Characterization of Home-Made and Industrially Produced Niger Seed Oils by Fluorescence Spectroscopy

By: Minda Regasa Tulu

A Thesis Submitted to the Applied Physics Program of School Of Applied Natural Science, Office Of Graduate Studies, Adama Science And Technology University In Partial Fulfillment of the Requirments for the Degree of Masters of Science in Physics

Advisor: Alemu Kebeda Hordofa (PhD)

Adama, Ethopia
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Declaration

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: Minda Regasa Tulu

Signature: _______________

Advisor’s approval sheet

This is to certify that the thesis entitled “Characterization **of Home-Made and Industrially Produced Niger Seed Oils by Fluorescence Spectroscopy**” submitted in partial fulfillment of the requirements for the degree of Masters of Science in Physics, the Graduate Applied Physics program, and has been carried out by **Minda Regasa Tulu**, ID/No PGR/18295/11, under our supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the Applied Physics program.

Name of Advisor	Signature	Date
Alemu Kebede Hordofa (PhD).	____________________	___________

Approval Board of Examiner

We, the undersigned, members of the board of examiners of the final open defense by **Minda Regasa Tulu** have read and evaluated his thesis entitled "**Characterization of Home-Made and Industrially Produced Niger Seed Oils by Fluorescence Spectroscopy"** 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 masters of Science in Applied physics (Laser Spectroscopy)

Advisor	signature	date
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External Examiner	signature	date
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Program chairperson	signature	date
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Contents page

List of Figures

Abstract

Home-made and industrially produced Niger seed oils were characterized by florescence spectrometer in order to analyze the impact of industrial process on the physical and chemical properties of the oil. Fluorescence spectra were collected in the visible range of spectrum using Cary eclipse Luminescence spectrometer. Excitation wave length was varied from 350nm–380 nm with an incremental step of 10 nm and the slit width was kept at 5nm. Spectral analysis was done using origin 8 software. Experimental results indicated that the major component in oil samples (home-made, unrefined and refined industrially produced) were Vitamin E, poly unsaturated fatty acid and chlorophyll. The emission spectra recorded in the range of band between 400 nm - 500nm are related to poly unsaturated fatty acids, emission spectra recorded in the range of band between 500nm-550nm with peak around 548nm is related to vitamins E and the band of emission wave length between 650nm - 725nm is related to chlorophyll. It was also observed that refined oil had very small vitamin E peak. This could be associated with the heating process of refining, as vitamin E is heat unstable. Moreover, the emergence of new peaks in the spectra of refined oil between 400nm - 450nm was assigned to fat-soluble vitamins and lipids that emerge as a result of oxidative reaction due to heating process. The oxidative stability of the unrefined oils was better than that of refined oils, thus it has a longer hypothetical stability and are more suitable for frying. It was also found that as the pH value of the sample goes more acidic the more peak would be the fluorescent spectra at a particular excitation wave length. Increase in excitation wave length also resulted in blue shift of the emission spectra. The quantum yield of the fluorescence of the oil was 10.4%.

1. Introduction

1.1 Vegetable oils

Vegetable oils are the main source of fatty acid, which are predominantly triglycerides (95% - 98%), the remaining (2% -5%) consists of complex mixtures of minor compounds in a wide range of chemical classes [1]. The proportion of saturated and unsaturated fatty acid has an important role on the behaviors of vegetable oils. The degradation of vegetable oil quality is essentially caused by the unsaturated fatty acid oxidation which is a complex phenomenon that generates mainly hydro peroxides but also volatile compounds through a three-phase process initiation, propagation and termination [2].

Niger seed (Guizotia abyssinica) is one of the most important edible oil crops in Ethiopia providing 50% to 60 % of the indigenous edible oils [3]. The use of Niger seed (Guizotia abyssinica) as a source of edible oil, after production at household level through pounding it by wooden mortar and pestle of lightly roasted seed to gather with added boiling water and transferring the pounded mash into an earthen pot which is rotated manually in order to let the oil migrate to the top and separated by decanting the oil has been practiced since ancient time. The preparation is still continued to be practiced at households of rural setting. This long-established use of fresh Niger seed (Guizotia abyssinica) oil in the Ethiopian cuisine could not be as advanced as it is demanded by consumers. This may be partly because of low stability nature of Niger seed (Guizotia abyssinica) oils against oxidation resulting in rapid development of rancid off odor upon relatively short storage period [4,5]. The higher proportion of polyunsaturated fatty acids, its relative low amount of unsaponifiable matter, its low total phenol and polar lipids, have been reported to be among the possible reasons for Niger seed oils slow oxidative stability [6].

Oxidized lipids not only result in objectionable flavors and odors, loss of color and nutrient value, but also generate potentially toxic compounds which may be detrimental to the health of consumers [7]. One of the most effective ways of retarding lipid oxidation and hence increasing shelf life of oils and oil products is to incorporate antioxidants which may be defined as substances that when present at low concentration compared with those of oxidizable substrates significantly delay or prevent the oxidation of that substrate. In doing so, antioxidants slow down the rate at which oxidation occurs. Application of natural antioxidants as food additives was also raised because of their potential health benefits. It was reported that natural antioxidants decrease risks of heart disease than their counter synthetic antioxidant [8].

The addition of antioxidants to reduce oxidation and hence the loss of color, taste and contents require great deal of knowledge of food chemistry and sophisticated equipment [9]. In addition, controlled environment and state of the art laboratory is essential. Thus, the researcher is more interested in the photo-physical analysis of the factors that are responsible for shelf life effects indicated above using recently developed food characterization technique which uses fluorescence property of the sample under investigation.

1.2. Statement of the problem

Ethiopia is located around the tropical region. Thus, the weather makes a suitable environment for the growth of vegetable plant. There are lots of vegetables growing in different regions. Besides, most Ethiopian people use home-made vegetable oils. But the oil quality they use deteriorate due to different factors [10]. However, factor affecting the vegetable oil quality are not well known scientifically. Therefore, the researcher tries to identify the factors that are responsible for deterioration of oil quality.

1.3. Objectives of the study

1.3.1. General objective

The general objective of this study was Characterization of Home-Made and Industrially Produced Niger Seed Oils by Fluorescence Spectroscopy.

1.3.2. Specific Objective

The specific objectives of this study are:

- To determine the fluorescence wavelength and fluorescence peaks of the refined and unrefined Niger Oils
- To identify the types of fluorescence compounds found in refined and unrefined Niger seed oil
- To determine fluorescence quantum yields of local vegetable oil.

1.4. Significance of the study

This study was conducted on the dilatory item, an Ethiopian vegetable oil that is daily consumed, liable to quality deterioration as a result of mismanagement and know how. Thus, the finding of this research is so significant for the consumers, traders and scientific community in the following ways:

1. It gives valuable information to producers, consumers, and traders on the factors and ways to handle vegetable oils during the process of production, storage and distribution.
2. It helps future researcher as important source of information about the main constituent compounds found in the Niger oil and the spectral emission wave length, the time and temperature dependence, etc.

2. Literature Review

2.1. Types and Significance of Vegetable Seeds oils

Many vegetable oils are produced from seeds (such as soybean oil, palm oil, Niger seed oil, and rapeseed oil). Seeds and beans are an important food source for humans and animals due to their high nutritive value. They consist mainly of proteins, carbohydrates (which are the major source of energy), vitamins and minerals. Usually, the higher the oil content in the seed or bean, the lower the protein content, and vice versa. Vegetable Seeds are produced world-wide but of specific interest to this study are oil seeds produced in Ethiopia. Vegetable oils find wide use in pharmaceutical, nutritional and cosmetic products. These include products such as cooking oils, salad, food coatings, paints, plasticizers, printing inks, medicines, face masks, hand creams, shower gels, soaps, detergents and many more too numerous to mention here [11]. Vegetable oils are complex mixtures of different compounds; those are the major components while the minor components comprise of among others polyphenols, aldehydes, sterols and a variety of volatile compounds. The major components are of importance for their nutritional value and also for differentiating between different types of oil. Several studies have been carried out on the health aspects of vegetable oils especially olive oil [12].

