UV - Vis SPETROSCOPIC ANALYSIS OF HONE ADULTERATION WITH STANDARD SUGAR SOLUTIONS FROM LOCAL HONEY MARKETS IN DEBRE MARKOS CITY, ETHIOPIA

MSc IN LASER SPECTROSCOPY PHYSICS

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BY

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This is to certify the thesis entitled *UV – VIS spectroscopic analysis of honey adulteration with standard sugar solutions from local honey markets in Debre Markos city, Ethiopia submitted* authors by Barkilign Simachew in partial fulfillment of the requirements for the degree of Master of Science in physics with specialization in Laser spectroscopy. Therefore I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

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We, the undersigned members of that the Board of Examiners of the final open defense by Barkilign Simachew have read and evaluated his thesis entitled UV-VIS spectroscopic analysis of honey adulteration with standard sugar solutions (glucose) from local honey markets in Debre Markos City, Ethiopia and examined the candidate. This is, therefore, certifying that the thesis has accepted in partial fulfillment of the requirements for the degree.

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I hereby declare that this MSc specialty or equivalent 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.

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This MSc Specialty or equivalent thesis has been submitted for examination with my approval as thesis advisor.

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Lists of Abbreviations

UV-Vis	Ultra Violate Visible
MO	Molecular Orbital
AO	Atomic Orbital
AU	Absorbance Unit
EMR	Electromagnetic Radiation
LOD	Limit OF Detection
LOQ	Limit Of quantification
IR	Infrared
NIR	Near Infrared
MIR	Mid Infrared
FIR	Far Infrared
NMR	Nuclear Magnetic Resonance
FT-IR	Fourier Transform Infrared

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ABSTRACT:

This research was designed to evaluate the quality and detect adulteration from commercial honey available in the market around Debre Markos city and the surrounding areas using UV-Vis spectroscopic technique. For that, pure (real) honey samples were collected directly from the hives and commercially available honey samples were bought from the supermarket. In this study more concerned qualitative and quantitative analyzed using UV-Vis spectrometer were discriminated pure honey from adulterants honey bought from super market. For the reference 3 pure honey collected from hive were used. Calibration curve was generated by adulterating the pure honey with known concentrations of glucose syrup (0% - 30%). After that the absorbance of glucose, which is maximum at wave length of 490.56nm, was measured on the honey samples obtained from market and converted to glucose concentration using the calibration curve. The results show that the sample bought from market has more glucose concentration compared to pure honey. The order of average concentration of glucose obtained honeys collected in varies market places are as follows: 6.214% (6ADB) < 20.53% (4MHR) < 23.878% (3LUM) < 24.417% (8DE) < 25.934% (1DZ) < 26.146% (2SHW) < 27.016% (5MKO) < 28.597% (7GW).The sample from 6ADB (6.214%) show lower glucose concentration than others and more close to pure honey. The experimental results are validated in terms of the widely used analytical parameters for method validation such as precision, linearity, limit of detection (LOD) and limit of quantification (LOQ).

Key words: *honey, adulteration, Beer-lambert's law, UV-Vis spectroscopy*

CHAPTER ONE

1. INTRODUCTION

1.1 Background of Study

Honey is defined as the excretions of insects sucking on the parts of plants. Honeybees are the most well-known plant-sucking insects and can collect and transform honey or combine with specific substance of their own (enzymes), and deposit, dehydrate, store and leave honey in the honeycomb to ripen and mature. Honeybees collect pollen and nectar from a variety of flowering plants and convert it into the wax and honey. Only worker honeybees forage for food, consuming as much nectar from each flower as they can. After foraging, worker honeybees return to the hive/comb and pass the collected nectar to the other worker honeybees. This worker holds the nectar on her tongue until the liquid evaporates, creating honey. The honey is then stored in a cell within the hive/comb[1]. Various physical types (pressed, centrifuged and drained) and forms (comb, chunk, crystallized or granulated, creamed and heat-processed) of honey are on the market [2]. It contains unique natural color, aromas and flavors. Honey is an energy source that has the major carbohydrates by dry weight (glucose, fructose, maltose, and sucrose), lesser amounts of water, and a large number of minor components such as proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols[3]. Adulterate honey is produced directly by adding syrup and indirectly by feeding bees with syrup. Authentication of honey has primary importance for both industries and consumers. Besides good quality honey cannot contain more than 18% water. High water level can cause the process of fermentation of honey and loss of its quality. Honey is adulterated in the practice by cheaper, commercially available sugar syrups with similar composition. Among the different techniques optical spectroscopy techniques are more preferable and more accurate in identification and quantification of these adulterates in food and drinks. Spectroscopy is the interaction between waves originated in the electromagnetic spectrum and molecules present in the sample matrix under analysis [4]. Some of these spectroscopic techniques include FT-IR, Raman spectroscopy, and UV-Vis spectroscopy. From these techniques in my study UV-Vis spectroscopic technique is implemented to study sugar adulteration of commercially available honey. UV-Vis spectroscopy is a sensitive method in molecular spectroscopy that uses ultraviolet and visible light in the wavelength range between 200nm and 800nm [5].

1.2 Statement of the Problem

Quality is an important aspect for both domestic and international market as it helps to achieve competitive premium prices and promote human health[6]. There have been serious reports of poor quality honey on the market and their safety is also increasingly being questioned [7]. There is common perception that honey which is sold in markets of Ethiopia is poor quality and suspected of adulteration with different adulterants to increase quantity there by maximizing profit from sales in the market. Such kind of honey may have poor physicochemical, organoleptic and hence poor functionality. Reports have indicated that such adulterant honey does not have any nutritional and medical values[3]. Unfortunately not much work has been reported on the quality and adulteration, detection techniques especially for commercially available honey in Ethiopia with exception of few studies on local traditional test practiced by Beekeepers and customers. The studies done on the Ethiopia honey were focused on the physicochemical and botanical origins of honey sample (PH, free acidity, Ash content, color, electrical conductivity, HMF, sugar content) [8]. At present there is no enforcement by authorities to prevent adulterate honey from regulating in the market in Ethiopia as there is a lack of data on the honey to identify, characterize and differentiate between natural (pure) and adulterate honey. Beekeepers, honey processors and customers heavily rely on their own experience and observation to determine whether the honey meets the quality requirement for the above and many other reasons, it is important to investigate and understand the detection of honey adulterate and this study aim at the authentication of quality and determination of adulteration in honey based on Ultra violet visible spectroscopy analysis for the consumers or regulatory authorities to check before buying or prior approval of honey to be sold in the region of East Gojjam specially Debra Marko's city and surrounding area.

1.3. Objectives of the study

1.3.1General Objective

The general objective of this study is to characterize sugar adulteration of honey samples collected from markets in Debre Markos city and the surrounding areas using UV-Vis spectrophotometer.

1.3.2 Specific Objectives

- Compare the change in glucose content in pure and deliberately sugar adulterated honey.

- Determine the presence and concentration of adulterant in the commercially available honey bought in the market from specified areas above.
- Compare the results with literature values.

1.4 Significance of Study

Honey is one of the most commercialized bee products. The study is carry out keeping in view the recently emerging concern of low quality due to addition of various substances on natural honey to increases volume of honey on the market. This research will design to evaluate the quality by detecting and quantifying sugar adulteration from commercially available honey in Debra Marko's a city and surrounding area. So the result of this study will provide information to the consumers and also pave the way for researchers to perform similar works at different geographical locations. It also initiates the authorities to give attention on the matter. Quality control of honey is important to determine its suitability for processing and to meet the demand of the market. Honey is used for nutritional and medical purpose. Therefore keeping honey quality for the honey producer, consumers and foreign exchange many countries.

Finally, this research paper is providing information on selected pure honey from hive and selected from supermarket honey samples from selected districts of east Gojjam Debre Marko's city, Ethiopia

1.5 Scope of the study

- ✓ The study will concentrate on Debre Markos City and its surrounding area Ethiopian honey from distinct geographical regions rather than attempting to encompass honey from various global areas. This targeted approach allows for a more detailed and comprehensive analysis of a specific pure and unknown super market honey region.
- ✓ The research paper will provide information on selected pure honey from hive and supper market honey samples only from selected districts of east Gojjam Debre Marko's city, Ethiopia.
- ✓ Measure absorbance of the different concentrations prepare from the various intensities measured at the respective wave lengths where maximum absorbance occur using spectrometer.
- ✓ Plot a concentration versus absorbance curve (calibration curve) for standard sugar (glucose) solution.

- ✓ Validate the experimental results in terms of linearity, limit of detection and limit of quantification, and relative standard deviation.

