Ohio State University also discovered that not all lycopene molecules are created equally. In its original state, it has a molecularly linear formation (in a straight line). However, when testing the levels in the bloodstream, these researchers found that the lycopene molecules in the bloodstream were nearly all "bent." [19] .

Using heat while cooking with foods rich in lycopene causes their molecules to bend and, therefore, absorb more efficiently into your body. Fat is another necessary part of your body's process to absorb lycopene. Heat processing of lycopene-containing plant foods induces "cis" isomerization of lycopene, which has a beneficial effect on the absorption of lycopene. Lycopene in the natural "trans" form is poorly absorbed from fresh koshim fruits. In 2004, Donaldson reported that lycopene is less absorbed from fresh koshim than cooked koshims. The scientific literature lacks information on the effects of time and temperature on koshim extraction and characterization. Commonly used times (30-120 minutes) and temperature (20-80)^{0}C ranges which use koshim juices were experimental parameters in this study. It's worth the effort because this amazing phytonutrient has a host of impressive benefits [20].The benefits are:

[1]. One of the Most Powerful Antioxidants in the World

Antioxidants are so important for many reasons, especially in a world where processed food has eliminated most of what gives your body the ability to prevent and fight disease. Lycopene is an antioxidant that might as well be worth its weight in gold for the incredible things from which it protects your body. Fortunately, lycopene's antioxidant

capabilities can protect your body from the damage induced by pesticides. Studies show that it can protect your liver from common corruption by dichroic and also reverse or protect from destruction to your adrenal cortex by atrazine. Not only can lycopene help with infections, but its antioxidant properties even have been found to repair damage in the blood-spinal cord barrier in cases of spinal cord injury. This incredibly recent research is groundbreaking, as destruction of that barrier is part of what causes paralysis to people who suffer spinal cord injury [21].

[2]. Helps Prevent Cancer

Also related to its powerful ant oxidative qualities, lycopene plays a role in preventing and slowing several types of cancer, making any foods containing it cancer-fighting foods. Similar to its effect on breast and prostate cancer, lycopene also stunts the growth of renal cell carcinoma, the most common type of malignant kidney tumors. This also suggests it has a crucial part in prevention of this cancer. Another cancer-related treatment use of lycopene is its use against HIV infection, a major cause of uterine cancer. People who supplement their diets with additional lycopene recover faster from this cancer-causing infection than people with low lycopene levels [22].

[3]. Keeps Your Eyes Healthy

Lycopene also protects your eyes from oxidative stress that causes common eye diseases, making it one of the stronger eye vitamins you can consume. In experimentation with cataract development by the Department of Pharmacology at the All India Institute of Medical Sciences, it was discovered that lycopene may have the ability to prevent or delay cataracts in a large majority of cases. Lycopene also has great effect on the chemical processes that lead to age-related macular degeneration, the leading cause of blindness in the elderly [23].

[4]. Alleviates Neuropathic Pain

Neuropathy, or neuropathic pain, is a complex pain condition caused by nerve damage often accompanied by soft tissue damage. Many things can cause neuropathy, from alcoholism to limb amputation to diabetes. It sometimes occurs idiopathic ally, meaning there's no obvious cause. There are few effective treatments for neuropathy, although for some cases, treating the related disease (like diabetes or HIV/AIDS) can help

alleviate some of the pain. Increased lycopene dietary intake has powerful potential to help alleviate the chronic pain those with neuropathy suffer.

[5]. Good for Your Brain

Lycopene also has compelling neurological benefits. For example, treatment with lycopene has been explored as a possible option to delay the onset and progression of Alzheimer's disease by correcting cell corruption and protecting healthy cells [24].

[6]. Improves Heart Health

From start to finish, lycopene also proves to be a tool in protecting the heart from a large number of common conditions. It's one of the nutrients recommended for lowering high blood pressure levels. It prevents several cardiovascular diseases, such as coronary heart disease, myocardial ischemia (reduced blood flow to the heart caused by arterial blockages) and atherosclerosis [25]. Specifically related to research on coronary heart disease, tomato nutrition in particular was named as a determining factor in prevention. Overall, high levels of lycopene in the bloodstream are associated with a lower mortality rate in people with metabolic syndrome, a combination of disorders that lead to heart disease [26].

[7]. Keeps Your Bones Strong

Vitamin K and calcium aren't the only things that keep your bones strong. Lycopene also helps relieve oxidative stress in bones that cause brittle and weak bone structure. It slows the apoptosis (cell death) that causes bones to weaken and reinforces the cellular architecture of bones, keeping them healthier and stronger [27].

2.3 Lycopene: Structure and Properties

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. It is a carotenoid, an acyclic isomer of β-carotene. Lycopene is a highly unsaturated hydrocarbon containing 11 conjugated and 2 unconjugated double bonds.

It undergoes *cis-trans* isomerization induced by light, thermal energy, and chemical reactions [28]. The color of lycopene is due to its many conjugated carbon double bonds. Each double bond reduces the energy required for electrons to transition to higher energy states, allowing the molecule to absorb visible light of progressively longer wavelengths. Lycopene absorbs most of the visible spectrum. If lycopene is

oxidized (for example, by reacting with bleaches or acids), the double bonds between carbon atoms will be broken, cleaving the molecule into smaller molecules each double-bonded to an oxygen atom. Although C=O bonds are also chromophore, the much shorter molecules are unable to absorb enough light to appear colorful. A similar effect occurs if lycopene is reduced; reduction may saturate (convert the double bonds to single bonds) the lycopene molecule, diminishing its ability to absorb light. In the common variety of fruits and vegetables lycopene is found predominantly in the all-*trans* configuration, the most thermodynamically stable form and at concentrations of 3.1 to 7.7 mg/100 g of ripe fruit [29].

In human plasma, lycopene is present as an isomeric mixture, with 60% of the total lycopene as *cis* isomers. The molecular formula of lycopene ($C_{40}H_{56}$) was first determined when presented their study showing that lycopene is an isomer of the carotenes [30]. Published the chemical structure of lycopene, which was subsequently confirmed by identifying its degradation products following chromic acid oxidation [31]. The molecular weight of lycopene is 536.85, with the general structure being an aliphatic hydrocarbon with 11 conjugated carbons–carbon double bonds, which imparts coloration as well as fat- and lipid-soluble characteristics. Lycopene absorbs light in the visible range, and hexane solution of lycopene has maximum absorption λ_{max} at 472 nm and a differential emission wavelength [32]. As a result of the 11-conjugated carbon–carbon double bonds in its backbone. The acyclic structure of lycopene makes it more soluble in organic solvents such as chloroform, hexane, benzene, methylene chloride, and acetone and petroleum ether. Structure and physical properties of lycopene are shown in Table 1.

Table 1: Physical properties of lycopene. Adapted from: African Journal of Food Science, vol. 2(2), pp. 037-044, 2011.

Molecular Formula	$C_{40}H_{56}$
Molecular Weight	536.85
Melting point	172-175^{0}C
Crystal form	Long red needles separate from a mixture of carbon disulfide and ethanol
Powder form	Dark reddish brown
Solubility	Soluble in Chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil; insoluble in water, ethanol, and methanol
Stability	Sensitive to light, oxygen, high temperature, acids, catalysts and metal ions

Lycopene, whose structural formula is reported in Figure 2, is a fat-soluble carotenoid with 11 conjugated double bonds in the molecule, and it is a precursor of the β carotene.

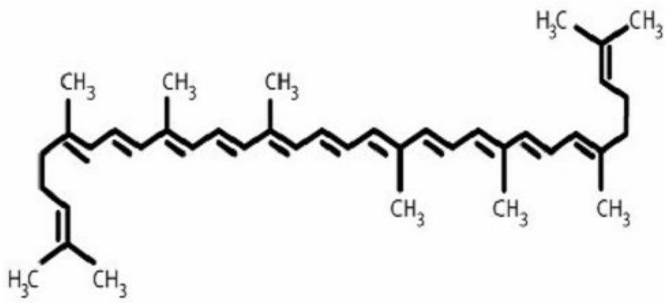

Figure 2: Structural formula of Lycopene. Adapted from: African journal of food science,Vol.2(2),pp.037-045

Lycopene can be easily degraded by atmospheric oxygen and by light and converted from the *all trans* to the *cis* forms that show a decreased biologic activity. For these reasons, it must be stored at a very low temperature (-70°C) away from the light and from the atmospheric oxygen. In the last years the importance of lycopene rapidly increased due to its pharmacological and anti-carcinogenic properties and antioxidant activity. Epidemiological studies indicate a protective effect of lycopene against some types of cancer, e.g., stomach and prostate cancer, so the demand for this molecule and for the carotenoids in general, is rapidly growing [33]. In the literature, different synthetic pathways for this molecule have been reported, but in all cases the synthesis of lycopene seems to be a very expensive and economically not convenient procedure. A great variety of food products of plant and animal origin, contain a wide range of lycopene.