2.2. Oilseeds processing in Ethiopia

Several oil seeds grown in Ethiopia including sesame, soybean, Niger seed, cotton seed, linseed, rapeseed. Some of the oil crops like sesame are one of the leading export items for the nation. With this regard, Ethiopia has a large number of local small-scale vegetable oil processing industries. Since the majority of the oil seeds are becoming export commodities, raw material scarcity is becoming a problem for the local oil processors. Hence, the country is importing edible vegetable oils from abroad, palm oil taking the major share. Niger seed is an oil seed crop, indigenous to Ethiopia where the crop is found in greatest diversity. It is mainly cultivated in Ethiopia and India. It is also cultivated in small scale in other African countries such as Sudan, Uganda, Tanzania, Zimbabwe and Malawi [13]. In Ethiopia, Niger seed is grown in the average temperature ranges from 15 ^{0}C to 23 ^{0}C and annual rainfall ranges from 500 mm to 1000 mm [14]. Gojam, Gondor, Shoa and Wollega are the major Niger producer regions and Wello, Harerge, Arsi and Bale regions also cultivate this crop [15]. Niger seed (Guizotia abyssinica) can grow on a wide variety of soils, buts appears to thrive best on clayey loam or sandy clays within a pH range 5.2-7.3. Niger seed (Guizotia abyssinica) is characterized by indeterminate growth habit. Correct time of harvesting of Niger needs (Guizotia abyssinica) consideration because ripe buds and young flower may be present on the same plant at harvest which result in uneven ripening that cause loss from seed shattering. Niger (Guizotia abyssinica) seed is a major source of edible oil in Ethiopia. It also contributes in edible oil production in some African and Asian countries, mainly India. Niger seed (Guizotia abyssinica) oil is not only used for edible purposes but also used for industrial purposes in preparation of soaps, paints, illuminants and lubricants and for cleaning machinery [16].

2.3. Characteristics of vegetable oils

Oils and fats are macronutrients for humans, who need to consume about 20 to 25 Kg per capita per year, as recommended by the World Health Organization. For good health, one needs a balanced diet, including oils and fats that supply energy and essential fatty acids. There are many sources of oils and fats, but vegetable oils including Niger seed (Guizotia abyssinica) oil, soybean oil, palm oil, sunflower oil, and rapeseed oil contribute 60% to 70% of the world’s oil production [17].

2.4. Refined and Unrefined Niger seed oils.

Refining Niger seed (Guizotia abyssinica) oil produces edible oil with characteristics that consumers desire such as bland flavour and odour, clear appearance, light color, stability to oxidation and suitability for frying. Refined oil is an output that we attain after clarifying and sifting the raw, crude oil by eliminating the toxic and harmful ingredients existing in it. It is very obvious that good quality refined or sifted oils are way better than the preliminary raw oils. The refined version of oils is very light, clear, pure and more apt for consumption by humans. Other positive points in favour of refined oils are, it is safe, free from any bad odor, it generates minor lamp black while cooking which does not leave heavy smoke patches or stains on the kitchen walls and it is easy to store.

Unrefined oils are the oils which are not much perceived to the heat. In other words, these are the kinds of oils which have minimal frontage to the heat. They are conserved in the natural aspect once taken out from the seeds. They are not deodorized or washed out after the extraction. In common terms, the unrefined oils are much in the need of the customers as correlated to any other type of oils because they are authentic in their color, taste, smell and flavor. Also, due to the existence of good percentage of nutrients in them, the unrefined oils enjoy much preference and acceptance in the market. They have a good proportion of oleic acid in them. We make use of oils in almost every domain of being. Be it our kitchen, medical purpose, cosmetic purposes or any other basis, oils are very valuable. We have learned the very basic notions of using refined and unrefined oils in our food [18].

2.5. Spectroscopic Analysis of Vegetable Seed Oils

Fluorescence spectroscopy has been used in the past for determining the authenticity of olive oils. However, few papers have been published in recent years on the use of fluorescence in vegetable oils. [20] Applied fluorescence spectroscopy for detecting hazelnut oil adulteration in virgin olive oils. This technique has some advantages such as its speed of analysis, that it is reagent less, and that small amounts of sample are required. [21] Showed that emission fluorescence spectra of virgin olive oils between 400 - 700 nm measured at excitation wavelength 365 nm have clear differences compared to the spectra of other vegetable oils. Virgin olive oils present two low peaks at 445 and 475 nm (related to conjugated hydro peroxides), one intense peak at 525 nm (due to vitamin E) and another peak at 681 nm (due to chlorophylls). The very low intensity of the peaks at 445 and 475 nm is due to their large content on monounsaturated fatty acids and phenolic antioxidants, which provide more stability against oxidation. All refined oils show only one intense peak at 445 nm. It is due to fatty acid oxidation products formed as a result of the large percentage of polyunsaturated fatty acids present in these oils. In recent years, instrumental improvements and the availability of software specially designed to extract the information contained in spectra have contributed to the development of fluorescence spectroscopy. Hence, it is possible to record one fluorescence excitation-emission matrix for each sample, i.e. a set of emission spectra recorded at several excitation wavelengths.

For some oils, namely linseed, rapeseed, Niger seed (Guizotia abyssinica) oil and olive oil indicate theoretical from literature that the wavelength band is observed with excitation at about 350 nm–420 nm can be divided into three regions associated to the emission of oxidation products in region I (between 400-500 nm) [19, 20, 21], vitamin E in region II (between 500-600nm) and emission at about 650 nm–730nm. A long-wavelength band is observed with excitation at about 350 nm–420 nm and emission at about 650 nm–730 nm. This band was attributed to pigments of chlorophyll group, based on its excitation and emission characteristics [22].

Niger seed oil appears to be nutritionally valuable, as the high content of linoleic acid is thought to prevent cardiovascular diseases and is a precursor of structural components of plasma membranes and some metabolic regulatory compounds. Linoleic acid may also decrease arrhythmias and improve insulin sensitivity [23].

2.6. Fluorescence Spectroscopy

A hot body that emits radiation solely because of its high temperature is said to exhibit incandescence. All other forms of light emission are called luminescence. When luminescence occurs, the system loses energy and if the emission is to be continuous, some form of energy must be supplied from elsewhere. Thus, the radio luminescence emitted from a luminous clock face is supplied by high energy particles from the radioactive material in the phosphor and the electro luminescence of a gas discharge lamp is derived from the passage of an electric current through an ionized gas. Other such phenomena include chemical luminescence, derived from the energy of a chemical reaction, and this is called bioluminescence when the reactions take place within living organisms, for example, glow-worms and fireflies. When the external energy supply is by means of the absorption of infrared, visible or ultraviolet light, the emitted light is called photoluminescence and this is the process that takes place in any fluorometric analysis. To account adequately for the processes of absorption and emission of light, it is necessary to assume that radiant energy can only be absorbed in definite units, or quanta. The energy, E, carried by any one quantum is proportional to its frequency of oscillation, that is

$$E = hv = \frac{hc}{\lambda} \quad \text{..} \quad (1)$$

Where ν is the frequency, λ the related wavelength and h = Planck's constant (6.624 x 10^{-27} ergs/sec). The energy of a single quantum is too small for convenience and it is usual to talk of the energy associated with N quanta (where N = 6.023 x 10^{23} the number of single molecules in a gram molecule), which is called an Einstein. Thus, if in a photochemical reaction one molecule reacts for each quantum absorbed, then the absorption of one Einstein is sufficient energy for the reaction of one-gram mole. Since the amount of energy per Einstein is proportional to the frequency of the radiation, it varies enormously over the range of the electro-magnetic spectrum [24].