1.6 Limitations of the study

Due to time and budget constraints, the honey samples of only Three(3) pure honey and Eight(8) bought randomly from super market available in local Ethiopian markets will be addressed.

CHAPTER TWO

2. THEORETICAL BACKGROUND

This chapter is sub divided in to five parts. In the first part I will present general principles of spectroscopy, in the second part the interaction of EMR with molecules, in the third part the UV-Vis spectroscopy including its principle, instrumentation and applications for detecting food and drink adulteration, particularly adulteration of honey by sugar is discussed and the fourth the composition of honey and the common adulterants of honey and fifth some of the recent works that are related to this work are reviewed.

2.1. Spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation with matter involving either absorption, emission or scattering of radiation by the system under study. Atomic and molecular spectra can provide detailed information about the structure and chemical properties of the system. Nowadays spectroscopic tools are routinely used in quantitative and qualitative chemical analysis and the characterization of new molecules and materials. It plays an essential role in diverse fields as the elaboration and testing of theoretical models, synthetic chemistry, the study of reaction mechanisms or biochemistry and materials science. The most common types o f spectroscopic techniques that are used for chemical analysis include atomic spectroscopy, UV-Vis spectroscopy, infrared spectroscopy, Raman spectroscopy and nuclear magnetic resonance(NMR)[9].

2.1.1. Electromagnetic Radiation

According to elementary physics a charge is surrounded by an electric field and a moving charge that is an electrical current also generate a magnetic field. Besides this accelerated charges emit electromagnetic radiation, while radiation accelerates charged particle. Maxwell's equations condense all these phenomena describing the dynamics of free charges and current and providing the foundation of classical electromagnetic theory and the interaction of light and matter. These equations describe macroscopically the behavior of charges in electric and magnetic fields. Among this propagation of light in vacuum is easier to describe, since light in matter is constantly absorbed and re-emitted. Solution of Maxwell's equations without sources (charges or current) leads to the equations of propagating electromagnetic wave[10]. Electromagnetic radiation (EMR) is form of energy whose behavior is described by the properties of both wave and particle. Properties like

reflection, refraction, diffraction, etc. describing wave nature of EMR on the other hand properties such as absorption, emission, scattering is better describing particle property of EMR as discussed below.

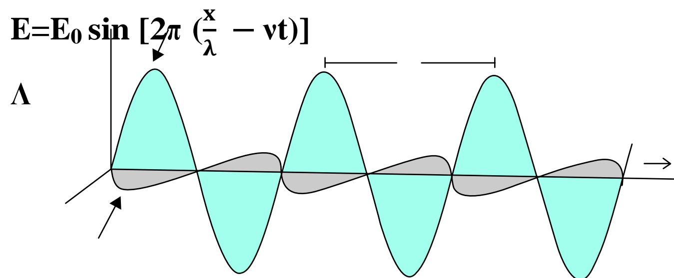
Wave Properties of Electromagnetic Radiation

Electromagnetic radiation can be describe as a wave phenomenon formed by the combination of electric(E) and magnetic(H) fields which oscillate in phase orthogonal to each other and orthogonal to the direction of propagation as well. And it is characterized by several fundamental properties including velocity (c), amplitude (A), frequency (v), phase angle (ϕ) [11]. The radiation and matter usually interact through the electric components. The relation between frequency and wavelength is given by:

$$v = \frac{c}{\lambda} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.1$$

Where, v is the frequency, inverse of wave period (s^{-1} or Hz), λ is wavelength (nm) and *c* is the velocity of propagation of the wave.

$E=E_0 \sin [2\pi (\frac{x}{\lambda} - \nu t)]$



$H=H_0 \sin [2\pi (\frac{x}{\lambda} - \nu t)]$

Figure 2.1 Perpendicularity of electric and magnetic field.

E_0 and H_0 correspond to the amplitude that is maximum value of the electric and magnetic fields, respectively. Wave number is reciprocal of wavelength, the relation is

$$\tilde{v} = \frac{1}{\lambda} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.2$$

It is known as wave number (ṽ) and is usually given in unit's cm^{-1}. The relationship between wavelength and frequency is

$$\lambda = \frac{c}{v} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.3$$

Particle Properties of Electromagnetic Radiation

When matter absorbs electromagnetic radiation it undergoes a change in energy. The radiation consists of a beam of an energetic particle called photons. When photon absorbed

by a sample is absorbed and its energy acquired by the sample [12]. The energy of photons is related to its frequency, wavelength and wavenumber.

$$E=h\nu=\frac{hc}{\lambda}=hc\tilde{\nu} \qquad 2.4$$

Electromagnetic spectrum

Depending on its frequency and wave length an electromagnetic wave is divided. The electromagnetic spectrum covers large range of radiation frequencies, which the visible region only a small part.

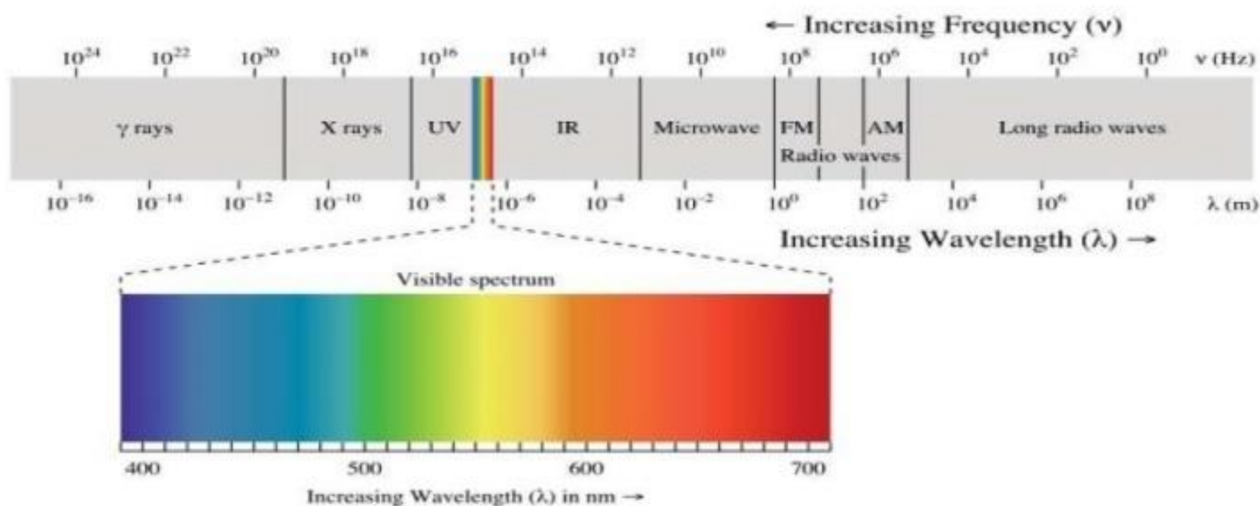


Figure 2.2 Electromagnetic spectrum of range. The color insert show the visible spectrum source modified from Zedh (www.commons.Wikipedia.org).

2.1.2. Molecular Structure

Molecular structure plays a crucial role in spectroscopy as different molecular arrangements and functional groups affect how molecules interact with electromagnetic radiation. Atoms have discreet atomic orbitals; similarly molecules have also molecular orbitals. Understanding the nature of these orbitals is crucial to understand the properties of these atoms and molecules. When two or more atoms combined to forms a molecule, each atom will share their atomic orbitals and electrons and forms a molecular orbital. The simplest molecular orbital is the hydrogen molecule. Each hydrogen atoms contribute their *s* orbitals and forms the bonding σ and anti-bonding σ* molecular orbitals of the hydrogen molecule. The bonding orbital is located at lower energy than the atomic orbital of the individual hydrogen atoms, while the anti-bonding orbital is relatively at higher energy than the *s* atomic orbitals of the

individual hydrogen atoms as shown in the figure below.