While most studies focus on the high lycopene content in food nutrition, there are several foods high in lycopene content that you can introduce into your daily diet. Tomatoes, Gac (a Vietnamese fruit), Watermelon, Grapefruit, Guavas, Papaya, Asparagus, Red Cabbage, Mango, Carrots [34]. This molecule is also responsible for the color of these vegetables, and lycopene content in the range between 5.40-1500 mg/kg in this vegetables fruits (wet weight) and between 20- 30 mg/kg in vegetables peels as raw material were determined, respectively. The United States Department of Agriculture (USDA) has quantitated the lycopene content of various foods consumed by

Americans. Table 2 estimates the lycopene content of major food items found in the American diet.

Table 2:Lycopene content of various fruits and vegetables. Adapted from: African journal of food science, Vol.2

Food	Lycopene content(mg/100g)
Tomato Foods	
Tomatoes, raw	0.9-4.2
Tomatoes, cooked	3.7-4.4
Tomatoes sauce	7.3-18.0
Tomatoes paste	5.4-55.5
Tomatoes soup	8.0-10.9
Tomatoes juice	5.0-11.6
Catsup	9.9-13.4
Other fruits and vegetables	
Apricots, fresh	0.005
Watermelon, fresh	2.3-7.2
Papaya, fresh	2.0-5.3
Grapefruit, pink/red	0.2-3.4
Guava, raw	5.3-5.5
Vegetable, juice	7.3-9.7
Vietnamese fruit	5.5-6.5

It is worth noting that conventional methods for the extraction of lycopene from natural vegetal origin sources use great quantities of organic solvents and the extraction procedure is usually followed by a complex purification step [35]. According to (Allen et al., 2002), lycopene is not synthesized in the human body but fluctuations in the presence of serum greatly affects human health. Several studies found that lycopene has potent antioxidant activity. Levy et al. (1995) mention that lycopene is able to

inhibit the growth of endometrial cancer, breast cancer and lung cancer in cell cultures with higher activity than α and β-carotene. Lycopene is found able to inactivate free radicals and inhibiting compounds of hydrogen peroxide and nitrogen peroxide [36]. Naturally lycopene in plants is in the form of the trans configuration are thermodynamically stable form. With the warming effect can be turned into a form of trans isomers cis mono or poly. In general, the cis isomers are more polar, have a lower tendency to be crystalline, more soluble in oil and a hydrocarbon solvent, it is easier to join the lipoprotein or the structure of lipid subcellular, making it easier to enter the cell and are less stable than trans isomer. It is therefore necessary to streamline product innovation for the public consumption of lycopene. The results showing that consumption of tomato sauce more effective to improving bioavailability of lycopene in the body than eating fresh tomatoes. Furthermore, Allen et al. (2003) states Lycopene is found in the mucosal cells in larger quantities in individuals who consume fruits and vegetables. It shows that the presence of lycopene increases in processed fruit products than in fresh vegetables [37].

2.4 Solvents

The selection of the solvent to promote the extraction is a very important issue since it determines the degree of affinity to the chemical composition of the substances to be extracted. A solvent, by definition, is a substance that has the power to form a homogeneous solution with other substances. The solvent should be selective, with a high dissolving capacity, a low boiling point and low viscosity. The pigment polarity is a determining factor to choose the extraction solvent. When the lycopene is nonpolar hexane is an efficient solvent. Methanol is able to extract polar lycopene, while a nonpolar solvent such as hexane lead to crystallization. Methanol or a mixture of Methanol, acetone and other more a polar solvent is usually used for extraction procedures. A solution was used by Hart and Scott (1995) for lycopene analysis of raw, dry and heated vegetables and fruits. Table three shows valuable information on the selection of solvents for lycopene extraction as well as the molar extinction coefficient (L/mol.cm) of the lycopene which is used for the calculation of the lycopene concentration.

Table three Relative molar absorption coefficient ε(L/mol.cm) of lycopene in organic solvents [38].

Table 3: Relative molar absorption coefficient ε(L/mol.cm) of lycopene. Adapted from: Journal of Agriccultural and Food Chemistry, vol. 42 (2), pp. 208-213, 1993.

Solvent	Lycopene			
	λ_{max} nm	Absorption coefficient ε(L/mol.cm)	λ_{max} nm	Absorption coefficient ε(L/mol.cm)
Acetone	446	2540	452	2559
Methanol	442/444	2629	450	2540
Hexane	444	2589	448(450)	2592
Hexane	445	2589	453	2592

3. Electromagnetic Wave Theory

The description of theoretical background is briefly presented under this chapter. In the first two sections of this chapter the base for electromagnetic theory is reviewed. Starting with Maxwell's equations, the wave equations in material media are described by classical electromagnetic theory to show the various optical phenomena exhibited by medium like absorption and dispersion of the light. The last two sections of this chapter deal with the mathematical derivation of electromagnetic radiation through vacuum and material medium and the application of Beer-Lambert's law for testing the concentration of lycopene will be presented. At the end of this chapter the property and function of ultraviolet-visible spectroscopy will be provided [39].

3.1 The Electromagnetic spectrum or wave

Electromagnetic waves are waves which can travel through vacuum and material medium. Electromagnetic waves are created by the vibration of an electric charge. This vibration creates waves which has both an electric and magnetic component. An electromagnetic wave transports its energy through a vacuum at speed of 3.00×10^8m/s. However, the propagation of an electromagnetic wave through material medium occurs at net speed which is less than 3.0×10^8m/s. The mechanism of energy transport through a medium involves the absorption and reemission of wave energy by atoms of materials. When an electromagnetic wave impinges up on the atoms of a material, the energy of that wave is absorbed [40]. The absorption of energy causes the electrons within the atoms to undergo vibrations. After a short period of vibrational motion, the vibrating electrons create a new electromagnetic wave with the same frequency as the first electromagnetic wave. While these vibrations occur for only a short time, the delay motion of the wave through the medium. Once the energy of the electromagnetic wave is re-emitted by an atom, it travels through small region of space between atoms. When it reaches the next atom, the electromagnetic wave is absorbed, transformed into

electron vibrations and then re-emitted as an electromagnetic wave. While electromagnetic wave will travel at speed of c ($3x10^8$m/s) through the vacuum of inter-atomic space, the absorption and reemission process causes the net speed of the electromagnetic wave to be less than c [41].

3.2 Maxwell's and the Wave Equations

All the macroscopic aspects of the static and dynamics of the electromagnetic field in the presence of the material media are described by four Maxwell's Equations. These equations are the most fundamental description of electric and magnetic field. The differential form of Maxwell's equations is given by

$$\vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t}, \tag{1}$$

$$\vec{\nabla} \times \vec{H} = \frac{\partial \vec{D}}{\partial t} + \vec{J}, \tag{2}$$

$$\vec{\nabla}.\vec{D} = \rho, \tag{3}$$

$$\vec{\nabla}.\vec{B} = 0. \tag{4}$$

The four quantities which describe the electromagnetic nature of matter at a given point are the electric charge per unit volume (ρ), the electric dipole moment per unit volume or polarization (P), the magnetic dipole moment per unit volume or magnetization (M), and the electric current per unit area or current density (J). The material media responses are expressed by the material relation $\vec{D} = \epsilon_0\vec{E} + \vec{P}$, and $\vec{B} = \mu_0\vec{H} + \mu_0\vec{M}$, where $\vec{D}$ is the electrical displacement , $\vec{E}$ is the electric field strength , $\vec{H}$ is the magnetic field strength, $\vec{B}$ is magnetic induction and ϵ_0, μ_0 are permittivity and permeability in vacuum respectively. For non-magnetic and non-conducting media, the

volume density of electric charge and magnetization is zero, thus the Maxwell's equations expressed in Eqn. (1) to (4) take the form of the following [42]:

$$\vec{\nabla} \times \vec{E} = -\mu_0 \frac{\partial \vec{H}}{\partial t}, \tag{5}$$

$$\vec{\nabla} \times \vec{H} = \epsilon_0 \frac{\partial \vec{E}}{\partial t} + \frac{\partial \vec{P}}{\partial t}, \tag{6}$$

$$\vec{\nabla}.\vec{E} = 0, \tag{7}$$

$$\vec{\nabla}.\vec{H} = 0. \tag{8}$$

From the above Maxwell's equations, the general wave equation for electric field can be obtained by taking the curl of Eqn.(5) and time derivative of Eqn. (6) and eliminating the magnetic field [42].Thus,

$$\vec{\nabla} \times \left(\vec{\nabla} \times \vec{E}\right) + \frac{1}{c^2} \frac{\partial^2 \vec{E}}{\partial t^2} = -\mu \frac{\partial^2 \vec{P}}{\partial t^2} \tag{9}$$