Luminescence is the emission of light from any substance and occurs from electronically excited states. Molecular fluorescence is a type of luminescence in which the molecules emit light from excited singlet states after the absorption of visible or UV radiation (around 200-900 nm). In excited singlet states, the electron in the excited orbital is paired (of opposite spin) to the second electron in the ground-state orbital. Consequently, the return to the ground state is spin-allowed and occurs rapidly by the emission of a photon. Therefore, the emission rates of fluorescence are typically of 10^{-8}s, so the typical fluorescence lifetime of a fluorophore (i.e. the average time between excitation and the return to the ground state) is around 10ns [24, 25].

The singlet ground, first and second electronic states are depicted by S_0, S_1, and S_2, respectively. At each of these electronic energy levels, the fluorophore can exist in a number of vibrational energy levels (v = 0, 1, 2). Absorption typically occurs from molecules with the lowest vibrational energy. After light absorption, several processes usually occur. A fluorophore is usually excited to some higher vibrational level of either S_1or S_2. Then there is a relaxation to the lowest vibrational level of S_1. This process, called internal conversion, generally occurs in 10^{-12}s or less. As fluorescence lifetimes are typically near 10^{-8}s, internal conversion is generally complete before the emission. Fluorescence emission therefore generally results from the lowest-energy vibrational state of S_1. Molecules in the S_1 state can also undergo a spin conversion to the first triplet state, T_1 (i.e. the electron has the same spin as that in the ground-state orbital). Emission from T_1 is named phosphorescence and is generally shifted to longer wavelengths (lower energy) than fluorescence. Conversion of S_1 to T_1 is called intersystem crossing. Transition from T_1 to the singlet ground state is forbidden, so rate constants for triplet emission are several orders of magnitude smaller than those for fluorescence [25]. The processes that occur between the absorption and emission of light are usually illustrated by the Jablonski diagram as shown in Figure 2.1.

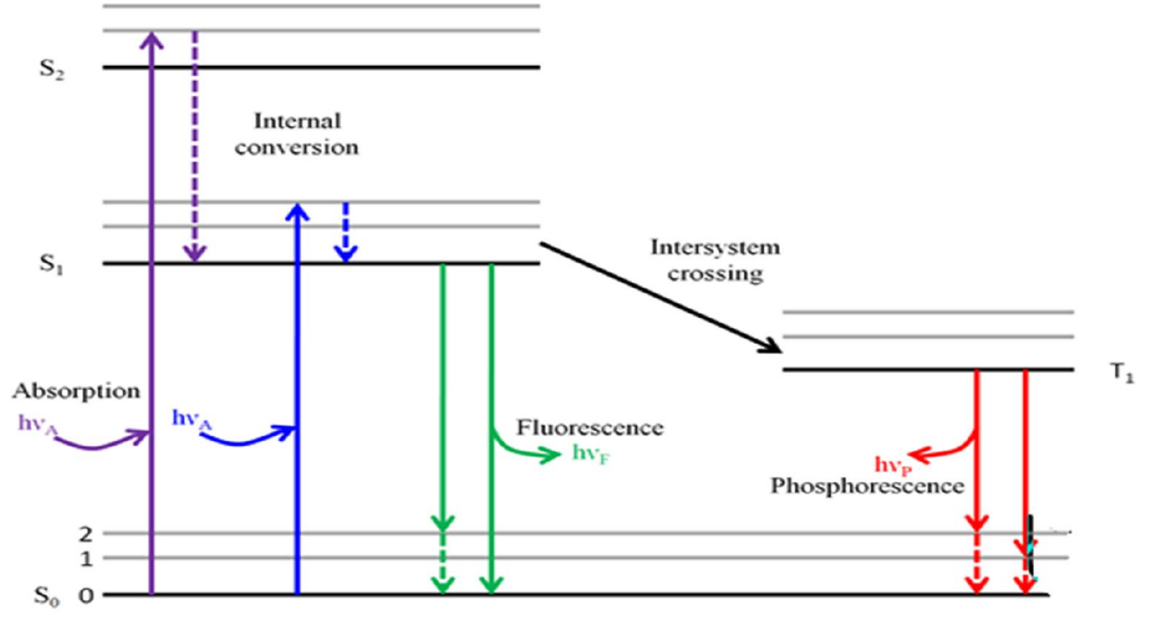

Figure: 2.1. A simplified scheme of the Jablonski diagram.

Fluorescence intensity is a simultaneous function of the excitation and emission wavelengths. The intensity values of a fluorescence emission spectrum are obtained by keeping the excitation wavelength (λex) constant while the emission wavelengths (λem) are scanned. Similarly, the intensity values of a fluorescence excitation spectrum are obtained by keeping the λem constant while the λex are scanned. When measuring several emission spectra at different λex (vice versa), a three-dimensional fluorescence landscape, the so-called fluorescence excitation-emission matrix is obtained [25].

Fluorescence is almost always the result of a transition between the lowest energy level of the first excited state (S_1) and some of the ground state (S_0). The part of the molecule responsible for the fluorescence is known as the fluorophore. The quantum energy of the emitted photon is equal to the difference in energy between these two levels. It follows that the quantum energy and therefore wavelength of the emission are independent of the wavelength of the photon producing excitation.

Examination of the Jablonski diagram reveals that the energy of the emission is typically less than that of absorption. Because of that, the wavelength of the emission is longer than that of excitation. From the Jablonski diagram, although it is rare for molecules to enter an excited triplet state directly from the ground state, in many molecules there is an efficient process where by an excited singlet state may be converted to an excited triplet state. This process is called intersystem crossing. It is immediately followed by vibrational relaxation where by the molecule falls and return to the ground state from the triplet state by emission of a photon. This is called as phosphorescence. Phosphorescence decay is very much longer, typically milliseconds to seconds; therefore, the wavelength of phosphorescence is generally longer than fluorescence. Another process which has similar spectral but different temporal distribution is called delayed fluorescence. The delay is due to a double intersystem crossing, from singlet to triplet and back to singlet. It is a form of luminescence which takes place over a time scale similar to the phosphorescence but otherwise has the nature of fluorescence [26].

The most common type of fluorescent sample is a dilute, transparent solution that absorbs light following the Beer-Lambert law and emits corresponding fluorescence intensity directly proportional to the concentration, the absorption coefficient and the fluorescence quantum yield of the fluorescent species or fluorophore. The light intensity I can now be described as:

$$I= I_o e^{-\alpha l} \dots\dots\dots\dots (2)$$

Where l is the path length of light within the absorbing medium. The Beer-Lambert law results directly from equation (2)

$$I= I_o e^{-\varepsilon c l} \dots\dots\dots\dots (3)$$

Where ε is the molar absorption coefficient and c the concentration of the absorbing compound. The Beer -Lambert law is usually expressed in its logarithmic form:

$$=A=\varepsilon c l \dots\dots\dots\dots (4)$$

Conventional fluorescence spectrometer has both excitation and emission wavelength selectors and a spectrum is collected by fixing the wavelength of one of the selectors and scanning the other wavelength selector over a range. When the excitation wavelength is fixed and the emission wavelength is scanned, the resulting spectrum is termed an emission spectrum. When the emission wavelength is fixed and the excitation wavelength is scanned, the resulting spectrum is termed an excitation spectrum [27].