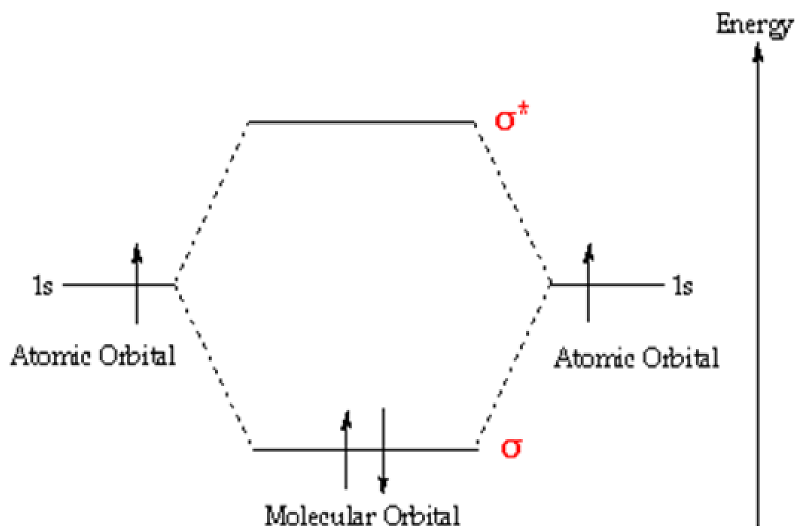


Figure 2.3 Molecular orbitals of hydrogen molecule.

When electrons occupy the bonding orbital they will form a stable molecular bond between the atoms as the charge density between the atoms is large. On the other hand, when electrons occupy the anti-bonding orbital the charge density between the atoms will be minimum and their will not be stable bond between the atoms and as a result the molecule will dissociate in to atoms. When several atoms form large molecules, their molecular orbital becomes more complicated. In addition to the σ molecular orbitals, there will be also π orbitals, n-orbitals (non- bonding orbitals).

In molecules in addition to the electronic energy levels (molecular orbitals) they also have vibrational and rotational degrees of freedoms which results vibrational and rotational energy levels of the molecules.

According to Born-Oppenheimer approximation, the total energy of a molecule is the sum of translational, rotational, vibrational and electronic energies, i.e. $E_{tot} = E_{tran} + E_{rot} + E_{vib} + E_{el}$. It is found that the translational energy is negligibly small. Hence Born-Oppenheimer approximation can be written as $E_{tot} = E_{el} + E_{vib} + E_{rot}$……………………………………………………………2.5

The amount of energy a molecule possesses in each form is not a continuum but a series of discrete levels or states. The differences in energy among the different states are in the order: $E_{electronic} > E_{vibrational} > E_{rotational}$

2.1.3. Interaction of Electromagnetic radiation with molecules

The knowledge of the physics of molecules, atoms, nuclei and elementary particles is obtained through interaction of matter with electromagnetic radiation [13]. The electromagnetic radiation emitted or absorbed results in transitions between bound states of the quantum system. Another way in which we can acquire knowledge of the physics of matter particles is through scattering experiments. The interaction of electromagnetic radiation with matter can result absorption, emission and scattering processes. Therefore the principle of spectroscopy arises from these processes. Spectroscopy methods can be categorized depending on the types of radiation, interaction between the energy and the material, the type of material and the applications the technique is used for [14]. Depending on the material that interacts with electromagnetic radiation, spectroscopy can be categorized as atomic spectroscopy, molecular spectroscopy, and solid state spectroscopy.

2.2. Atomic and Molecular spectroscopy

Atomic spectroscopy deals with the interaction of electromagnetic radiations with atoms. Since atoms have sharp energy levels, the spectroscopy measured in atoms is sharp. Since atoms do not vibrate or rotate, only transitions between electronic energy levels will occur. Such transitions will result a sharp peak spectra.

Molecular spectra, on the other hand, are observed when a molecule absorb or emit electromagnetic radiation with a resulting increase or decrease in energy. These processes obey the laws of quantum mechanics as pair of energy levels can participate in energy changes and as to the extent of the radiation absorbed or emitted. Unlike atoms in which the quantization of energy results only from the interaction of the electrons with the nucleus and with other electrons, the quantization of molecular energy levelscontainsadditionalinteractionwiththeelectronsandthenucleiofotherconstituent atoms of the molecule and the resulting absorption or emission of radiation involving these energy levels encompasses several mechanisms [15]. This interaction results in transition between rotational, vibrational and electronic energy levels. Symmetry plays a fundamental role in molecular spectroscopy [16]. The symmetry of molecules provides the basic information required for the classification of molecular states and determines the electron and intensity rules several types of spectroscopies. The rotational, vibrational and other energies of molecules are quantized. A particular molecule can exist in a variety of energy levels and can move from one level to another only by a sudden jump provided the appropriate amount of energy.

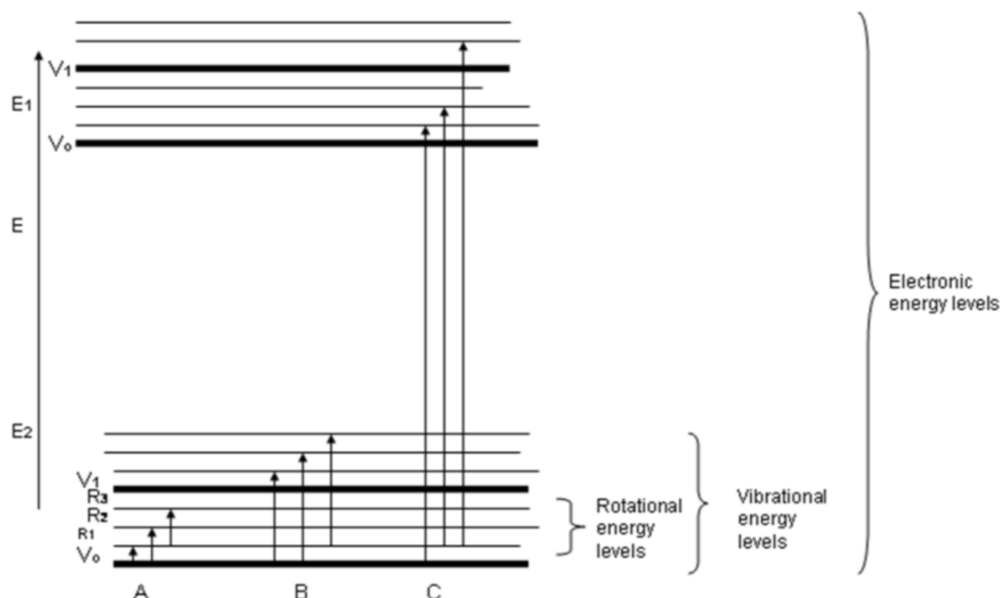


Figure 2.4.Molecular orbital structures encompassing electronic, vibrational and rotational orbitals

Table 2.1 Different type's spectroscopy

Type of spectroscopy	Type of transition	Wavelength range
Gamma ray	Nuclear	$(10^{-10} - 10^{-14})$ m
X – ray	Inner K and L Shell electrons	$(10^{-9} - 6*10^{-12})$ m
Ultraviolet rays	Valance and middle shell electrons	$(3.8*10^{-7} - 6*10^{-10})$ m
Visible	Valance electrons	$(7.8 - 3.8)*10^{-7}$ m
Infrared	Molecular rotations and vibrations	$10^{-3} - (7.8*10^{-7})$ m
Microwave	Molecular rotations	0.3 m- 1mm
Radio waves	Spin	Few Kms – 0.3 m

1. Rotational spectroscopy (microwave spectroscopy): The rotational transition energies of molecules in gas phase are measured using microwave radiation. It accomplishes this through the interaction of the electric dipole moment of the molecules with the electromagnetic field of the exciting microwave photon. The reason why the molecules must be in the gas phase is due to intermolecular interactions hindering rotations in the liquid and solid phases of the molecule. Concerning the magnitude of the various effects that govern a rotational spectrum, it should be noted that the frequency (energy) of a rotational transition is mainly determined by the rotational constant, *B*, where**, J** is rotational quantum number.

$$E_R = B J (J+1) \qquad 2.6$$

with their values ranging from less than 1 GHz for large molecules up to 1300 GHz for a molecule as small. Therefore, detections in the millimeter and sub millimeter wavelength range are usually limited to the study of small to medium-sized molecules [17]. While measurements in the centimeter wave region allow the investigation of larger molecules.

2. Vibrational Spectroscopy (Infrared spectroscopy): when electromagnetic radiation in the infrared region of the electromagnetic spectrum interacts with a molecule it can cause vibrational excitation of a molecule. The vibrational energies of the molecules are usually modeled using quantum harmonic oscillator where the discreet molecular energy states are given by:

$$E_n = h\omega \left(n + \tfrac{1}{2}\right) \qquad \text{2.7}$$

Where, n is vibrational quantum number.

Vibrational spectroscopy is one of the most versatile techniques available for the measurement of molecular species in the analytical laboratory today. The classical view of infrared spectroscopy was based on what is termed the mid-infrared. This region covers the fundamental vibrations of most of the common chemical bonds featuring the light- to medium-weight elements. In particular, organic compounds are well represented in this spectral region. Today, the mid-infrared region is normally defined as the frequency range of 4000 cm^{-1} to 400 cm^{-1} the region below 400 cm^{-1} is now generally classified as the far infrared, characterized by low frequency vibrations typically assigned to low energy deformation vibrations and the fundamental stretching modes of heavy atoms. There is only one IR-active fundamental vibration that extends beyond 4000 $cm\text{-}^{1}$.