By general identity of vector calculus, the first left hand side of Eqn.(9) can be expressed as follows:

$$\vec{\nabla} \times \left(\vec{\nabla} \times \vec{E}\right) = \vec{\nabla}\left(\vec{\nabla}.\vec{E}\right) - \vec{\nabla}^2 \vec{E} \tag{10}$$

Substituting Eqn. (10)in Eqn.(9) the general wave equation for electric field becomes

$$\vec{\nabla}^2\vec{E} - \vec{\nabla}(\vec{\nabla}.\vec{E}) - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = \frac{1}{\epsilon_0 c^2}\frac{\partial^2\vec{P}}{\partial t^2} \tag{11}$$

$$\mu_0\epsilon_0 = \frac{1}{c^2}. \tag{12}$$

Eqn. (12) represents the speed of light in vacuum, on the other hand Eqn.(11) Is a Partial differential equation with the term on the right side a source term shows the presence of polarization within the medium. The way in which the propagation of light is affected by the source is revealed by the solution of the wave equation when the source term is included. For non-conducting (a neutral isotropic dielectric media) the polarization term is important. This term leads to an explanation of many optical effects including dispersion and absorption. For solenoid field Eqn.(11) Becomes

$$\vec{\nabla}^2\vec{E} - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = \frac{1}{\epsilon_0 c^2}\frac{\partial^2\vec{P}}{\partial t^2} \tag{13}$$

Thus, Eqn. (14) is the fundamental electromagnetic wave equation. In order to make any use of it, it is necessary to specify the polarization. However, this cannot be done solely with in the frame work of Maxwell's equations, since polarization is the property of the material media. We need to know how a dipole moment density is produced in the medium. First however, this section would be completed with a discussion of the solution of the Maxwell's equation in vacuum, where a wave equation (13), does not contain the polarization term [42].

$$\vec{\nabla}^2\vec{E} - \frac{1}{c^2}\frac{\partial^2\vec{E}}{\partial t^2} = 0 \tag{14}$$

For a wave propagating in the z-direction in free space the solution of wave equation becomes

$$\vec{E}(\vec{z},t) = \vec{\epsilon}E_0 \cos(wt - kz) \tag{15}$$

Where the wave vector and phase velocity are defined as follows

$$k_0^2 = \frac{\omega_0^2}{c^2} \tag{16}$$

$$\upsilon_p = \frac{\omega}{k_0} = c \tag{17}$$

3.3 Ultraviolet Visible Absorption Spectroscopy (UV-Vis)

The optical absorption is resulted from the interaction of the material and light. When the frequency of light is in resonance with energy difference between states where the transition allowed or partly allowed by selection rules a photon is absorbed by the material. This results in a decrease of transmission or an increase in absorbance of light passing through the sample. By measuring the transmission or absorbance of sample as a function of the frequency of light, one can obtain a spectrum characteristic of the material. Ultraviolet/visible spectroscopy involves the absorption of ultraviolet light by a molecule causing the promotion of an electron from a ground electronic state to an excited electronic state. It is the study of how a sample responds to light. When a beam of light passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample. Most dissolved molecules absorb light in the visible or UV range at specific wavelengths. The absorption of light (Fig. 3) is a physical process where the amount of absorbed light depends on the concentration of the substance *c*, the thickness of the liquid layer *d* and a specific absorption coefficient α at a defined wavelength λ [43]. Different terms define the process of light absorbance.

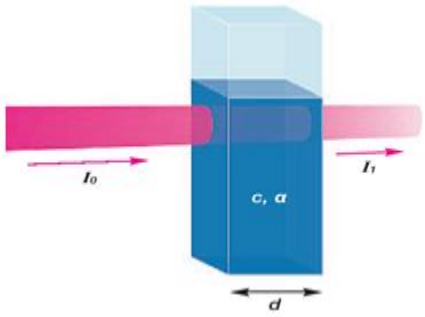

Figure 3: Light transmission through a homogeneous material. Adapted from: Bio Techniques,Vol.22

Transmission and transmittance are often used as synonyms. UV/Visible transmittance spectroscopy is based on transmission of light through matter and selective absorption at specific wavelengths, based on the electronic transitions in the matter. As per the Beer Lambert law, if the radiation of a particular wavelength passed through a homogeneous solution in a cell, the intensity of the transmitted radiation reduces by an amount that depends upon the thickness and concentration of the material. The UV/Visible spectra of lycopene have three major absorption bands with absorption maxima at 446, 472 and 503 nm or with extinction coefficients, ε_{max} = $1.206x10^5$, $1.850x10^5$ and $1.690x10^5$ M-1cm-1, respectively. These bands were observed when lycopene was dissolved in hexane [44]. In conjugated polyenes like lycopene and other carotenoids, from the ground state to excited state transitions of energy levels occur upon absorption of light energy of a particular wavelength. Any charge around a molecule will alter the energy difference between ground state and excited state. Therefore, an aqueous environment (being polar), reduces the ground state to excited state transition energy due to a small decrease in energy of the excited state orbitals, while the ground state orbital energies remain unchanged. This results in less energy for ground state to excited state transitions. Thus, a lower energy (longer wavelength) light can cause this transition, resulting in shift of absorbance band towards longer wavelengths in aqueous environment, called the colored shift. Therefore, the absorbance peaks of lycopene and other carotenoids are colored shifted in aqueous environments (such as in fruit tissues) as compared to the peaks of lycopene in hexane [45].In spectroscopy, the physical phenomenon of light transmission through a sample can be

described by the fraction of light at a specific wavelength λ that passes through the sample. If I_0 are the intensity of the incident radiation and $I_t$the intensity of the transmitted radiation, then the transmittance T can be defined as:

$$T = \left({}^{I_t}/_{I_0}\right) \tag{18}$$

In parallel, the transmittance can be described by the light absorbance A_λ :

$$A_\lambda = -log_{10}\frac{I_t}{I_0} \tag{19}$$

The higher the optical density of the sample, the higher the absorption and the lower is the transmission of light. Often the transmission is given as a percentage. Although the absorbance or optical density does not have true units, it is customarily reported in absorbance units (a.u). The absorbance or optical density depends on the wavelength λ and the path length *d* (Fig. 3). In order to be able to compare measurements the optical density is often calibrated to a defined path length, typically 1 cm. Optical densities above 1.0 should be avoided in a sensitive measurement as even large differences in the optical density readouts represent only minor modifications in the transmission. It is recommended to dilute samples with a high optical density in order to achieve results below 1.0 [46].

3.3.1 Beer- Lambert's law

The absorption of light in a sample solution is dependent on the concentration *c* of the dissolved molecule, the specific molar extinction coefficient α at a defined wavelength λ and the path length *d*. With known extinction coefficient and path length, the concentration of a sample can be determined by using the Beer-Lambert's equation (20). As well, Beer-Lambert's law can be used to determine the molar extinction coefficient (21).

$$A_\lambda = -log_{10}\frac{I_t}{I_0} = \alpha_\lambda cd \tag{20}$$

C Concentration of the dissolved absorbing molecule. Typically defined as $[mol.dm^{-3}]$ or $[mol.l^{-1}]$,

α_λ Molar extinction coefficient which is for the absorbing molecule a specific dimension at a defined wavelength λ.The dimension of the extinction coefficient is $\left[\frac{dm^3}{mol.cm}\right]$ or $\left[\frac{l}{mol.cm}\right]$

d Distance light has to pass through the material $[cm]$.Determination of the molar extinction coefficient [47]

$$\alpha_\lambda = \frac{A_\lambda}{c.d} \tag{21}$$

A plot of the sample absorbance against the concentration of the absorbing substance should give a straight line. In practice, a calibration curve is prepared by plotting the absorbance of a series of solution of samples as a function of their concentration. If the absorbance of an unknown sample is then measured, the concentration of the absorbing component can be assessed from this graph. Another consequence of the Beer–Lambert law is that it is possible to change the path length to affect the absorbance. This can be useful where lower detection limits are required as the path length can be increased (longer path length cuvettes are available) or, where the absorbance is too high to be measured on the instrument, the path length can be reduced. Alternatively, it is possible to reduce the absorbance by diluting the sample, but one has to take care when dealing with biologically active samples, particularly enzyme-based solutions, as this may have a profound effect on the activity [48]. Spectroscopic techniques are non-destructive and generally require small amounts of sample. The electromagnetic spectrum is composed of energy that may behave both as a particle and as a wave. When we describe this energy as a particle, we use the word photon. When we describe this energy as a wave, we use the terms frequency (v) and wavelength (λ) [49].