The fluorescence quantum yield gives the efficiency of the fluorescence process. It is defined as the ratio of the number of photons emitted to the number of photons absorbed.

$$\Phi = \frac{number\ of\ photon\ emitted}{number\ of\ photon\ obsorbed} \dots\dots\dots\dots (5)$$

The maximum possible fluorescence quantum yield is 1.0 (100%); each photon absorbed results in a photon emitted. Compounds with quantum yields of 0.10 are still considered quite fluorescent. Another way to define the quantum yield of fluorescence is by the rate of excited state decay:

$$\Phi = \frac{ki}{\sum_i kf} \quad \ldots\ldots\ldots\ldots \quad (6)$$

Where is the rate constant of spontaneous emission of radiation and the sum all rates of excited state. Other rates of excited state decay are caused by mechanisms other than photon emission and are, therefore, often called "non-radiative rates", which can include: dynamic collisional quenching, near-field dipole-dipole interaction (or resonance energy transfer), internal conversion, and intersystem crossing. Thus, if the rate of any pathway changes, both the excited state lifetime and the fluorescence quantum yield will be affected. Fluorescence quantum yields are measured by comparison to a standard [28].

2.6. Qualitative and quantitative fluorescence measurements

There are two general classes of measurements that are commonly performed by fluorescence spectrometry: qualitative and quantitative [29].

2.6.1 Qualitative Fluorescence Measurements

Qualitative fluorescence measurements are used to detect the presence of particular analyses, yielding a positive or negative answer. The excitation and emission wavelengths are often fixed at the peak maximum of the fluorophore to be detected. The observation of fluorescence intensity at the peak position that is greater than a set threshold, typically based on the noise level, e.g. the limit of detection, indicates a positive result. For more complex systems, e.g. where multiple fluorophores are present, the shape of the fluorescence emission spectrum or the value of the fluorescence lifetime can be used to determine which of the fluorophores of interest are present.

2.6.2 Quantitative Fluorescence Measurements

Quantitative fluorescence measurements are used to determine amounts or concentrations of analyses in unknown samples. These quantities may be determined in absolute units, such as moles or moles per liter, or in relative units, such as the ratio of the concentrations of two fluorescent analyses contained in a single unknown solution.

2.7. Instrumentation of fluorescence spectroscopy

Fluorescence measurements fall into two categories, the measurement of the emission spectra, called fluorimeter, and measurement of the time dependence of the emission, called fluorimeter. All fluorescence instruments consist of light source, wavelength selectors, sample compartment and detector system [29-31]. A schematic representation of essential components of fluorescence spectrometer is shown in Figure 2.2.

Figure 2.2. (a) Cary eclipse fluorimeter Luminescence spectrometer which has been used for experimental purpose.

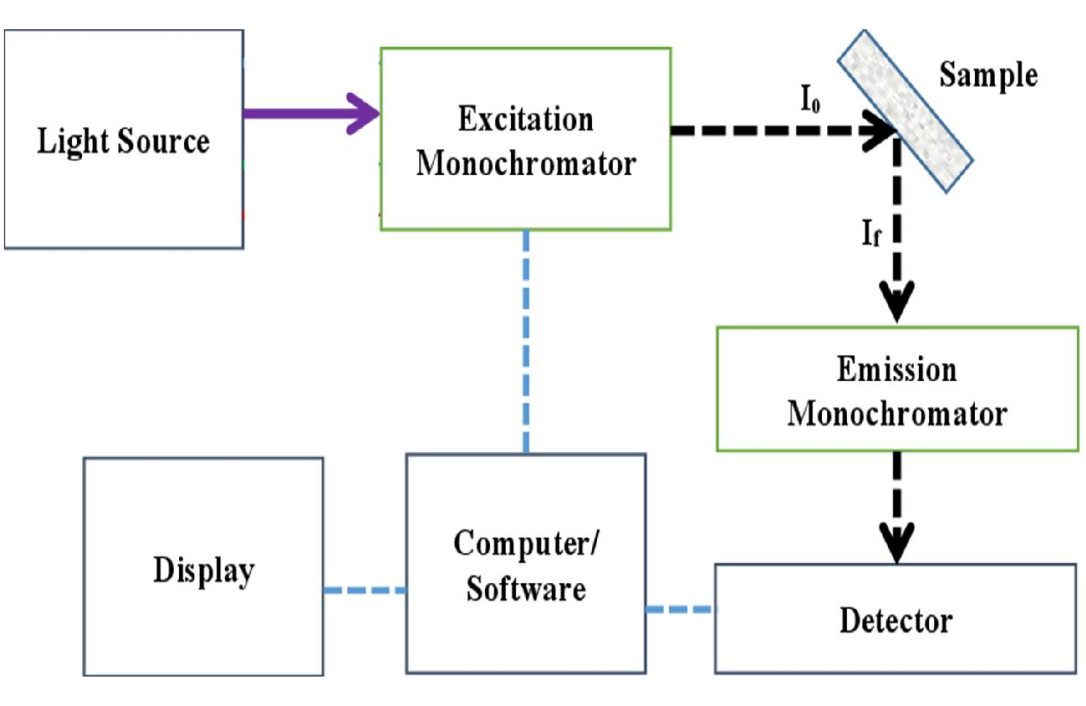

Figure:2.2 (b) Schematic representation of the instrumentation of a luminescence spectrometer.

1. Light sources

Several types of light source are available for spectroscopic studies such as tungsten incandescent, mercury, xenon, hydrogen and deuterium lamps. Since the total fluorescence observed is proportional to the intensity of the source excitation, it is necessary to have a significant amount of energy available in the absorption region of the sample to be determined. The type of light source chosen depends primarily on whether a fluorescence excitation spectrum or fluorescence emission is to be measured. The most common incandescent lamp used in spectroscopic studies is the tungsten lamp. The disadvantages of incandescent lamps are their limited useful output which is covering only the visible range. Tungsten lamps therefore are useful for excitation in the visible range. However, since many compounds are excited by ultraviolet and short wavelength visible radiation, the tungsten lamp has found very little use in fluorescence instrumentation.

Xenon arc lamp on the other hand, employ a source whose output is a continuum on which are superimposed a number of sharp lines, allowing any wavelength throughout the ultraviolet and visible region of the spectrum to be selected. It is also the brightest and most powerful sources of ultra violet radiation. In this particular work, pulsed xenon was used because it produced a high output using a low voltage, resulting in longer lamp life with minimal ozone and heat production.

Moreover, the pulsed source reduces potential photo bleaching of the sample during analysis by several orders of magnitude over continuous sources.

2. Monochromators

The purpose of the monochromatic is to disperse polychromatic or white light into the various colors or wavelengths. It is a wavelength selector. The performance specifications of a monochromatic include dispersion, efficiency and stray light levels. Dispersion is usually given in nm/mm and this can be accomplished using prisms or diffraction gratings. The monochromators in most spectra fluorimeters use diffraction gratings rather than prisms. Prisms are not used in spectra fluorimeters instruments because they give their greatest dispersion in ultraviolet and not the visible where most measurements are made. In addition, a larger prism would be needed to obtain adequate sensitivity and this would be very expensive. Two types of monochromatic are used in luminescence equipment, filters and gratings. Filters allow a relatively wide range of wave lengths to excite the sample and to pass through to the photodetector. Meanwhile, monochromators which are usually diffraction gratings with slit arrangements inherently allow the passage of a smaller range of wavelengths which is ultimately determined by the optical characteristics of the diffraction grating. However, it is possible to allow a wider range of wavelengths to pass a monochromatic by manipulation of the slits.