3. Electronic Spectroscopy: when EMR in Ultra violet and visible region of the EM spectrum, interact with the molecule will undergo a transition from its ground state to the excited state. The spectroscopic technique that used this principle is called electronic spectroscopy. One of the common techniques in this category is the UV-Vis spectroscopy which will be the focus of this study. Electronic transition a molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition from lower to higher energy levels.

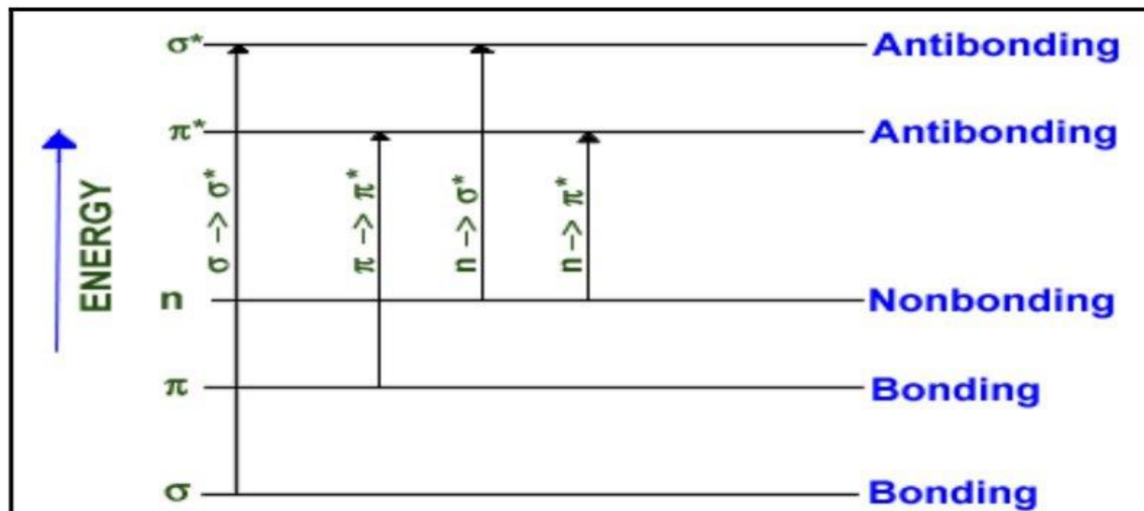


Figure 2.5 Electronic transitions of σ, π and n electrons

1.σ to σ∗ transitions: An electron in a bonding s orbital is excited to the corresponding anti-bonding orbital. The energy required is large. For example, methane (which has only C-H bonds, and can only undergo σ to σ∗ transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ to σ∗ transitions are not seen in typical UV-Visible spectra (200 - 700 nm).

2. n to σ∗ transitions: Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of n to σ∗ transitions. These transitions usually need less energy than σ to σ∗ transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm. The number of organic functional groups with n to σ∗ peaks in the UV region is small.

3. n to π∗ and π to π∗ transitions: Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π∗ excited state. This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm). These transitions need unsaturated group in the molecule to provide the π electrons. Molar absorptivity's from n to π∗ transitions are relatively low, and range from 10 to100 L mol-1 cm-1. π to π∗ transitions normally gives molar absorptivity's between 1000 and 10,000 L mol-1 cm-1[18].

2.3. UV-Vis spectroscopy

UV-Vis spectroscopy is an important physical tool which exploits light in ultraviolet, visible, and near infrared range of electromagnetic spectrum. UV-Vis molecular spectroscopy stands out due to its dependable, quick, and affordable technology that is common in routine analysis laboratories. UV-Vis spectroscopy provides a chemical fingerprint that can show structural and chemical information and allows quantitative and qualitative examination of complicated samples [19].

UV-Vis spectroscopy can also be employed for determining the concentration of the absorbing species for a fixed path length [20]. Instrument employed for ultraviolet-visible (UV-Vis) spectroscopy is called UV-Vis spectrophotometer. It measures the intensity of light passing through sample (I) and compares it to the intensity of the light before it passes through the sample (I_o). The ratio is called the transmittance and usually expressed as a percentage [21]. This technique is also used to assess the concentration or amount of a given species using the Beer-Lambert law. This law relates the absorption of a radiation to properties of the material which is passing through sample. There is a logarithmic dependence between transmission (T) of light and the product of the absorption coefficient of the substance α, and distance the beam travels through the material (i.e. the path length).This can be used to analyze liquids, gases and solids by using radiation energy corresponding to ultraviolet (UV), visible (Vis) and near infrared (NIR) regions of electromagnetic spectrum. Consequently, predetermined wavelengths in these regions have been defined as: UV 300 - 400 nm; Vis: 400 - 765 nm; and NIR: 765 - 3200 nm.

2.3.1 UV–Vis Instrumentation

In UV–Vis spectroscopy, light from a UV–Vis source is passed through a monochromatic to isolate a specific group of wavelengths, and then passed through the sample where a specific wavelength is absorbed, and finally, the light reaches the detector. The main function of a spectrophotometer is to measure the absorbance or transmittance of a sample as a function of the wavelength of electromagnetic radiation. The basic components of a spectrometer are:

1.Source of energy or light source:-Tungsten filament radiation, Deuterium lamp, Hydrogen lamp, Tungsten lamp, Xenon discharge lamp, Mercury arc lamp, continuous over UV region is generally used as light source and Hydrogen-Deuterium lamps are most widely use and suitable in continues light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations more specifically they emit the radiations of 375 nm [22]. UV- visible spectroscopy requires a continuous source and use hydrogen lamp (160-380 nm), Deuterium lamp (160- 450 nm) or tungsten lamp (330-900 nm).

2. Monochromatic: -is composed of prisms and slits. It is the wavelength selector or device for isolating a narrow range of wavelength. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms.

3. Sample stage (sample holding) and reference cells: - One of the two divided beams is passed through the sample solution and second beam is passing through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz cuvette. Glass can't be used for the cells as it also absorbs light in the UV region.

4. **Detector:** - Detectors are photosensitive devises that convert (transforms) photon or light energy to electric current or voltage. There are two broad classes of **spectroscopic** and **thermal** detector and photon detector.

Thermal detector: is used for infrared spectroscopy. The absorption of infrared photons by a thermal transducer increases its temperature, changing one or more of its characteristic properties. Finally a detector electrical signal is sent to a signal processor where it is displayed in a form that is more convenient for the analyst.

Photon detector: contains a photosensitive surface that absorbs radiation in the ultraviolet, visible, or near IR, producing an electrical current proportional to the number of photons reaching the detector. On the other hand, since infrared photons do not have enough energy to produce a measurable current with a photon detector

The transmitted radiation falls on the detector which determines the intensity of radiation absorbed and detector employed are, Photo voltaic cell, phototubes or photomultiplier tube.

There are two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the 13 times radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells. Two type of detector using in UV –visible spectroscopy, the photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. They have fast response times. Intense light damages photomultipliers, they are limited to measuring low power radiation.

The Photodiode detector: It is an example of a multichannel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously**.**

Amplifier: The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servo meter. The current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

5. Recording devices: -Computer stores all the data generated and produces the spectrum of the

desired compound. Signal output to display the results conveniently for the analyze [23].

Double beam spectrophotometer is an analytical instrument in which the light beam coming from the light source splits into two fractions. One fraction acts as the reference (the reference beam) and the other fraction passes through the sample (sample beam) under investigate.

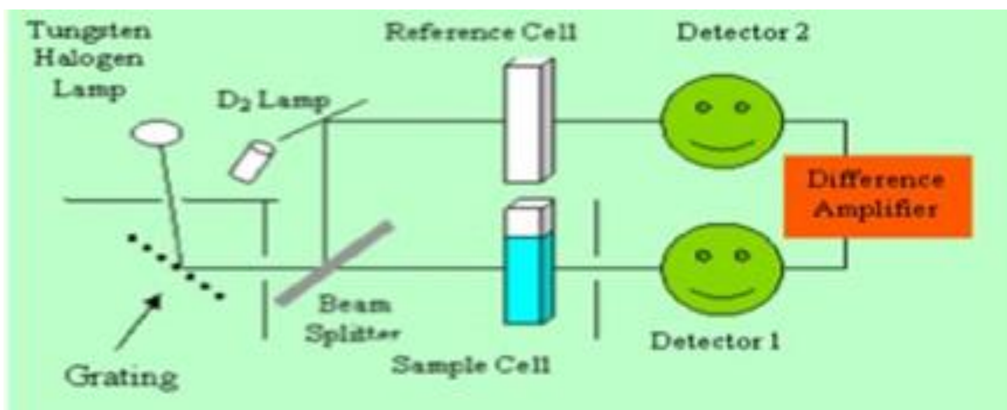


Figure 2.6 Basic instrumentation of double beam UV-Visible spectrometer

2.4. Beer – Lambert Law

Beer-lambert law states that absorbance of solution is directly proportional to the concentration of the absorbing species in the solution and the path length.