Frequency is the number of wave troughs that pass a given point in a second and wavelength is the distance from one crest of a wave to an adjacent crest. Frequency and

wavelength are inversely related, according to the equation E = h v = h c / λ. Therefore, as frequency increases, wavelength decreases. The UV/VIS range of the electromagnetic spectrum covers the range 190–700 nm (most instruments are capable of measuring at longer wavelengths than this, depending on their detector type). For clinical analysis this is useful, as water (most assays are in aqueous solution) is almost completely transparent in this region. Most clinical assays are concerned with quantitation rather than identification (there are more powerful techniques available to perform the latter) as absorption spectra tend to be feature less – they lack the fine structure which is found. The most important principle in absorption analysis is the Beer–Lambert law [50]. Spectrophotometer is a quantitative measurement method. This uses the reflection or transmission properties of a material as a function of wavelength. Lycopene was determined according to method developed by Fish et al. (2002). The lycopene content was estimated as mg/100g fruit koshim based on the following Eqn.(22) [51]

$$\mathrm{Lyc}\left({}^{\mathrm{mg}}/_{100\mathrm{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W} \qquad (22)$$

Where,

A_λ = is the absorbance of the solvent layer containing lycopene

α_λ = molar extinction coefficient of lycopene in solvents, L/mol.cm

536.9= is the molecular weight of lycopene, g/mole

v = Volume of the solvent, ml

w = weight of koshim, *g*

d= distance light has to pass through the material, cm

4. Materials and Methods

In this chapter, the materials and methods of the thesis were and to isolate lycopene from koshim juice (*Dovyalis Abssinica Warb)*, solvent extraction was performed on koshim juice. The first section of this chapter describes the various chemicals, samples and instruments used to carry out this research. Moreover, the basic components of UV-Vis Spectrophotometry and working principles of the optical systems were discussed in details. In the second section of this chapter the different methods of extraction of lycopene were presented.

4.1 Materials

In this section the different chemicals, laboratory apparatus, and the components of UV-Vis Spectrophotometry with its working principles were discussed.

4.1.1 Chemicals and Samples

This chapter summarizes the materials and methods used in this research. The experimental work was divided into the following sections:

- Selecting solvents and extracting lycopene
- Isolating and characterizing the extracted lycopene

4.1.1.1 Selecting Solvents

The solvent used for extracting lycopene should have the following characteristics:

- Low cost.
- Low toxicity and considered safe for food use.
- Stable during the extraction process.
- Low detrimental effect on the environment.
- Low flammability (for safety) [52].

The following solvents or mixture of solvents were used to identify the best solvent for extracting lycopene from koshim juice. All solvents were analytical grade and purchased from Ranchem Industry and Trading Pvt, Addis Ababa, Ethiopia.

- n-Hexane 95%
- ethanol 98%
- methanol 99%
- acetone 99.9%
- Carbon tetrachloride
- Benzene
- distilled water

4.1.2 Apparatus and Instrument

The apparatus used in the experiment were Glass beakers, Volumetric flasks, Magnetic stirrer with hot plate, measuring cylinders, pipettes, Electronic beam balance, Universal Drying Oven, Spatula, Tong, Sample holder, funnel, separator funnel, filter paper, Rota evaporator.

4.1.3 Basic Components of UV-Vis Spectrophotometry and Working Principles

The instrumentation for measuring the absorption of ultraviolet or visible radiation are made up of the following components as; - *Sources (*UV and visible), Monochromator, sample container (Cuvette), Detector and Signal processor and Read out.

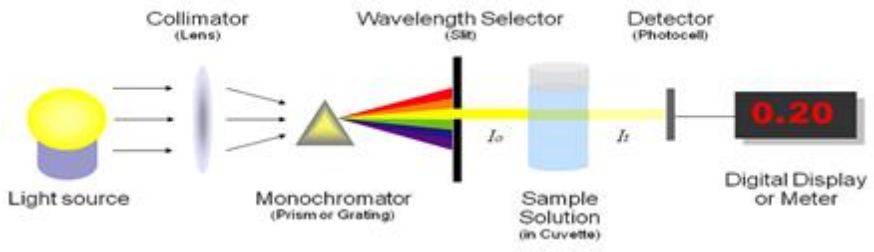

Figure*4*: Shows the optical components of spectrometry and its working principles. Adapted from: academic Journals, Vol.7

There are two types of radiation sources. For the UV region, the radiation source is deuterium discharge lamp that emits polychromatic UV radiation which can then be filtered into monochromatic UV radiation. For the visible region, the radiation source is tungsten filament. The monochromator or wavelength selector disperses the light from radiation source into its separate wavelength. The radiation of only a particular wavelength leaves the monochromator through an exit silt. It has double monochromator which offers the advantage of low levels of stray light that is significant to measure a high value of absorbance using 1cm cuvette. The monochromatic light that emerges from exit slit is pulsed by a chopper and split into sample and reference beam by the beam splitter. A reference beam passes through a sample holder or a quartz cuvette that contains only a solvent. The sample beam passes through a quartz cuvette that contains a sample solution. The radiation beams that passes through the detectors is amplified by difference amplifier and finally reaches the recorder, where the results are recorded digitally in a personal computer attached to a spectrophotometer. The investigated compound was measured in the UV-Visible spectrum range of 200 to 600nm [53].

4.2 Methods of extracting lycopene

Solvent extraction was done using koshim fruit. During analysis, the sample was protected from heat, light and moisture uptake. Under this the sample preparation and different extraction methods with different solvents were performed [52].

4.2.1 Koshim juice preparation

A800*g* fresh matured koshim was collected from ASTU campus and stored at 4 ^{0}C for 48 hours (two day) in chemistry laboratory. After collecting, the fruits were washed under tap water and stored in bamboo basket and dried on table by wind. The matured colored fruits were cut in to small pieces with sharp knives and diluted 1:1(w/v)/15 g: 15 ml/ in distilled water before blending. The colored fruits samples of 800*g* was divided in to three (400 g, 200 g and 200 g) and homogenized with distilled water in electric juice blender then covered by aluminum foil. All samples were pureed using Electric Homogenizer to produce uniform slurry with particles. All samples were

protected against heat and were covered with aluminum sheet to prevent the exposure to light. This process was done before the methanol, hexane and tri-mixture extraction solvents were added. The homogenized samples were centrifuged at 400 g, 200 g, and 200 g for 30 minutes at 4°C before being filtered under suction. The sample is stored at -20°C fridge until use within a week [54].

Figure*5*:Blending of Dovyalis fruits(koshim)

4.2.2 Extraction and characterization of lycopene using methanol as solvent

Twenty-seven-gram koshim Juice was weighed into a 100-mL flask and placed on a magnetic stirrer hot plate at 20^0C for 10minutes. Then 49ml methanol was added to the cooled flask to dissolve lycopene. This mixture was immediately shaken vigorously to prevent the formation of hard lumps. After 2 hours, the thick suspension was filtered; the dark red cake was shaken for another 10 min with 25ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetrachloride phase was transferred to a separator funnel; added 5ml of water and shake well. After phase separation, the carbon tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1 ml of boiling methanol was added in portion, then crystals of crude lycopene were appeared immediately and the crystallization was completed by keeping the liquid at room

temperature and ice bath, respectively. The crystals were washed 5 times using benzene and boiling methanol. Long, red lycopene prisms were observed under the microscope with some colorless impurity substances. After complete evaporation of solvent, the residue was dissolved in 2 ml benzene. After recrystallization using boiling methanol, no colorless substances observed. Crystalline lycopene is not isomerized but has a tendency to autoxidation (or air oxidation), especially in light, so it was kept in dark evacuated glass tubes prior to use. Identification of the optical properties of the isolated lycopene was done using UV-Vis spectroscopy [55].