3. Slits

The slit can best be described as volume controls for the fluorescence intensity. For optimal instrumental performance, the excitation slit width automatically controls the sample photomultiplier tube voltage. This control provides an optimum signal-to-noise ratio as function of sample intensity. The slit width is the most important factor in determining the resolution of the instrument. The distribution of energy as a function of wavelength for the light passing through the exit slit of a monochromatic can be represented as an isosceles triangle, if the entrance and exit slits are of equal width the middle wavelength (peak transmittance) is called the nominal wavelength and is the value read on the dial of the instrument. The smaller the slit widths employed, the narrower is the range of spectral band pass and the greater is the analytical selectivity. However, a smaller slit width results in a decrease in the intensity of the transmitted light and therefore results in a decrease in the analytical sensitivity. For weakly fluorescing samples it is advantageous to increase the band, pass and collect more light. For highly fluorescent samples the narrow band pass is recommended to avoid exposing the detector to too high signal levels. Generally, a wider slit setting causes higher fluorescence signal measurements. However, because of the fluorescence rationing system used in the fluorescence machine, widening the excitation slit width will not increase the reported fluorescent signal ratio, but does increase sample fluorescence signal, resulting in an improved signal-to-noise ratio.

4. Cuvette

The majority of fluorescence assays are carried out in solution, the final measurement being made upon the sample contained in a cuvette. The cuvette must be fluorescence free. Cuvettes may be circular, square or rectangular, and must be constructed of a material that will transmit both the incident and emitted light. Square cuvettes were found to be most precise since the parameters of path length and parallelism are easier to maintain during manufacture. Figure 2.3 shows the method of measuring fluorescence. The cuvette reader excites the sample over the entire path length and reads the emitted light at right angles

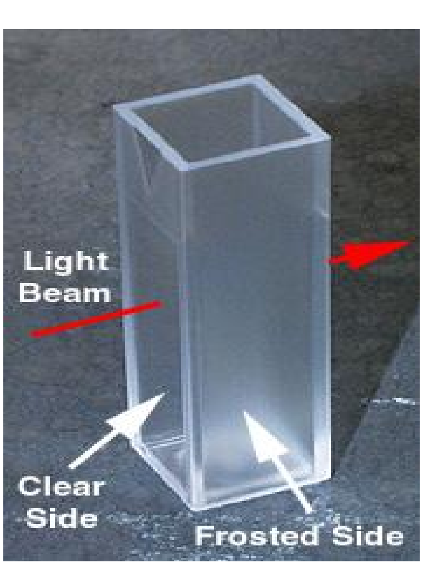

Figure 2.3: Cuvette Reader

For measurements involving ultraviolet radiation, quartz or silica cells are necessary. Parker has shown that various types of quartz cuvettes emit an appreciable amount of fluorescence. In this work, quartz cells were used instead of silica and glass cuvettes. This is because those materials have some fluorescence which may contribute appreciably to routine measurements.

5. Detectors

All commercial fluorescence instruments use photomultiplier tubes as detectors because of their extreme sensitivity and fast response. A photomultiplier is a phototube capable of effecting photocathode emission with multiple cascade stages of electron amplification to achieve a large and linear amplification of primary photocurrent within the tube itself. Upon exposure to light, the electric current produced by a photomultiplier is then amplified by an amplifier to a measurable level. The photomultiplier surface contains a thin layer of one or more elements possessing a low ionization potential. As a result, valence electrons are easily released when struck by photons. The alkali metals are commonly used and are plated on an Ag/Ag2O cathode. Photomultiplier tubes can be made to respond to different wavelengths by varying the elements of the photosensitive surface. The limit of sensitivity of a photomultiplier is normally governed by the level of dark current which is the signal derived from the tube with no light falling on it.

A photomultiplier dark current is acquired prior to the onset lamp pulse and is subtracted from that pulse for correction of phototube dark current. The instrument measures and corrects every flash of the lamp to improve sensitivity at low levels of fluorescence, making it possible to measure samples in room light, thus freeing the user from working through septa in light-tight compartments.

6. Read-out devices

The output from the detector is amplified and displayed on a readout device which may be a meter or digital display. It should be possible to change the sensitivity of the amplifier in a series of discrete steps so that samples of widely differing concentration can be compared. A continuous sensitivity adjustment is also useful so that the display can be made to read directly in concentration units. Digital displays are most legible and free from misinterpretation. Improvement in precision is obtained by the use of integration techniques where the average value over a period of a few seconds is displayed as an unchanging signal. Microprocessor electronics provide outputs directly compatible with printer systems and computers, eliminating any possibility of operator error in transferring data.

2.8. Factors affect fluorescence

A. Cuvettes

One of the advantages of fluorescence procedures compared to equivalent absorption techniques is that routine measurements may usually be carried out in inexpensive test-tubes rather than precision cuvettes without appreciable loss in precision. This benefit is derived from the geometrical layout of simple fluorimeters where only a small central area of the cuvette is actually viewed by the detector, so that the overall dimensions of the cell are less important. However, this statement needs careful qualification, since the use of laboratory grade test-tubes will result in deviation arising from other sources. Variations in cell wall width can give rise to errors by distorting the lens action of the round cell wall from one cell to another and the native fluorescence of the material used to manufacture the cell will often produce large blank values. Obviously, the cell must be fabricated from a material which will transmit both the excitation and emission wavelengths of interest and, if these are both in the visible region, savings can be made by the use of glass or even plastic sample containers. Only those cells recommended by the manufacturer of the instrument should be employed and every new batch should be carefully checked to make quite sure that their native fluorescence at the analytical wavelength chosen is minimal. Even the highest grades of silica and glass have some fluorescence, although it should not contribute appreciably to routine measurements. Cells and other glassware used for fluorometric analysis should be carefully cleaned, preferably by boiling in 50% nitric acid followed by thorough rinsing in distilled water.

B. Solvents and Reagents

It is essential to examine the quality of solvents periodically since small traces of contaminants may be enough to produce high black values. If the solvent blank is appreciable it can usually be reduced to a reasonable value by distillation or washing with acid, base or water.

- Water is the most common solvent and deionized-distilled water should always be employed.
- Fluorescence derived from contaminants should not be confused with the Raman scatter from the solvent itself.

- All other reagents used in the assay should be carefully controlled and high-quality grades are to be recommended.
- Reagent blanks should always be carried through the analytical procedure and the actual value of the blank determined before the instrument is re-zeroed.
- High or changed blank values should immediately cast suspicion upon the solvents and reagents employed.

C. Other contaminants

Soaking of glassware in synthetic detergent solutions is gaining ground in many laboratories as a general method of cleaning. Most commercial preparations are strongly fluorescent. Before use, the fluorescence characteristics of a dilute solution of the detergent should be measured to make quite sure that the fluorescence is minimal at the chosen analytical wavelengths. Inorganic cleansing agents are more suitable for fluorometric work. Stopcock grease is another common contaminant with strong native fluorescence. The growth of micro-organisms in buffer or reagent solutions will affect blank values by both their fluorescence and light scattering properties. Filter paper can be another source of contamination due to residues of phenols present from the original wood.

D. Adsorption

Loss of organic substances by adsorption onto the walls of the container becomes particularly troublesome at the sub-microgram level. New glass surfaces should be thoroughly cleaned in acid before use and measurements of aromatic substances in non-polar solvents should be avoided. Often the addition of a small quantity of a polar solvent to a non-polar one will greatly reduce adsorption losses. Protein precipitation produces a large, freshly prepared, adsorptive surface and choice of precipitation agent is critical to prevent adsorption of the fluorophore with subsequent loss. Each assay may require a specific precipitation agent and analytical procedures should not be changed without investigation.

E. Photo-decomposition

Fluorimeters employ intense light sources to produce high sensitivity and in some cases the level of incident light may be sufficient to decompose the sample under investigation. A shutter is essential so that the solution is only irradiated during the short period of measurement. The decomposition only occurs at the point where the excitation beam is focused onto the sample and if, after closing and then reopening the excitation shutter, the value of fluorescence rises to the original value, photo-decomposition is almost certainly taking place. This phenomenon is due to the diffusion of unaffected, fresh molecules into the light path whilst the shutter is closed, with subsequent renewal of fluorescence. Photo-decomposition may be reduced by choice of another excitation wavelength or by reducing the excitation intensity by use of narrower slit widths or a neutral density filter.