Mathematically it is given by: **A=εcl** where, **c** is the concentration of the sample, **l** is the path length through which the light travels in the sample, and **ε** is the molar extinction coefficient at a particular wavelength. UV-Vis-NIR spectrometer can monitor absorbance or transmittance in UV–visible wavelength range. The relation between incident light of intensity 'I_o' and transmitted light of intensity 'I' is described as follows.

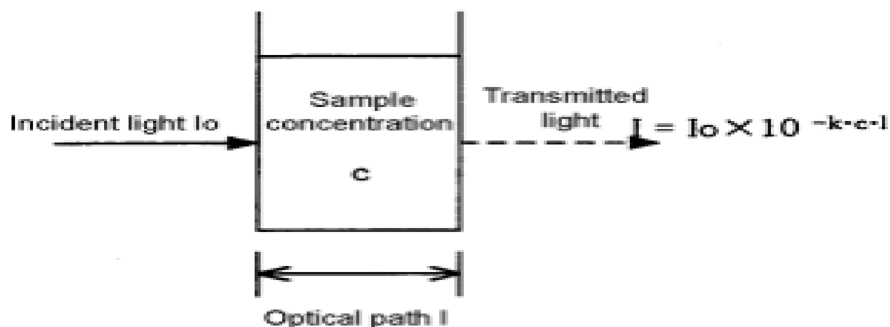


Figure 2.7 Schematic view of Beer-Lamberts law

Transmittance (T) is given by I/I_o and $(I/I_o)*100$ gives transmission rate (T %). Absorbance (abs) is the inverses of transmittance and given by $\log\frac{1}{T} = \log\left(\frac{Io}{I}\right)$

$T = I/I_o = 10^{-kcl}$

$$Abs = \log(1/T) = \log(I_o/I) = -kcl \text{ or } Abs = \log_{Io} / I = \log 1/T = -\log T = kcl$$

Here, k represents constant of proportionality. Transmittance does not depend on sample concentration, whereas absorbance shows proportionality with concentration of sample (Beer's law) and optical path. Bouguer's law additionally, when optical path is 1cm and concentration of targeted material is 1mol/l; k is described as molar absorption coefficient and denoted as 'ε'.

2.5. Application of UV-Vis for detecting adulteration

There is an increasing need for real-time analytical tools to monitor bioprocess and fermentation in biological and food applications [24]. The driving force behind this transition from traditional laboratory analysis to process monitoring is the need for more rapid process control information to comply with safety and environmental guidelines and reduce the costs of production [25]. Spectroscopy is the interaction between waves originated in the electromagnetic spectrum and molecules present in the sample matrix under analysis. The two main spectroscopic techniques used in food analysis are atomic and molecular spectroscopy [4]. The development and implementation of these spectroscopy methods in the field of food analysis [26] are based on the interactions between matters and light that resulted in absorption, emission, and scattering events characteristic of the sample [4]. In food analysis, these applications are based on a variety of spectroscopic methods and techniques that use the benefits of different wavelength ranges including ultraviolet and visible (UV-Vis), near-infrared (NIR), mid-infrared (MIR), far infrared (FIR), Raman, microwaves, radio waves, and NMR. UV-Vis spectroscopy is a sensitive method in molecular spectroscopy that uses ultraviolet and visible light in the wavelength range between 200nm and 800nm. As stated above this spectroscopic method based on the absorption, scattering, diffraction, refraction and reflection properties of the sample analyzed. The absorption of UV and Vis light is restricted to certain

molecular functional groups called chronophers in which electrons are excited at different frequencies. As in many spectroscopic applications, the Beer–Lambert law describes the correlation between light absorption by the molecule, the light path length of the sample, and the concentration of the absorbing molecules in the liquid medium. Therefore, on the basis of the absorption measurement, the presence and concentration of analysts in the food matrix as a consequence of its chemical and physical properties can be determined and quantified[5].

2.6. Honey and Chemical composition of Honey

Honey is one of humankind's oldest food products that preserve human health and shields them from various diseases, such as cancer, a cold, sore throat, etc. [27]. Composition of Honey is a product with complex chemical composition: it contains plant pigments (carotenes, xanthophyll's and chlorophyll), mineral substances, sugars, and various impurities. The main components of honey are sugars, which are presented by fructose (37.20%), glucose (31.28%), sucrose (1.31%), maltose (7.31%), etc. The composition of honey also reflects the contaminants which are present in the area of bee activity. Besides, honey contains more than 200 substances including organic acids such as acetic acid and gluconic acid [28]. Vitamins and minerals are present in a very small quantity, particularly iron and copper which are responsible for the redox properties of honey and potassium, being the most abundant. Honey also contains trace amounts of niacin, calcium, copper, riboflavin, iron, magnesium, and zinc [29]. Among honey, constituents are also amino acids, hydroxyl methyl furfural and phenolic compounds. Flavonoids present in honey are comprised of flavones, while phenolic acids are substituted cinnamon acids and benzoic acids. These compounds are the main contributors to the color, taste, and aroma of honey [30].

2.6.1. The common Adulterants of Honey

The concentration of sugars can be used as a criterion of honey adulteration, which is provoked with the artificial addition of syrup, sucrose that is hydrolyzed with acids, starch or beetroot treacle in honey [31]. That is why precise quality evaluation of honey has long been the goal of many investigators and specialists who are related to honey-breeding [32]. Adulterates honey is produced directly by adding syrup and indirectly by feeding bees with syrup. Honey adulteration does not pose significant health problems, but authentication of honey has primary importance for both industries and consumers. The common commercial sugars used for honey adulteration are cane sugar, beet, maltose syrup, corn syrup (CS), high-fructose CS (HFCS), glucose syrup (GS), sucrose syrup,

inverted syrup (IS), and high-fructose inulin syrup (HFIS) [33].Sugar, ripened banana, wheat flour, potato, maize flour, pollen, empty combs, melted candy, molasses, and hot water[8].

In this MSc. study UV-Vis spectroscopic analysis of honey adulteration with standard sugar solution from local honey markets in Debre Markos city and surrounding area is studied.

2.7. Review of related Literatures

Honey adulteration can be direct or indirect. Direct adulteration means that substance is added directly to honey. Indirect adulteration happens when honey bees are fed adulterating substance. Indirect adulteration of honey is accomplished by feeding honey bees with industrial sugars at the stage when broods become naturally available. Such indirect adulteration is extremely difficult to detect. Methods of detecting indirect adulteration Cordella et al. (2005) describe the development of high performance an-ion exchange chromatography with Pulsed Amperometric Detection (HPAEC-PAD) for the analysis of honey to detect adulteration combined with chemo metric techniques for processing chromatograms for better discrimination of pure and adulterated honey. This method was investigated using honey samples containing between10%and40%ofdifferentindustrial sugar syrups used for the feeding of honeybees [34].