Figure *6:* When the residue part of the sample separated using separating funnel and extracted lycopene

4.2.3 Optimization of extraction of lycopene, using hexane as solvent

Hexane was used as extraction solvent and optimum extraction of dry koshim was determined. The lycopene content of the extracts was characterized by spectrophotometer. Sample (0.6g powder) was weighed in a beaker, 5 mL acetone solution, 5 mL methanol and 10 mL hexane was added. The beaker was placed in a bowl of ice on a magnetic stirring plate, stirred for 10 min and added 3 mL distilled water. It was shaken for 5 min on ice and heated at 25^0C for 20 min to allow the separation of

both phases. The upper layer containing lycopene was isolated by means of a pipette and collected in a test tube. The tubes containing lycopene extracts were covered with aluminum foil and stored in the refrigerator until further analysis [55], [56].

4.2.4 Optimization of Acetone - Ethanol –Hexane extraction of lycopene

Two tri-mixture (acetone: ethanol: hexane) were used as extraction solvent, using the optimum extraction time and temperature. The lycopene content of the extracts was characterized by spectrophotometer.

4.2.4.1 Extraction of lycopene at 50^0c

100 ml of solvent was also carried out with a tri-mixture of 50:25:25 (v/v/v) hexane: acetone: ethanol to extract lycopene from the koshim juice. 100 ml of tri-mixture solvent was added to 200 g (wet weight) samples of koshim juice in conical flasks and mixed with a stirrer for 30-min at 50°C. Then stand for 10-min in a cooling water bath, the solution was separated into two layers. Separating funnel was used to separate these layers. Upper organic layer contains lycopene [57].

Figure 7: Stirring the blend with magnetic stirrer and separating with separator funnel

4.2.4.2 Extraction of Lycopene at 80^0C

Extraction was performed according to the method of Fish et al., 2002 with minor modifications. Fresh unpeeled Koshim were chopped and homogenized using an electric blender. Koshim juice (200 g) was weighed into a 250-mL flask wrapped with aluminum foil to protect from light exposure and placed in an oven set at 80^0C for 1 hour. Ternary mixture of hexane, acetone and ethanol in 2:1:1 v/v was added to the cooled flask to dissolve lycopene. The mixture was stirred with a magnetic stirrer for 1 hour at 4^0C. A 30-mL distilled water was added and stirred for further 30 min. The solution was left undisturbed for layer separation. The upper organic nonpolar layer containing lycopene was separated, and the hexane was allowed to evaporate under reduced pressure at room temperature, leaving behind solidified orange-red droplets of lycopene. The lycopene was kept at 4^0C refrigerator until use [58].

4.3 Analysis of extracted lycopene

The data of UV-Vis from Addis Ababa University Chemistry Laboratory was organized and summarized by appropriate methods. This Data was analyzed by using Origin 8 computer software program and Microsoft Excel. The graph of the extracted lycopene and its optical properties were discussed. The spectrophotometric analysis allowed to identify the lycopene molecule, by comparing the maximum peak absorbance from the extracted lycopene with the relative values reported in literature and some Journals.

5. Results and Discussion

In this chapter, the results and discussion of the thesis are presented. In the first two sections of this chapter the molar extinction coefficients, absorption cross-section of lycopene in Methanol, Hexane-Acetone-Ethanol and Hexane and the contents of lycopene in koshim calculated by Beer-Lambert's law were presented. UV–Vis spectra of the extracted lycopene were measured in organic solvents over a scanning range of λ_{max} of the compounds were determined.

5.1 Extraction and isolation of lycopene with Methanol

Lycopene was extracted from koshim by simple liquid-liquid extraction using as minimum organic solvent as possible. The main problem was purification of the extract. One simple and easy to use purification methods, namely recrystallization. Crystals obtained by each method was observed. Presence of colorless substances indicated the extent of impurity in crystals obtained by three times recrystallization. Recrystallization method gave completely pure lycopene crystals as no colorless substances were seen. Crystals obtained by triple recrystallization showed a few characterized lycopene peaks. The amount of pure lycopene was also good compared to those obtained from other studies.

5.1.1 UV-Vis analysis of lycopene with a methanol extraction method

The lycopene in the extracting solvent by methanol was determined by UV-Vis spectrophotometric measurement at room temperature. A double-beam UV-Vis and Quartz cells of 1-cm path length were used. Fig 8 shows the UV-Vis absorption spectrum of lycopene in methanol extracting in the wavelength regions of 350-550 *nm* at room temperature. In this region UV-Vis spectrum of lycopene shows four peaks located at 374 nm, 397 nm, 444 nm and 450nm (figure 8). These are the maximum wave lengths of lycopene in methanol extraction. The peak absorbance of lycopene observed in methanol at these wavelengths are quite similar with one reported by Mohamed et. al, 2013. Their Spectral Data for lycopene in UV-Vis methanol extraction are given by 293, 360, 442, and 456 [59]**.** Optical analysis of lycopene done by UV-

Visible spectrophotometer [60]. UV-Vis Spectrum is shown in figure 8, the maximum wavelength is 444 nm and 450nm, which is the maximum wavelengths of lycopene in this methanol extraction of koshim.

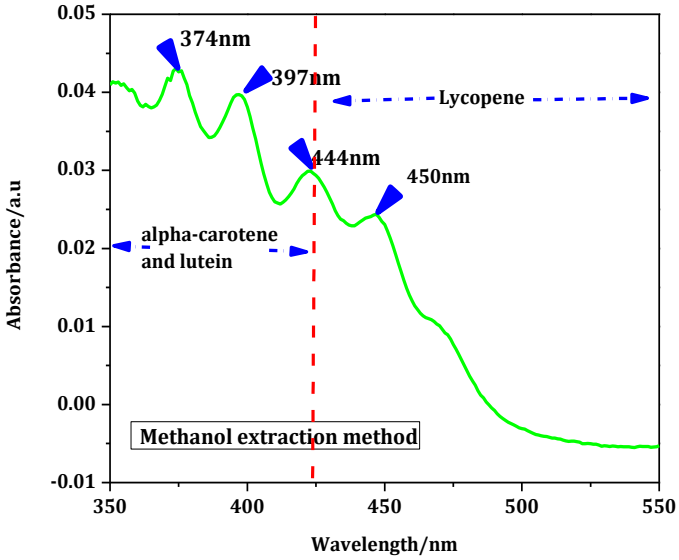

Figure *8*: UV-Vis Absorption Spectrum of lycopene in methanol extraction sample

By analyzing the absorption spectra of lycopene presented in the literature it is found that alpha-carotene and lutein were the most important contribution in the absorption spectrum bands at 374 nm and 400 nm. Lycopene in methanol extraction is the most important contribution to the absorption band at 444 nm and 450 nm. A simple liquid-liquid extraction method was employed to extract lycopene in minimum organic solvent. This test initially helps us to identify lycopene in the residue. We got colored crystals shows that they were crystals of lycopene because literature tells that impurity gives colorless crystals [61].

The lycopene content was estimated as mg/100g fruit flesh based on the above Equation(22).Lycopene concentration in the methanol extracts were calculated as follows:

a)

$$\mathrm{Lyc}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$Lyc\left(\frac{mg}{100g}\right) = \frac{A_{444} \times 536.9 \times V}{\alpha_{444} \times d \times W}$$

$$Lyc\left(\frac{mg}{100g}\right) = \frac{0.023866 \times 536.9 \times 99}{2629 \times 1 \times 27}$$

$$Lyc\left(\frac{mg}{100g}\right) = 0.1787 mg/100g$$

b)

$$Lyc\left(\frac{mg}{100g}\right) = \frac{A_{\lambda} \times 536.9 \times V}{\alpha_{\lambda} \times d \times W}$$

$$Lyc\left(\frac{mg}{100g}\right) = \frac{A_{450} \times 536.9 \times V}{\alpha_{450} \times d \times W}$$

$$Lyc\left(\frac{mg}{100g}\right) = \frac{0.0234735 \times 536.9 \times 99}{2540 \times 1 \times 27}$$

$$Lyc\left(\frac{mg}{100g}\right) = 0.1819 mg/100g$$

5.1.2 UV-Vis Calibration analysis of lycopene with a methanol extraction method

A calibration for lycopene was also done by UV-Vis spectra. The absorbance of lycopene standard was linear in the concentration range 0.001-0.01mg/100g [62]. Figure 9 could be represented by the following equation: Absorbance = 0.021 lycopene mg/100g +0.019, R^2 =0. 956. When the calibration curve extended to 0.28mg/ 100g, the relationship was also linear (Figure11). Absorbance = 0.021 lycopene mg/100g, R^2 = 0.956.