F. Oxidation

The presence of trace oxidizing agents, for example, dissolved oxygen or traces of peroxides, can reduce fluorescence intensity.

G. Non-linearity

The proportional relationship between light absorption and fluorescence emission is only valid for cases where the absorption is small. As the concentration of fluorophore increases, deviations occur and the plot of emission against concentration becomes non-linear. With right-angle viewing, the principal distortion arises from the absorption of the excited light before it can penetrate to the heart of the cell, where the emission produced is accepted by the detector optics. Generally, other sources of non-linearity are usually associated with the instrument's signal handling (for example, amplifiers) and display (especially meters) systems.

H. Temperature effects

Changes in temperature affect the viscosity of the medium and hence the number of collisions of the molecules of the fluorophore with solvent molecules. Fluorescence intensity is sensitive to such changes and the fluorescence of many certain fluorophores shows temperature dependence. In such cases the use of thermostatic cell holders is to be recommended. Normally it is sufficient to work at room temperature with the proviso that any sample procedure involving heating or cooling must also allow sufficient time for the final solution to reach ambient before measurement.

I. pH effects

Relatively small changes in pH will sometimes radically affect the intensity and spectral characteristics of fluorescence. Accurate pH control is essential and, when particular buffer solutions are recommended in an assay procedure, they should not be changed without investigation. Most phenols are fluorescent in neutral or acidic media, but the presence of a base leads to the formation of non-fluorescent phonate ions.

J. Inner-filter effects

Fluorescence intensity will be reduced by the presence of any compound which is capable of absorbing a portion of either the excitation or emission energy. At high concentrations this can be caused by absorption due to the fluorophore itself. More commonly, particularly when working with tissue or urine extracts, it is the presence of relatively large quantities of other absorbing species that is troublesome. The purpose of extraction procedures is usually to eliminate such species so that the final measurement is made upon a solution essentially similar to the standard.

K. Quenching

Decrease of fluorescence intensity by interaction of the excited state of the fluorophore with its surroundings is known as quenching and is fortunately relatively rare. Quenching is not random. Each example is indicative of a specific chemical interaction, and the common instances are well known. Quinine fluorescence is quenched by the presence of halide ion despite the fact that the absorption spectrum and extinction coefficient of quinine is identical in 0.5M H_2SO_4 and 0.5M HC.

3. Materials, Instrument and Method

3.1. Sample Preparation

Mature, normal and dried Niger seed (Guizotia abyssinica) produced at household level were bought from the local market. The seeds were washed and roasted before undergoing the process; then pounded by wooden mortar and pestle. Both refined and unrefined Niger seed (Guizotia abyssinica) oils products were purchased from the local oil manufacture.

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Fig. 3.1 .sample of home-made and industrial produced Niger seed oil

3.2. Instruments

The fluorescence spectra of Home-made and purified and unpurified Niger seed (Guizotia abyssinica) oil samples were characterized by using fluorescence spectrometer (Cary eclipse - MY18490002) with spectral range of detection ranging from 200nm up to 900nm.The system was controlled by a software operating within the Window’s environment. A continuous xenon arc lamp is used as the excitation source and the photomultiplier was used as the detector.

3.3 Methods

The lightly roasted and pounded to gather with added boiling water and transferring the pounded mash into an earthen pot which is rotated manually in order to let the oil to migrate to the top to separate the oil by decantation. The decanted oil was further filtered by sieve to avoid solid suspensions. The purified samples were stored in glass bottle and kept at room temperature for experimentation. Different molar solution/concentrations were prepared and emission spectra were collected using Cary Eclipse photo spectrometer in the visible region of spectrum. The excitation wave length ranged from 350nm to 380nm with an increment of 10 nm. The widths of the slits were fixed at 10 nm for excitation and 5 nm for emission monochromators. To ensure the validity of the comparison between the different measures, each spectrum represents four scans recorded after 120 s for each sample excitation. We use these parameters for all the samples in order to measure in the same experimental conditions. Data analysis was done using origin pro 8 software.

4. Results and Discussions

4.1. Spectral differentiation between home-made, unrefined and refined Niger oils

In order to analysis the three types of Niger seed (Guizotia abyssinica) oils samples (home- made, unrefined and refined oil samples) their emission spectra were recorded by using Excitation wavelengths ranging from 350nm–380nm. The choice of the excitation wave length was deliberate as the cuvette used was plastic that doesn't absorb light in the visible spectrum.

4.1.1. Analysis of home-made Niger seed oils

Excitation wave length was varied from 350nm–380 nm with an incremental step of 10 nm. The corresponding emission spectra were observed to show fluorescence peaks in the range λ_{em}: 400nm – 725nm. Different peaks and bands of fluorescence were observed for each excitation wave lengths. In order to differentiate the different spectra colors blue, red and black colors were assigned to each excitation wavelength and their corresponding spectra: green color for spectra excited at λ_{ex}: 380nm, blue color for spectra excited at λ_{ex}: 370nm, red color for spectra excited at λ_{ex}: 360 nm and black color for spectra excited at λ_{ex}: 350 nm, respectively. From literature values it was concluded that emission spectra recorded in the range of band between 400 nm - 500nm are related to poly unsaturated fatty acids. On the other hand, emission spectra recorded in the range of band between 500nm-550nm with peak around 548nm is related to vitamins E and the regions of emission wave length band between 650nm - 725nm is related to chlorophyll present in the Niger seed (Guizotia abyssinica) oil. The resulting fluorescence spectra are indicated by Fig. 4.1 below.

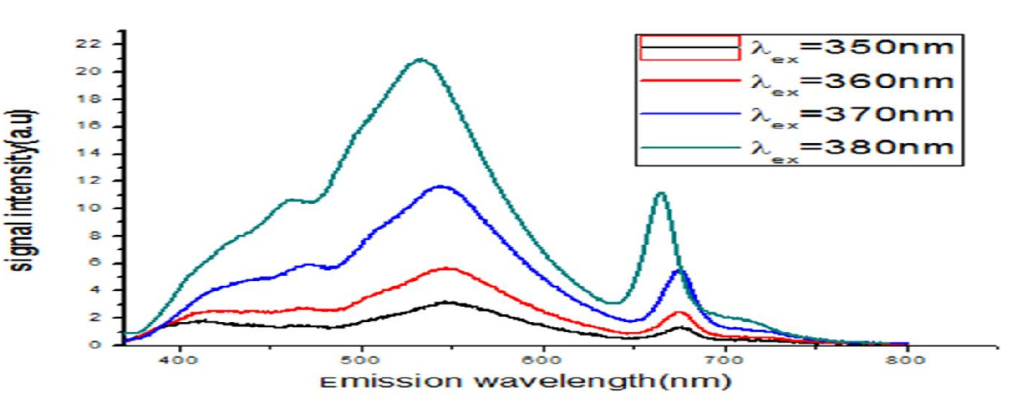

Figure 4.1: homemade Niger seed oil fluorescence

It was observed that as the wave length of excitation increases from 350nm to 380nm, the emission spectra underwent blue shift towards shorter wave length and higher intensity counts. This could be due to have high content polyunsaturated fatty acid. The emission peaks for vitamin E also shows it had higher proportion than chlorophyll.