Nunes A.et al. (2023) analyzed pure honeys by UV–Vis spectrophotometry all individual samples were heated in a water bath (15 min, 40 ◦C) for complete solubilization of the sugar crystals and homogenization of the honey constituents. Furthermore, 0.6 mg honey was weighed, added of 10 mL distilled water, following magnetic stirring (5 min) and centrifuging (2000 rpm, 5 min). The UV-VIS scanning spectra (n = 3/sample) were recorded over the ultraviolet (200–380 nm) and visible (380–800 nm) spectral windows, through a Biochrom Libra S22 UV-Vis spectrophotometer with a 2 nm/data point resolution. The spectra were obtained using the Biochrom software (Version 2.10.0.0). Honey adulteration tests by UV-Vis spectrophotometry For further adulteration tests, 42 pure honey samples were randomly chosen and added of glucose syrup according to their harvest seasons, as follows (2018–2019 harvest - 20 samples, 2019–2020 harvest - 15 samples, 2020–2021 harvest - 7 samples), comprising 30% of the samples from each harvest. Glucose syrup was added to the pure honey samples in concentrations ranging from 10% to 60% (w/w). The same UV–VIS scanning protocol applied to profile the pure honey was adopted for the adulterated samples. All the honey samples were analyzed in triplicate (n=3) totaling 756 analyzes with statistical and chemo metric analysis. The UV-Vis spectra recorded with support of the Biochrom software were exported as

an.xlsx-format data set to be further analyzed in the Microsoft Excel® environment. The average UV-Vis spectrum for each sample (n = 3) was calculated and further used for chemo metric analysis. For that, the UV-Vis data set firstly collected swept the 200–800 nm spectral windows, but since no relevant signals were found after 500 nm wavelengths, only the data collected in the region between 260 nm and 500 nm was further analyzed. Thus, principal components analysis (PCA) was applied to the UV-Vis data set considering different spectral regions, as follows: 260–500 nm (whole spectrum UV and visible absorbance regions), 260–360 nm (UV absorbance), and 280–300 nm (phenolic and flavonoid compounds absorbance). Importantly, other spectral windows were also analyzed. The visual analysis of the UV-Vis spectra revealed a significant discrepancy on absorbance intensities between the pure honey samples and the adulterated ones indeed, the adulterated samples exhibited prominent absorbance across the entire spectrum compared to pure honey, irrespective the amount of glucose syrup added to that food (i.e., 10–60%) for adulteration. Among the pure honeys, a very similar profile was observed, with only a difference being in absorbance [35].

Maja Benkovi et al (2022). Honey adulteration with cheap sweeteners such as corn syrup or invert syrup results in honey of lesser quality that can harm the objectives of both manufacturers and consumers. Therefore, there is a growing interest for the development of a fast and simple method for adulteration detection. In this work, near-infrared spectroscopy (NIR) was used for the detection of honey adulteration and changes in the physical and chemical properties of the prepared adulterations. Fifteen (15) acacia honey samples were adulterated with glucose syrup in a range from 10% to 90%. Raw and preprocessed NIR spectra of pure honey samples and prepared adulterations were subjected to Principal Component Analysis (PCA), Partial Least Squares (PLS) regression, and Artificial Neural Network (ANN) modeling. The results showed that PCA ensures distinct grouping of samples in pure honey samples, honey adulterations, and pure adulteration using NIR spectra after the Multiplicative Scatter Correction (MSC) method. Furthermore, PLS models developed for the prediction of the added adulterant amount, moisture content, and conductivity can be considered sufficient for screening based on RPD and RER values ($1.7401 < RPD < 2.7601$; $7.7128 < RER < 8.7157$) (RPD of 2.7601; RER of 8.7157) and can be moderately used in practice. The R2 validation of the developed ANN models was greater than 0.86 for all outputs examined. Based on the obtained results, it can be concluded that NIR coupled with ANN modeling can be considered an efficient tool for honey adulteration quantification [36].

Mohamed R. et al. (2024).This work evaluate fast and accurate analytical method model developed for the authentication of honey adulteration by sugar-feeding syrup using UV-Vis (Ultraviolet-visible) spectroscopy in absorbance mode together with chemo metric techniques of principal component analysis (PCA) and canonical discrimination analysis, thirty-five honey samples from three types of honey (18 samples of clover honey,14 samples of citrus honey and 3 samples of sugar-feeding honey samples), were measured by a spectrophotometer with recorded wavelength range from 200 to 900 nm, in two different extract solution (water and ethanol), the method of PCA was used to lower the number of inputs (wavelengths), then, the principal components were used with canonical discrimination analysis on the following wavelength ranges individually: UV (200-400nm), Vis (400-900nm) and UV-Vis (200-900nm) for water and ethanol extract honey samples. The results of the experiment clearly support for the UV-Vis spectral wavelengths with Chemo metrics Analysis can be reduced at large spacing interval, which allows easing data analysis as well as developing a simpler and cheaper sensor for honey discrimination in practice. PCA and canonical discrimination were successfully able to present a preliminary clustering pattern to segregate the feeding sugar syrup honey samples from the clover and citrus honey sample in UV and UV-Vis region spectroscopy by water extract [37].

S. Das, M.N. et al (2022). Honey has a lot of reputation because of its supposed medicinal properties. In this study, Hydroxyl methyl furfural (HMF), sugars, and Fructose/Glucose ratio of honey in Bangladesh were assessed for adulteration and authenticity evaluation. Methods: Seventy honey samples collected from different districts of Bangladesh were analyzed by High Performance Liquid Chromatography (HPLC) for HMF content and sugar profile. The samples were prepared by using Carrez I and Carrez II prior to injecting into HPLC. The samples were then filtered through syringe filter and taken in 1.5 ml vial for injecting into the HPLC system. Results: HMF values were ranging from 1.41 mg/kg to 2,063.90 mg/kg. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found 0.10 mg/kg and 0.33 mg/kg with R^2 =0.9994. The average values of fructose, glucose, and sucrose were in the range of 14.75 -52.44%, 8.19 -42.63%, and 0.10 -21.12%, respectively. From validation parameters, LOD values for fructose, glucose, and sucrose were 0.003, 0.008, and 0.004%, respectively; and LOQ values were 0.01, 0.028, and 0.015%, respectively with an excellent linearity with R^2 for fructose =1.0, glucose=0.9999, and sucrose=1.0. Conclusion: Some samples had higher HMF content which may be due to the storage time was increased and improper processing with high temperature or adulteration by High Fructose Corn

Syrup (HFCS), sugar cane syrup, rice syrups or rice molasses. The sugar profiles showed that the most of honey samples were nectar honeys [38].

Kerkvliet and Meijer (2000) analyzed 17 samples of honey and 6 samples of cane sugar to study the suitability of microscopic analysis to detect adulteration with cane sugar. Microscopic analysis of samples was performed quantitatively by counting parenchymal cells, round cells and epidermal stem cells from sugarcane in a 10g sample using polarized light microscopy. Repeatability of microscopic analysis was determined by calculating the standard deviation of 8 aliquots from the samples of honey. For all honey samples in this study, it was found that when the number of parenchymatous cells was more than 150 or the number of circular cells more than 10 in 10 g, the samples were adulterated with sugars derived from C4 plants (sugarcane or maize). Microscopic techniques were able to detect suspicious honey adulterated to concentrations below 7%. Overall, the microscopic procedure is a good screening method for detecting adulteration [39].

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Samples

Pure honey samples were directly obtained from hives 3 different artisanal beekeepers located in several areas of East Gojjam especially in Debere Maros city and surrounding woreda town such as Dibedabi, Debeza, Guay and8 commercially available honey samples were bought from markets in Dibiza (1DZ), Shiwaber (2SHW), Lumame (3LUM), Menahiria Akababi (4MHR), Monkorer (5MKO), Adebabay (6ADB), Gindewoyen (7GW) and Debre Elias (8DE). There are 11 total sets of pure and unknown super market honey samples.

1. Pure honey samples were collected from different beekeepers in different place (total = 3).
2. Unknown honey was bought from different supermarket randomly surrounding Debre Markos city (total= 8).

3.2. Reagents and chemicals

3.2.1. Chemicals

Some of the chemicals used in this work are; standard sugar solution, ($C_{12}H_{22}O_{11}$), distilled water (H_2O), sulfuric acid (H_2SO_4) and glucose ($C_6H_{12}O_6$).

Water: is chemical a simple to test involves adding water to honey, pure honey should dissolve completely without leaving any residue.

Phenol- sulfuric acid test: This test can be used to detect carbohydrates in honey leading to a color change that indicates the presence of sugars.

Standard sugar solution (glucose): Calibration Standard in the laboratory setting such glucose can be calibrating instruments for measuring sugar concentration such as spectrophotometers.

3.2.2. Reagents of honey

Reagents are a substance or a compound that is added to the system cause a chemical reaction or to test for presence of another substance. Reagents are commonly used in laboratory experiment and chemical analysis. Phenol-sulfuric acid method is one of the most common methods applied to the analysis of total sugar content. Some reagent and test for honey is phenol (C_6H_5OH).

3.3 Apparatus

The following apparatus are used in this study. Measuring cylinder (volumetric flasks), electronic analytical balance accurately weighted standard and samples, water path (hotter) to facilitated melting or diluting, separator funnel or filter paper to separate suspended from a solution, sample (test tube) holder, pipettes used to transferring small amount of solution during experiment, and cuvettes (quartz or glass) used to hold a sample solution to be analyzed in the light path of a spectrometer.