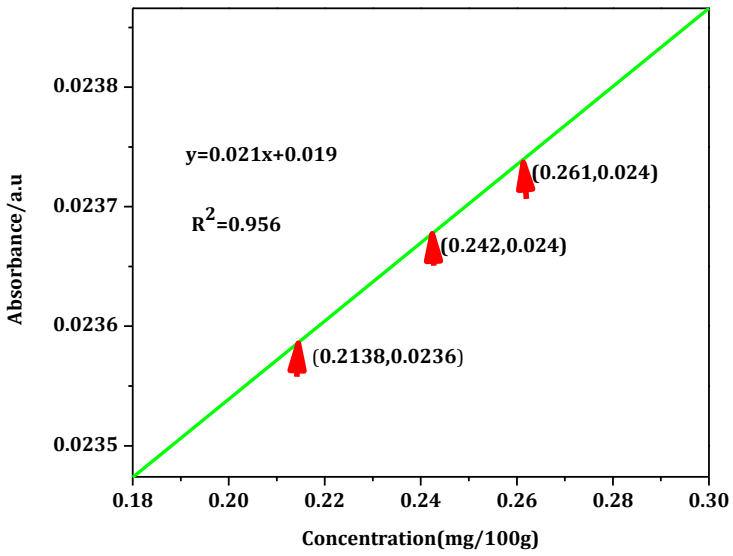

Figure 9: UV-Vis Calibration curve for lycopene in methanol extraction sample

5.2 Extraction and isolation of lycopene with Hexane method

UV-Vis spectrum of lycopene shows three peaks located at 424nm, 446nm and 472nm (Figure 10). Lycopene spectrum in the extracting solvents was determined by spectrophotometric measurements at room temperature in the wavelength range of 400–550 nm. The absorbance of the extract in hexane layer was measured in a 1-cm path length quartz cuvette at 472 nm. Absorbance at 472 nm has been selected for lycopene quantification to avoid interference from other carotenoids, which may be present in the sample [55]. The absorbance peaks of lycopene spectrum were at 446 nm and 472 nm. These absorbance peaks of lycopene match with the lycopene peaks reported in literature (Davies, 1976). Absorption determination for lycopene was made by using Spectrophotometer UV-VIS Perkin Elmer at Addis Ababa University Chemistry Laboratory. Lycopene in the koshim samples was extracted with hexane: methanol: acetone (2:1:1) (v/v) mixture following the method of Sharma and Le Maquer [55].

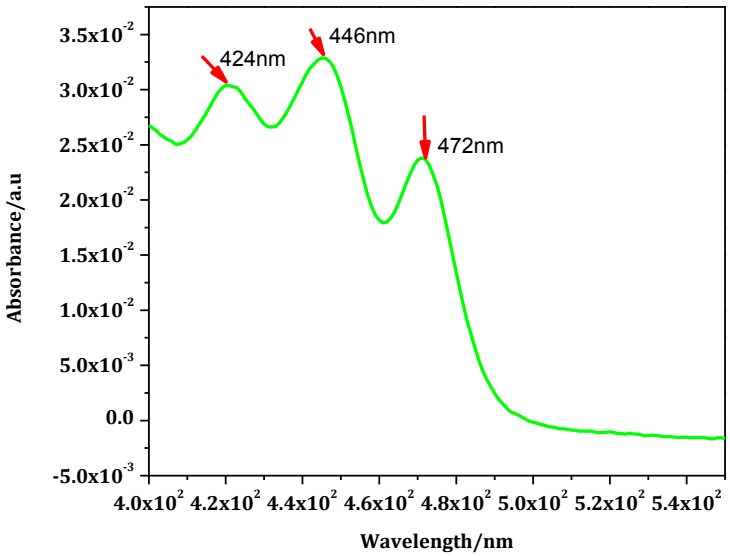

Figure *10*: UV-Vis Absorption Spectrum of lycopene in hexane extraction sample

5.2.1 UV-Vis analysis of lycopene with hexane extraction method

The extract was then analyzed for its lycopene content using UV-Vis spectroscopy technique. The data were recorded and the lycopene content (mg/100 g) was calculated as follows:

a)

$$\mathrm{Lyc}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\right) = \frac{\mathrm{A}_{\lambda} \times 536.9 \times \mathrm{V}}{\alpha_{\lambda} \times \mathrm{d} \times \mathrm{W}}$$

$$\mathrm{Lyc}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\right) = \frac{\mathrm{A}_{446} \times 536.9 \times \mathrm{V}}{\alpha_{446} \times \mathrm{d} \times \mathrm{W}}$$

$$\mathrm{Lyc}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\right) = \frac{0.032836 \times 536.9 \times 20}{120600 \times 1 \times 0.6}$$

$$\mathrm{Lyc}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\right) = 0.0487\mathrm{mg}/100\mathrm{g}$$

b)

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_{472} \times 536.9 \times V}{\alpha_{472} \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{0.023710 \times 536.9 \times 20}{185000 \times 1 \times 0.6}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = 0.0229\text{mg}/100\text{g}$$

Where A_{472}, A_{446} are the absorbance of the hexane layer containing lycopene. The Molecular weight of lycopene is 536.9 g/mol, molar extinction coefficient for lycopene in hexane is ($1.85*10^5$, $1.206x10^5$)L/mol.cm. Most of the other extinction coefficients reported for lycopene are subsequently within this range. Working with values of lycopene content expressed in terms of mg/kg (or, the equivalent, mg/100g) makes data handling easier and is a unit of concentration commonly used in the literature.

5.2.2 UV-Vis Calibration analysis of lycopene with hexane extraction method

A plot of the sample absorbance against the concentration of the absorbing substance should give a straight line (Figure 11). A calibration curve is prepared by plotting the absorbance of a series of solution of samples as a function of their concentration. The absorbance of lycopene standard was linear in the concentration range 0.001 0.01mg/100g [62]. Figure 11 could be represented by the following equation: Absorbance = 0.6675 lycopene mg/100g +0.0029, R^2 = 0. 9207. The calibration curve linearity increases, the accurate of the relationship also increases (Figure11). Absorbance = 0.6675 lycopene mg/100g, R^2 = 0.99207.

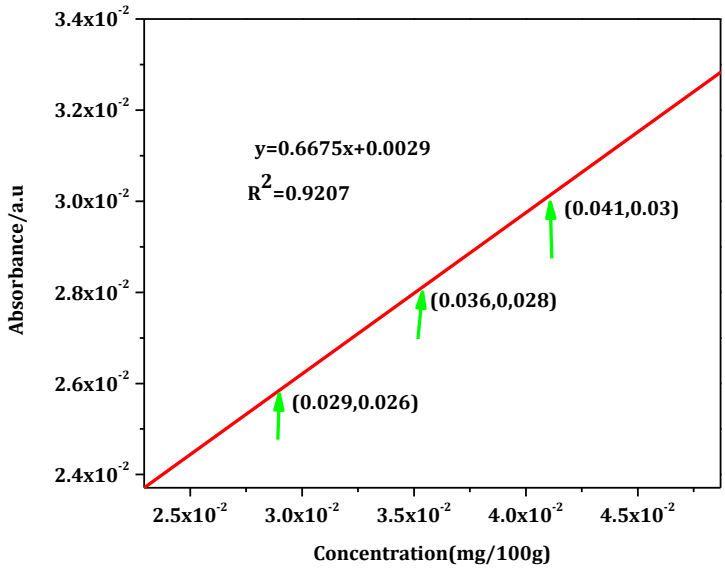

Figure *11*: UV–Vis Calibration curve for lycopene extracted in hexane extraction sample

5.3 Optimization of Acetone - Ethanol –Hexane extraction of lycopene

The total lycopene extraction from koshim was investigated to find the optimum extraction parameters. Solvent extraction method and UV-visible spectrophotometer were used for extraction and lycopene determination, respectively. Extraction duration and extraction temperature are assumed to be the most important factors affecting solvent extraction for determination of total lycopene. Two extraction temperatures of, 50°C and 80°C and two extraction time of 30 min and 60min were employed to choose the most suitable temperature and time for extraction of total lycopene from koshim fruit. The results are shown in Figures 12 and 14. The effects of time and temperature interactions were studied. In the present procedure of extraction, an initial of temperature at 80^0C for 1h given to koshim juice enhanced the lycopene yield. This is probably because heating breaks down the cell walls, disrupts the chloroplast membranes and reduces the cellular integrity, thus rendering lycopene more accessible to extraction (Shi and Le Maguer, 2000; Van den Berg et al., 2000).