4.1.2 Analysis of unrefined Niger seed oils

Similar to part 4.1.1, excitation wave length was varied from 350nm–380 nm with an incremental step of 10 nm. The corresponding emission spectra were observed to show fluorescence peaks in the range λ_{em}: 400nm – 725nm. Different peaks and bands of fluorescence were observed for each excitation wave lengths. In order to differentiate the different spectra colors blue, red and black colors were assigned to each excitation wavelength and their corresponding spectra: green color for spectra excited at λ_{ex}: 380nm, blue color for spectra excited at λ_{ex}: 370nm, red color for spectra excited at λ_{ex}: 360 nm and black color for spectra excited at λ_{ex}: 350 nm, respectively. From literature values it was concluded that emission spectra recorded in the range of band between 400 nm - 500nm unsaturated fat and fat□soluble vitamins. It was also observed that the fluorescence intensity increased by increasing exciting wavelengths from 350nm-380nm and there was also a blue-shift in the emission wave length. The peak of fluorescence for vitamin E around the band 548nm was found smaller compared to the home-made oil. This is expected to be the result of oxidation during factory process. The bands around 676 nm have been assigned to chlorophyll.

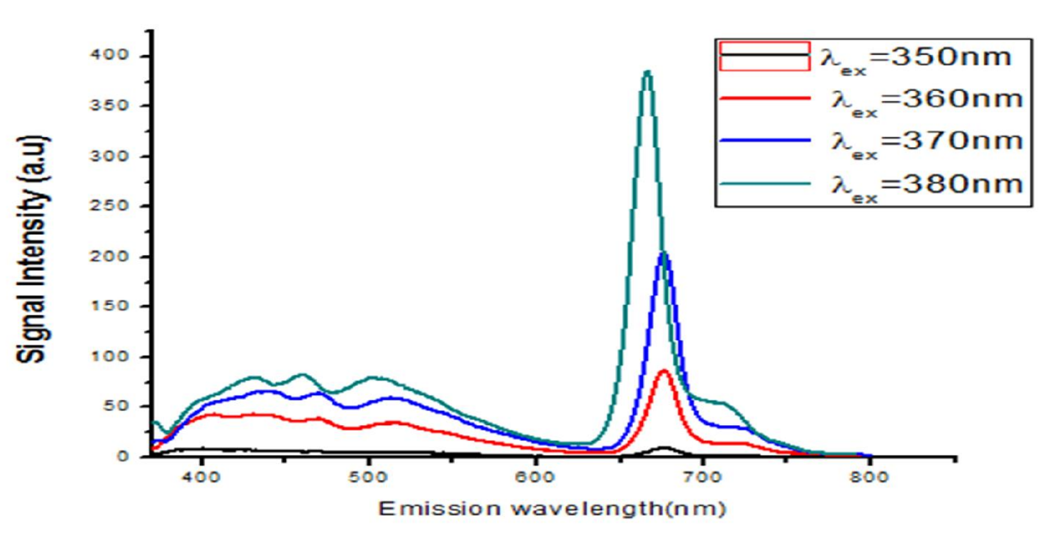

Figure 4.2: unrefined Niger seed oils

4.1.3 Analysis of refined Niger seed oils

A similar procedure was followed as in parts 4.1.1 and 4.1.2 to characterize refined Niger seed (Guizotia abyssinica) oil. Excitation wave length ranged from 350nm to 380 nm with an incremental step of 10 nm. Similar color naming was used for the emission spectra. A high intensity and many new peaks had emerged for the refined oil which could result from high temperature oxidation that induced chemical transformation. The band appearing between 400nm - 450nm was assigned to fat-soluble vitamins and lipids, hydroperoxides and the band appeared between 500nm - 550nm to vitamin E. The bands at 676 nm have been assigned to chlorophyll. Refined oils undergo bleaching so as to make it transparent, and heated at very high temperature to extract the maximum amount of oil from the seed. These processes could result in the loss of nutrients, color and taste. Refined oils are not good for health.

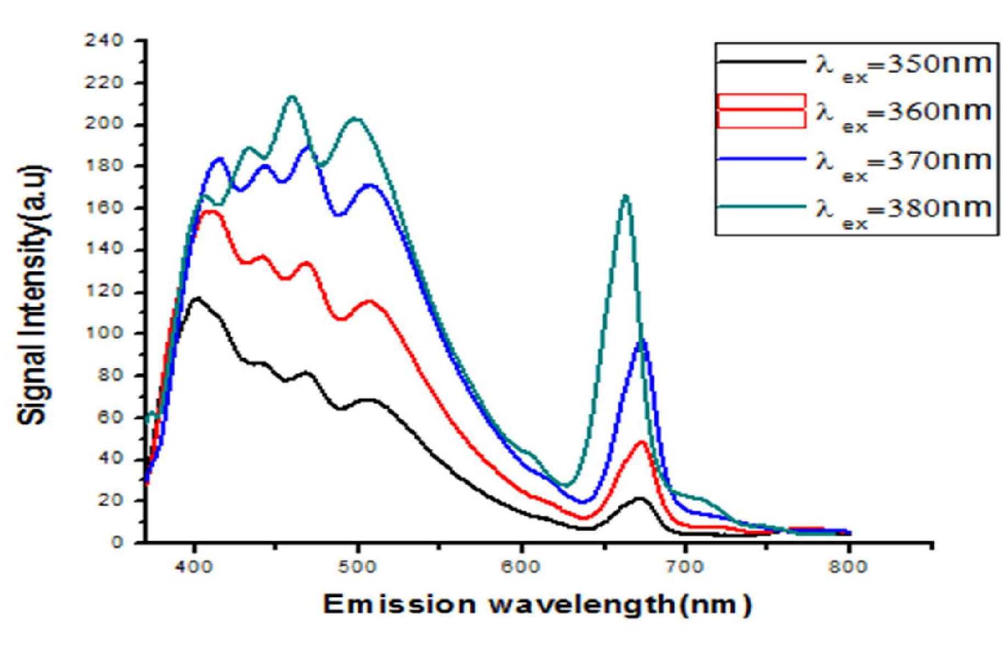

Figure 4.3: Refined Niger seed oil

4.1.4 Difference and similarity between homemade, refined and unrefined Niger seed oil

The major difference between Homemade, unrefined and refined Niger seed (Guizotia abyssinica) oils could be clearly observed from figure 4.4 below. The process of refining oil undergoes so many chemical processes, heating at very high temperature, and chemical bleaching so as to make it transparent and loose its odor. The oxidation during the heating process resulted in the emergence of new peaks and also loss of essential nutrients like vitamin and linoleic acid.

The unrefined oils retain many of the nutrients and antioxidants of the original seed/fruit. The only downside of unrefined oils is that they are often less stable than refined oils, meaning they are more prone to going rancid in a shorter time. It can depend on the temperature they are stored at and their exposure to light and oxygen. Oxidation occurs much faster at higher temperatures and with greater exposure. It was also observed that for the unrefined oils, the relative intensities of these lines were lower for the fat- and fat-soluble vitamins. However, both industrially produced oil samples, unrefined and refined oil samples, show similar behavior in fat- and fat-soluble vitamins. But since vitamin E is not heat stable, prolonged time and high temperature seem to have resulted in negligible pear in both samples. Thus, industrially manufactured oils may lack the antioxidant property of vitamin E. Homemade Niger seed (Guizotia abyssinica) oil is unrefined oil. It differed from the industrially produced oils in that it has larger peak and hence concentration of vitamin E. Unrefined Niger seed (Guizotia abyssinica) oil has high nutritionally valuable, as the high content of linoleic acid is thought to prevent cardiovascular diseases and is a precursor of structural components of plasma membranes and of some metabolic regulatory compounds. The unrefined Niger seed (Guizotia abyssinica) oil have low intensity of the peaks around 400nm - 475 nm. This may be due to their large content on monounsaturated fatty acids and phenolic antioxidants, which provide more stability against oxidation while a refined oil shows high intense peak around 445 nm - 475nm is due to fatty acid oxidation products formed as a result of the large percentage of polyunsaturated fatty acids present in these oils. The other the pH value of the home-made, unrefined and refined Niger seed (Guizotia abyssinica) oil were 5.7,5.4 and 5.1 respectively. So, the refined Niger seed (Guizotia abyssinica) oil has high acidity. As fluorescence is Ph sensitive the small variations in Ph values have resulted in spectral variation. This could be observed from fig. 4.4.