3.4 Experimental Instrumentation

In this work UV-Vis spectrometer (Instrument Model T80UV-Vis Spectrometer, PG Instrument Ltd) which uses quartz or silica glass cuvette, used to measure the absorbance spectrum of pure honey and standard sugar solution (glucose). It is double beam spectrometer with wave length range 200nm- 1100nm. The instrument can connect to PC and full instrument functionality and analysis by computer interfere.

3.5 Experimental Methods

Wave length selection of pure honey and standard sugar solution (glucose) spectra measured in the wave length range 200nm-800nm; from these measurements the wave length at which the maximum absorbance for standard sugar solution (Glucose) occurred is selected.

3.5.1 Sample preparation and measurement

Calibration for pure honey concentration: Preparation of pure honey for analysis, all individual pure honey samples were heated in water bath 15min, at $40^{o}c$ for complete solubilization of the sugar crystals and homogenization of the constituents. Furthermore 1gram (g) of pure honey was weighted and then added of 10ml distilled(purified) water and centrifuging 2000rpm, 5min used for dilute in well. The working standard solution of pure honey has been prepared in five (5) concentration that are 0%, 5%, 10%, 20% and 30% by serial dilution of standard solution in a separate 10ml volumetric flasks (test tube).

Table 3.1 Concentration pure honey and Glucose syrups uses in the adulteration test

Glucose concentration (%)	Pure honey (g)	Glucose syrup (g)
0	1	0
5	1	0.05
10	1	0.1
20	1	0.2
30	1	0.3

Then accurately added 1ml of phenol solution (correctly prepared) to the working solution and the prepared sample respectively and then quickly added 5ml of concentration of sulfuric acid mixed well in time and stand at water bath for 15min and leaved at room temperature for 20min. Distilled water was used as blank control.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Maximum Absorbance Wavelength Selection

The wave length where the maximum absorption occurs was determined by measuring the absorption intensity versus wavelength spectrum in the wave length range between 400nm and 800nm. From these measurements, the maximum absorption for standard sugar solution (Glucose) occurs at 490.56nm.

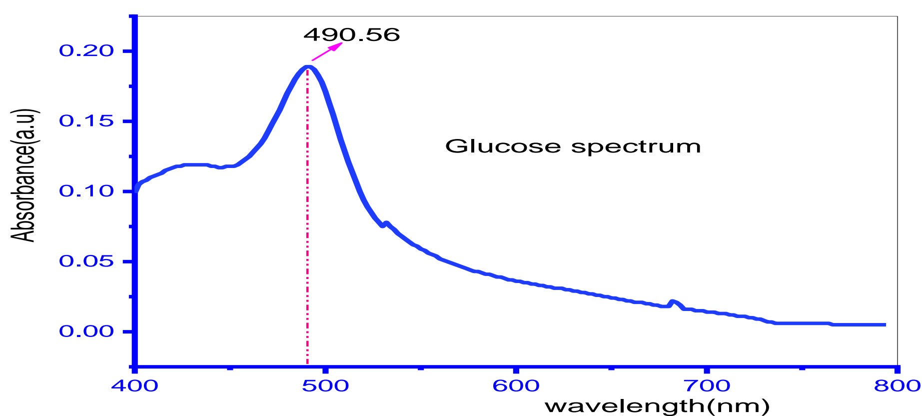


Figure 4.1 Standard Sugar (Glucose) Spectra

4.2 Calibration curve

Calibration curve were determined by measuring the absorbance of the difference concentration standard sugar solution (glucose) prepared from solution at fixed wavelength determined above i.e. at $\lambda_{max} = 490.56nm$.

Table 4.1 Calibration Curve data

Concentration (%)	Abs
0	0.698
5	0.747
10	0.814
20	0.96
30	1.065

Calibration curve of honey using UV-Vis spectroscopy involves measured the absorbance of honey sample to determine their concentration. From prepared a series of standard solution with known concentration of specific compound found in honey measure the absorbance of each standard solution at the selected wavelength using the UV-Vis spectrometer. The absorbance was measured at the maximum wavelength (λ_{max}=490.56nm) of each of the standard glucose solution calibration curve (absorbance vs. concentration curve) is plotted.

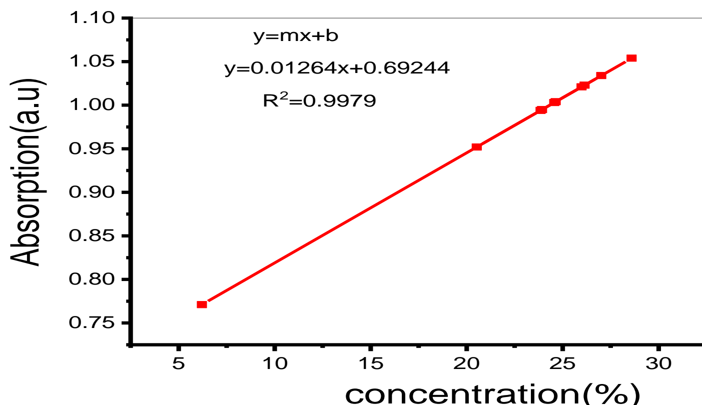


Figure 4.2 Calibration for standard sugar solution

As shown in the above figure 4.2 the absorbance and concentration show good linear relation with R^2 value of 0.9979.

4.3 Glucose concentration in different honey samples

As shown below table4.2 the results of experiment observed in different concentration in different location. The relation between honey sample its location in which the maximum concentration observed for 7GW (28.597%) and lower concentration on the 6ADB (6.214%).

The average concentration glucoses of different place are: 28.597% (7GW), 27.016% (5MKO), 26.146% (2SHW), 25.934% (1DZ), 24.617% (8DE), 23.878% (3LUM), 20.53% (4MHR) and 6.214% (6ADB). The lower value of honey sample concentration is 6.214 % (6ADB) and more related to the pure honey. It indicates good validation and also good precision at zero standard deviation.

Table 4.2 Glucose concentration in different honey sample including the average and standard deviation values

No	Location(ID)	Chemicals Conce (%)	Average (%)	Standard dev.
1	1DZ	25.908	25.934	
		25.908		
		25.987		0.045610671
2	2SHW	26.146	26.146	
		26.146		
		26.146		0
3	3LUM	23.852	23.878	
		23.852		
		23.931		0.045610671
4	4MHR	20.53	20.53	
		20.53		
		20.53		0
5	5MKO	27.016	27.016	
		27.016		
		27.016		0
6	6ADB	6.214	6.214	
		6.214		
		6.214		0
7	7GW	28.597	28.597	
		28.597		
		28.597		0
8	8DE	24.564	24.617	
		24.643		
		24.643		0.045610671