5.3.1 UV-Vis analysis of lycopene extracted at 80^{0}C

UV-VIS spectrum of lycopene shows four peaks located at 376nm, 395nm, 445nm and 453nm (Fig 12). Lycopene spectrum in the extracting solvents was determined by spectrophotometric measurements at room temperature in the wavelength range of 350–550 nm. The absorbance of the extract in tri-mixtures layer was measured in a 1-cm path length quartz cuvette at 453 nm. Absorbance at 453 nm has been selected for lycopene quantification to avoid interference from other carotenoids, which may be present in the sample. Absorption determination for lycopene content was made by using Spectrophotometer UV-VIS Perkin Elmer Lambda 900 at Addis Ababa University Chemistry laboratory. In the present study, the isolated lycopene was identified and characterized using UV–Vis spectroscopy. Lycopene in tri-mixtures had the characteristic peaks of lycopene absorbance at 445 and 453nm. The amount of lycopene was quantified at 453nm to avoid interferences from other carotenoids present in the sample [58].

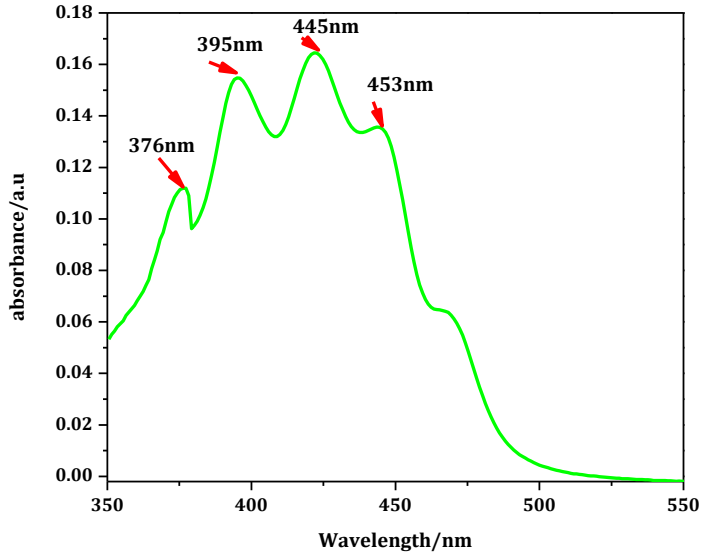

Figure *12*: UV-Vis Absorption Spectrum of lycopene extracted at 80^{0}C

The lycopene content was estimated as mg/100g fruit juice of koshim on the above Eqn.(22). The lycopene concentration was calculated using its specific extinction coefficient of 2589 in hexane at 445 nm and 2592 at 453 nm in literature review. The lycopene concentration was expressed as mg/100g product.

a)

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_{445} \times 536.9 \times V}{\alpha_{445} \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{0.13616437 \times 536.9 \times 100}{2589 \times 1 \times 200}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = 0.14118762\text{mg}/100\text{g}$$

b)

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_{453} \times 536.9 \times V}{\alpha_{453} \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{0.103064992 \times 536.9 \times 100}{2592 \times 1 \times 200}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = 0.106743004\text{mg}/100\text{g}$$

Several studies have demonstrated that extraction solvents affect the extraction of lycopene. Amongst all the solvents tried, hexane/acetone/ethanol mixtures in 2:1:1 resulted in the maximum yield of lycopene. In the extraction procedure followed in the present study, the lycopene yield varied from (0.023 to 0.23) mg/100g of Koshim. The results are not in complete agreement with some previous literature results, which may be due to the differences in the koshim variety, heating time and patterns, or to some other conditions applied. Studies characterizing total lycopene concentration from

koshim revealed that it contains carotenoids as well as noncarotenoid components. Lycopene is the primary constituent present in the carotenoid fraction [63].

5.3.2 UV-Vis Calibration analysis of lycopene extracted at 80^{0}C

If the absorbance of an unknown sample is then measured, the concentration of the absorbing component can be assessed from this graph(Figure13). The absorbance of lycopene standard was linear in the concentration range 0.001-0.01mg/100g [62]. Figure 13 could be represented by the following equation: Absorbance = 0.960 lycopene mg/100g +0.005, R^2 = 1. Absorbance = 0.96 lycopene mg/100g, R^2 = 1.

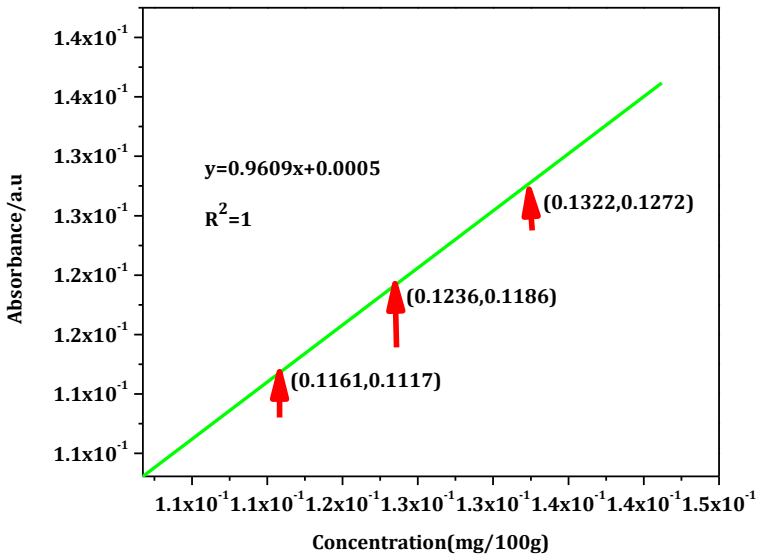

Figure 13: UV–Vis Calibration curve for lycopene extracted at 80^{0}C in tri-mixture

5.3.3 UV-Vis analysis of lycopene extracted at 50^{0}C

The hexane/ ethanol/acetone were used as extraction solvent, using the optimum extraction time at 30min and temperature at 50^{0}C. The lycopene content of the extracts was characterized by spectrophotometer. Spectrophotometer is a quantitative measurement method which uses the reflection or transmission properties of a material as a function of wavelength. Spectrophotometer is much less expensive. The UV-VIS spectrum of lycopene shows four peaks located at 376nm, 400nm, 445nm and 450nm (Fig 14).

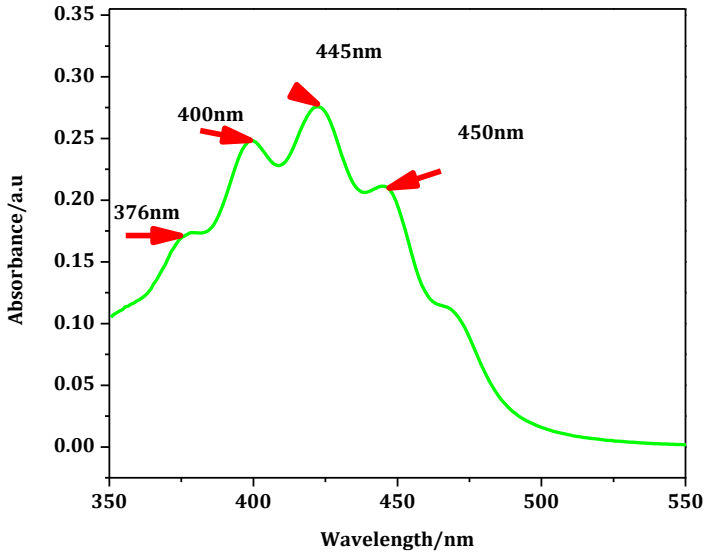

Figure *14*: UV–Vis absorption spectrum of at 50^0clycopene

In this study to determine the lycopene content, by spectrophotometer. A spectrophotometric method was used due to its cheaper and lesser environmental impact [64] . Molar extinction coefficient for lycopene in mixture of acetone: ethanol: hexane is (2589 at 445nm and 2592 at450nm) L/mol.cm (Zechmeister et al.,1943). The following equation is used for to determine the lycopene concentration extracted at 50^0C.

a)

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_{445} \times 536.9 \times V}{\alpha_{445} \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{0.211695795 \times 536.9 \times 100}{2589 \times 1 \times 200}$$

$$\text{Lyc} = 0.21950457\text{mg}/100\text{g}$$

b)

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_\lambda \times 536.9 \times V}{\alpha_\lambda \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{A_{450} \times 536.9 \times V}{\alpha_{450} \times d \times W}$$

$$\text{Lyc}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{0.188244 \times 536.9 \times 100}{2592 \times 1 \times 200}$$

$$\text{Lyc} = 0.19496181\text{mg}/100\text{g}$$

5.3.4 UV-Vis Calibration analysis of lycopene extracted at 50^{0}C

Another consequence of the Beer–Lambert law is that it is possible to change the path length to affect the absorbance. A plot of the sample absorbance against the concentration of the absorbing substance should give a straight line (Figure 15). The absorbance of lycopene standard was linear in the concentration range 0.001-0.01mg/100g [62]. Figure 15 could be represented by the following equation: Absorbance = 0.972x lycopene mg/100g +0.003, R^2 = 0. 9997.When the calibration curve linearity increases, the accurate of the relationship also increases (Figure 15). Absorbance = 0.9718 lycopene mg/100g, R^2 = 0.9997.