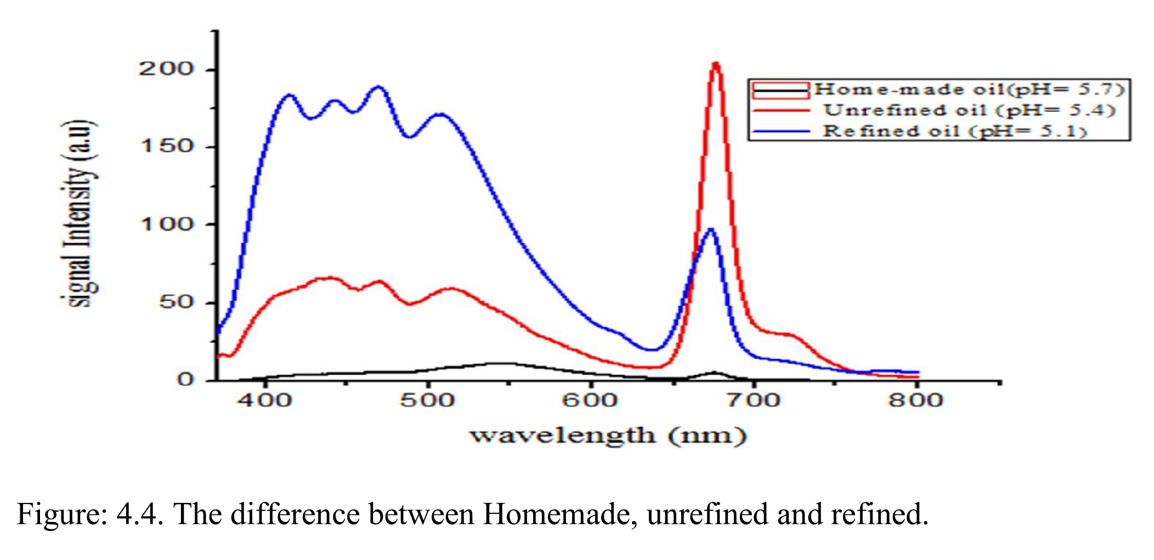

Figure: 4.4. The difference between Homemade, unrefined and refined.

4.2. **Determination of the fluorescence quantum yield**

Fluorescence quantum yield for Niger seed (Guizotia abyssinica) oils were determined the gradients of the integrated fluorescence area versus absorbance of Niger seed oils as shown in figure 4.5. Relative Fluorescence quantum yield values were calculated employing Eq (2). We used wavelength at 674 nm, the values industrial Niger seed (Guizotia abyssinica) oil to fix the value of quantum yield of reference sample 0.102. The linearity of the plot for industrial Niger seed (Guizotia abyssinica) oil indicates that it was possible to conclude that the sample taken reference has very good quantum yield.

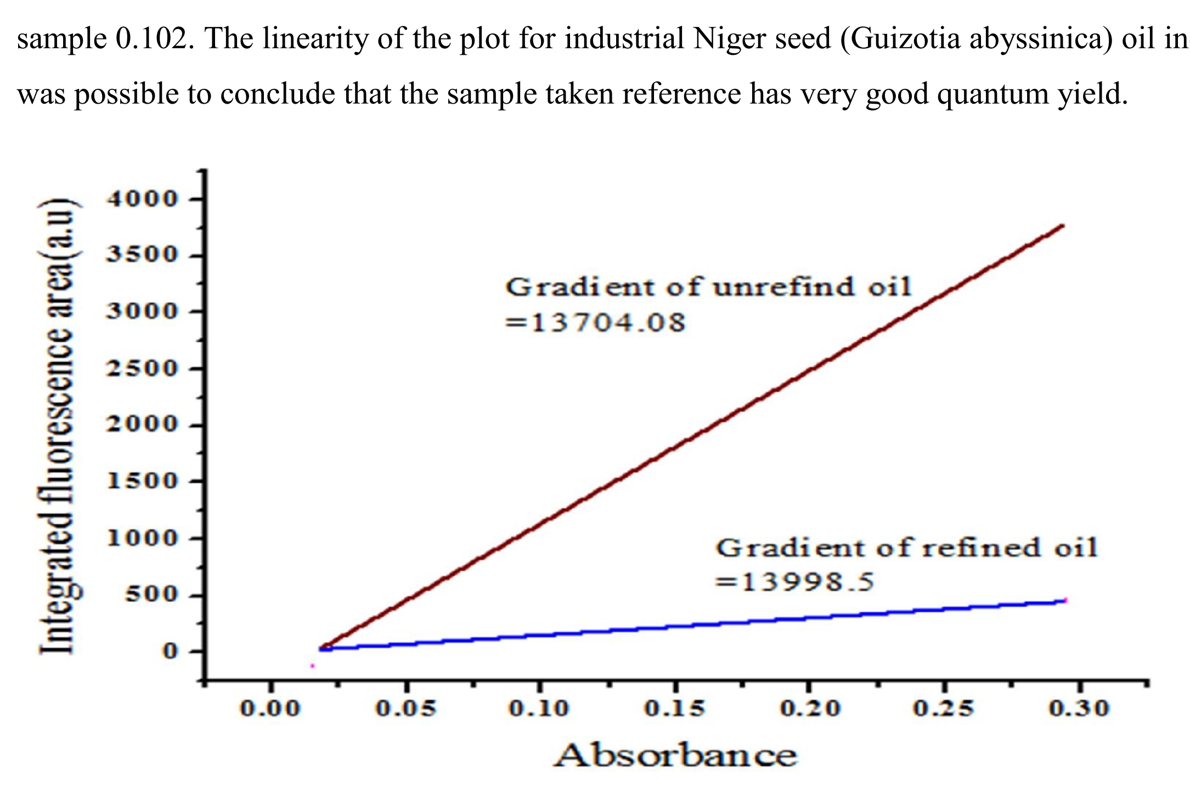

Figure 4.5. Integrated fluorescence intensity versus absorbance

Using the gradients obtained for the curves presented in Figure 4.5, fluorescence quantum yield was calculated using the formula described in the literature.

$$\Phi_{\text{refined oil}} = 0.102\left(\frac{13998.5}{137\ 0.4\ 8}\right) = 0.1042 = 10.4\%$$

5. Conclusions and Recommendations

5.1 Conclusions

The study successfully distinguished the major fluorescent compounds found in the home-made and industrially produced Niger seed oils, identifying the variations of each compound (oxidation products, vitamin E and chlorophyll) that result from the process of purification. The study proved the possibility of using fluorescence spectroscopy for analysis of the quality of vegetable oils. Fluorescence spectra for Niger seed oils with excitation wavelength varying from λ = 350nm-380nm by 10nm incremental steps resulted in corresponding emission spectra of major fluorophore compounds found in the samples. The major compounds found were fatty acids, vitamins and chlorophyll. It was also observed that heating oil results in oxidation of fatty acids that results in fluorescence of fat-soluble vitamins, but results in loss of essential antioxidant vitamins like vitamin E. It was also found that chlorophyll was in abundance in all the sample types, but with more intensity in the industrially produced oil samples. The calculated quantum yield of the fluorescence of the sample was 0.1042 or 10.4%. But this value could not be compared to theoretical value as related literature could not be found.

5.2 Recommendations

Depending on the findings and challenges faced during the research work, the researcher recommends the followings.

1. Thorough cleaning and sorting the grains are very essential to reduce the chance of quenching and reduction of chlorophyll concentration.
2. Low temperature and steady heating are essential to avoid the loss of essential vitamins and oxidation of fatty acids.

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