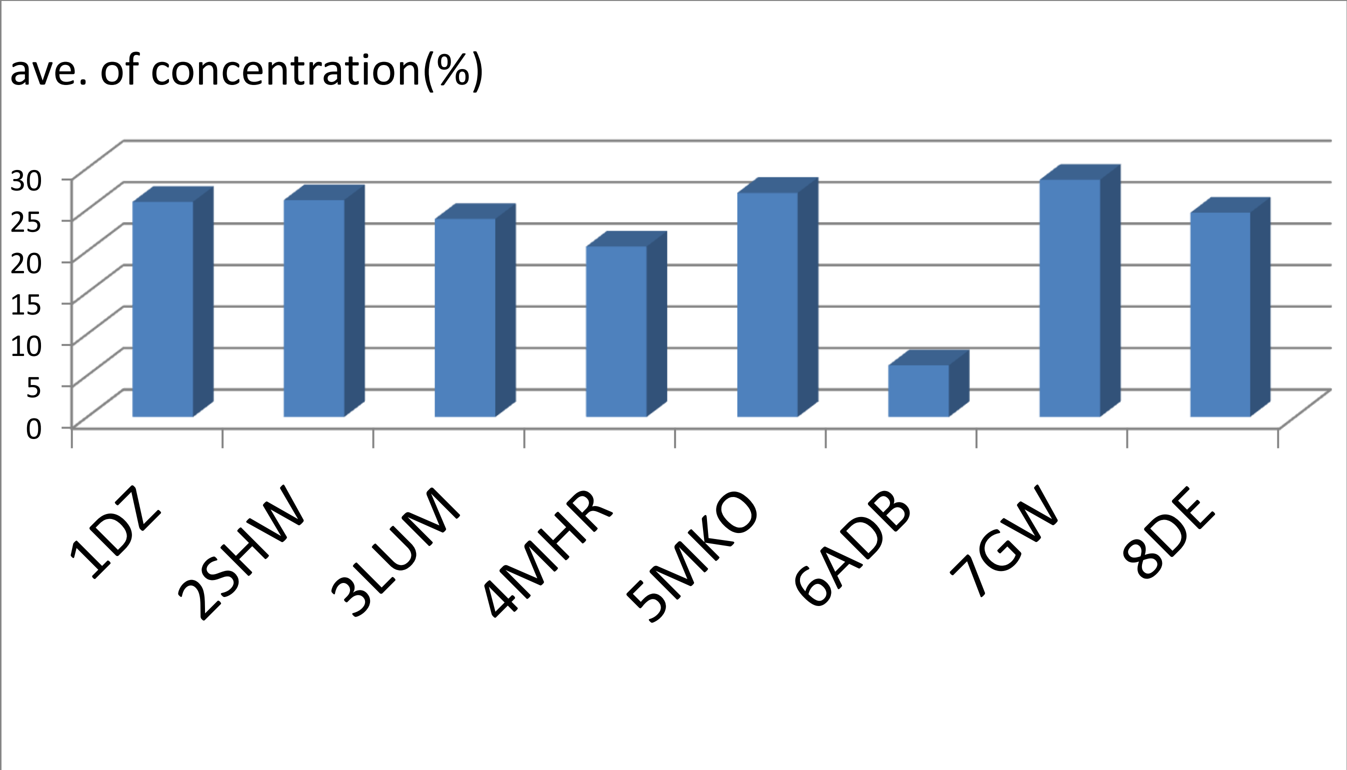


Figure 4.3 Glucose concentration of bar graph vs. in varies locations honey samples

Specifically, the maximum concentration was observed for the 7GW honey sample, while the lowest focus was recorded for the 6ADB honey sample.

4.4. Validation of Experimental results

The widely used analytical parameters for method validation are; linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) in terms of coefficient of determination (R^2). In scientific research, the validation of experimental results is paramount to ensure the credibility and accuracy of the findings. Various parameters play a crucial role in validating experimental outcomes.

4.4.1 Linearity

Linearity was assessed using a calibration curve constructed using a mixed sugar standards containing glucose. The linearity can be determined from the value of R^2in the calibration curve. The data is acceptable and considered accurate when the R^2 value is closed to or equal to 1. An R^2 value of 1 means the data is well fitted to the regression line of the calibration curve and good linearity. From this result constructed calibration curves for the sugar had linear calibration curves with $R^2 >$

0.9979. This shows that the concentration of sugar standard and peak intensity has high degree of correlation.

4.4.2 Limit of detection and limit of quantification

The LOD and LOQ were determined using 11 reagent blanks prepared following the same procedure used for the preparation of samples for the quantification of sugars in honey samples. The LOD and LOQ were measured based on the standard deviation of the blank solutions and the slope of the calibration curves. The low LOD is essential for detecting trace amounts of glucose in honey, which is identified adulteration and LOQ is lowest concentration of an analyze that can quantitatively measure with acceptable precision and accuracy. The LOD was obtained by the multiplication of the standard deviation by 3.3and diving by the slope (**LOD= 3.3δ/m**. Based on standard deviation (S.D) response and slope [40].

LOD=3.3×0.0456/0.0126=0.1368/0.0126=**10.857** (for 1 DZ),

LOD= 3.3×0/0.0126= 0 (2SHW)

LOD=3.3×0.0456/0.0126=0.1368/0.0126=10.857 (3LUM),

LOD=3.3×0/0.01126 =0 (5MKO), LOD=3.3×0/0.0126=0 (4MHR)

LOD=3.3×0/0.0126 =0 (6ADB), LOD=3.3×0/0.0126 =0 (7GW),

LOD=3.3×0 =0 (8DE) =, and LOQ**=10δ/m**

LOQ=10×0.0456/0.0126=36.190 (1DZ), LOQ=10×0/0.0126= 0 (2SHW)

LOQ=10×0.0456/0.0126=**36.190** (3LUM), LOQ=10×0/0.0126= 0 (5MKO)

LOQ=10×0/0.0126= 0(6ADB). LOQ= 10×0/0.0126 = 0(4MHR)

LOQ = 10×0/0.0126=0 (7GW) LOD = 10×0.0456/0.0126 = **36.190** (8DE)

The LOQ was obtained by the multiplication of the standard deviation by 10 and diving by the slope (**LOQ =10δ/m**).

4.4.3 Precision

Precision is a key aspect in assessing the reliability of results, can be determined by analyzing the standard deviation of the collected data points. A lower standard deviation value indicates a higher level of precision in the experimental results obtained; conversely, a high standard deviation indicates greater variability from given measurement. In this work the percentage standard deviations are in the range of between 0 - 0.0456% which suggested a good precession in the experiment. To validate the used method for quantification of sugars by the analytical figures of

merit were assessed to check the precision of the data obtained. When saw table 4.2 show that standard deviation is that become zero in different glucose concentration honey sample. The results indicate the device there is no fluctuated or no the device nosing in repeating measured value under the same conditions. This available to say a good precision and. Precision of the method was validated by determining the percent relative standard deviation (%RSD) of the samples ran in triplicate. The %RSD was calculated.

$\%RSD = \delta / x \times 100$

Where: σ = standard deviation, X = Mean

%RSD =0.0456×100 /25.934= 0.0456 /25.934 =0.00176

%RSD =0.0456×100 /24.617 = 0.0456/24.617 = 0.00185

CHAPTER FIVE

5. CONCLUSION AND RECOMEDATION

5.1. CONCLUSION

Honey is one of humankind's oldest food products that preserve human health and shields them from various diseases, such as cancer, a cold, sore throat etc. Honey is among the most adulterated foods worldwide, and fraud detection is difficult because it is a chemically complex matrix. In this study aimed to develop a simple, fast, and cheap method based on UV–Vis spectrophotometry for discriminating pure honey from adulterated ones. For that, pure (real) honey samples were collected directly from the hives and commercially available honey samples were bought from the supermarket. In this study more concerned qualitative and quantitative analyzed using UV-Vis spectrometer were discriminated pure honey from adulterants honey bought from supermarket. For that 3 pure honey that are collected directly from the hives were used as a reference. Individual pure honey sample correctly weighted and heated in water bath for 15min at 40℃ to solubilized and homogenize of the constituents. Furthermore 1g of pure honey was weighted and added 10ml distilled (purified) water then centrifuging 2000rpm for 5min used for dilute in well and accurately added 1ml of phenol, then quickly added 5ml of concentration of sulfuric acid mixed well in time and stand at water bath for 20minand leave at room temperature for 20min. Calibration curve was made by deliberately adding known amount of glucose, 0%, 5%, 10%, 20% and 30% to these pure honey samples and their absorbance was measured at a fixed wave length, which is the wave length where maximum absorbance of glucose occurred (490.56nm) by UV-Vis spectrophotometry. Then UV-Vis spectroscopic measurements were performed on the samples which are bought from random markets that are located in Debre Markos and the surrounding areas. The results show that the sample bought from super market have more concentration of sugar (glucose) compared to pure honey obtained directly from hives. The average concentration glucoses from honey samples obtained from various market places are: 6.214% (6ADB), 20.53% (4MHR), 23.878% (3LUM), 24.417% (8DE), 25.934% (1DZ), 26.146% (2SHW), 27.016% (5MKO) and 28.597% (7GW). The lowest concentration of glucose is found in sample from 6ADB (6.214%) which most probability is un-adulterated honey.

Validation of Experimental results the widely used analytical parameters for method validation are limit of detection, (LOD) limit of quantification (LOQ), accuracy, precision, and the linearity in terms of coefficient of determination (R^2) and Standard Deviation. The precision of the method with reached maximum a standard deviation of concentration is 0.0456, which demonstrates the reliability of that technique for detecting adulteration honey, The protocol described involves a simple sample preparation step, but difficult chemical or hazardous that harmful to health (toxic and overheating create) with fast scanning it is guided that used to analysis of honey helping to determine the quality of honey product. As it is fast and less expensive technology for monitoring fraud in honey.

5.2. Recommendations

Based on the results of this study, the following recommendations are made for future consideration.

- Given results honey adulteration can control by concerned sectors updating the Ethiopian honey's standards and specifications is required.
- The sucrose and praline content of some of the deliberately adulterated honey samples were similar to the pure honey. Besides, there is no standard specified for fructose, glucose, and maltose as well as need for updating for reducing sugar and sucrose.
- Training of beekeepers and other stakeholders on rapid test assessment to keep quality, identify the adulterated honey, and avoid adulteration.
- Strong legal framework and enforcement is required to reduce honey adulteration further research on the effect of honey adulteration on human and alternative control Systems should be encouraged, keep sustainable for the purpose of honey for, domestic and international market.

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