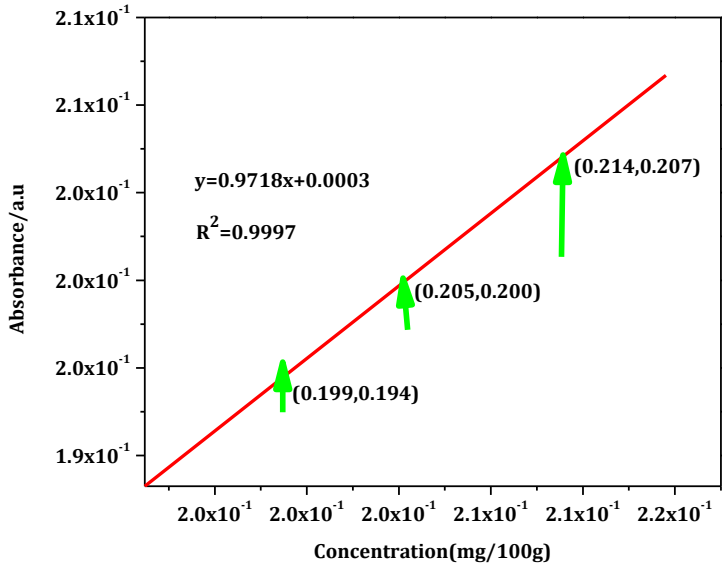

Figure 15: UV–Vis Calibration curve for lycopene extracted at 50^0C in tri-mixture

5.4 Effects of time and temperature

Four extraction temperatures of 20°C, 25°C, 50°C and 80°C and four extraction time of 10 min, 20 min, 30 and 60 min were employed to choose the most suitable temperature and time for extraction of total lycopene from koshim. The effect of extraction temperature on the total lycopene yield in koshim extract and results are shown in table four below. Extraction and concentration of lycopene is typically conducted at temperatures ranging from 20 to 80°C because temperatures above 80°C have been shown to cause rapid degradation. For example, in order to extract lycopene from koshim juice, added tri-mixture (acetone: ethanol: hexane) and heats the mixture to 50°C for 30 min. An increase in temperature increases the efficiency of the extraction since heat cause the cell walls permeable, increase solubility and diffusion coefficients of the lycopene to be extracted and decreases the viscosity of the solvent. Thus facilitating its passage through the solid substrate mass. However, the use of temperatures higher than 80°C decreases the total lycopene yield which is probably due to their degradation [65]. According to table four as extraction time increased (10 to 30 minutes), the yield of lycopene juice also increased. Times longer than 60 min have been shown to cause rapid lycopene degradation.

Table 4: Effect of Blending Time on Lycopene Extraction

Blending time of lycopene ext.	Methanol extra. mg/100g	Hexane extra. mg/100g	Tri-mixture ext. mg/100g	Extraction Temperature
10min	0.179-0.182			20^0C
20min		0.023-0.049		25^0C
30min			0.195-0.23	50^0C
60min			0.107-0.142	80^0C

Table four shows the concentration of total lycopene, after 10 min, 20min,30min and 1 h of methanol, hexane and tri-mixture extraction respectively. This table indicates that the amount of total lycopene (0.195-0.23 mg/100g) is higher for extraction at 50°C after 1/2 hours. Thirty minute was an optimum time for maximum extraction of lycopene from koshim. This result is in agreement with the results of Neda reported in 2012. In table four, the lycopene of koshim is 0.023-0.049 mg/100g of dry powder. Barth et al. (1995) reported that there is an optimum temperature of 50°C for extraction of lycopene from koshim fruit [65], [66].

6. Conclusion and Future Studies

The purpose of this study is to develop a simple method to extract and characterize the lycopene for solvent extraction from koshim (Dovyalis Abyssinica Warb) fruits juice. The results of studies can be improved when we use different solvents in extraction process. In the above discussed procedure methanol, hexane and mixture of hexane-acetone-ethanol were used to extract lycopene from koshim. In this study, the important lycopene's that are found naturally in koshim are used for nutritional and pharmacological interest have been extracted, under optimum condition, using the mentioned above solvent. Optimum solvent extraction conditions were determined for maximizing the extraction of total lycopene.

Extraction conditions such as temperature and stirring can affect the extraction results. Mechanical stirring results in continued suspension of particles in the solvent and homogenization of the medium, and has a positive effect on extraction yield. In the case of aqueous extraction, stirring can reduce resistance to the transfer of solutes at the solid liquid interface (boundary layer) and can increase the transfer coefficient. An increase in the concentration of lycopene in heated vegetables which could be due to changes in the cell structure and therefore the availability of the lycopene. Heating intensities and solvents to study the influence of these factors on lycopene isomerization and degradation during extraction. The heat, below the degradation temperature, facilitates the extraction of solute by permeabilization of cell walls, increasing the solubility of solutes, increasing of diffusion and decreasing the viscosity of the solvent extraction. Heat treatment changes the physical structure of the koshim tissue and the bioavailability of lycopene.

The heat treatment temperatures of 20°C, 25°C, 50°C and 80°C were applied for 10, 20,30 and 60 minutes. Their results showed that lycopene concentrations increased with increasing the temperature; however, using tri-mixture at 80°C resulted in lower yield of lycopene and comparing to that obtained at 50°C. This can be an indication for great isomerization that occurs when extraction is performed at high temperature with tri-mixture; however, the oxidative degradation is the principal reaction.

In case of the influence of the blending time (in 10, 20,30 and 60 minutes) on extraction yields, increasing the blending time from 10-30 for juice resulted in an increase of extraction efficiency and 30-minute was an optimum time for maximum efficiency, but an increase of blending time from 31-60 minutes progressively there was a degradation and isomerization of lycopene as a result of oxidative reduction process, resulted in decrease of extraction efficiency.

The primary identification and quantification of lycopene in the extract was performed using ultraviolet–visible (UV–vis) spectroscopy. Figure 8,10,12 and 14 shows the absorption spectrum of the total lycopene solution. The absorption spectrum demonstrated the two peaks characteristic of lycopene at 444nm and 450nm in methanol, 446nm and 472nm in hexane, 445nm, 450nm and 453nm in tri-mixture. The lycopene concentration was expressed as mg/100g product. In koshim extraction the lycopene content had the following values: in methanol approximately (0.179-0.182) mg/100g, in hexane approximately (0.023-0.049) mg/100g, in mixture of (hexane: acetone: ethanol) at 50^0C and 80^0C are (0.195-0.230) and (0.107-0.141) mg/100g respectively. Results indicated that tri-mixtures to be the best solvent for extraction and average lycopene content of the Koshim which was (0.126–0.151) mg/100g Koshim weight. There is an optimum temperature of 50°C for extraction of lycopene from koshim fruit juice. Under this optimal conditions, the amount of total lycopene in flesh koshim were (0.195 - 0.230) mg/100g. Results of the studies showed that the fruits analyzed having high concentration of Lycopene. In this study, the highest content of lycopene was observed in tri- mixture at 50^0C. The second highest content was found in methanol and the lowest content was in hexane.

Therefore, the objective of the thesis work is spectrophotometric characterization of these Dovyalis Abyssinica fruits(Koshim) in different solvents and based on this, develop reliable, fast, simple and inexpensive methods for determination of lycopene spectroscopic methods.

Future Studies

Areas for further study include:

- New, and environment friendly sorbent such as nano and bio materials can be used in the isolation and purification of lycopene from koshim juice.
- To explain the role of lycopene and to formulate guidelines for healthy eating and disease prevention.
- Studies can be carried out on the structure of lycopene in detail.

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