NEAR-INFRARED GLUCOSE SENSING IN ADULTERATED HONEY USING SMARTPHONE-BASED COLORIMETRIC DETECTION

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By

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CERTIFICATE

This is to certify the dissertation entitled "NEAR-INFRARED GLUCOSE SENSING IN ADULTERATED HONEY USING SMARTPHONE-BASED COLORIMETRIC DETECTION” is the bona fide record of research work done by Ms "FATIN NURSYAFIQAH BINTI ALIXSIUS " during the period of October 2024 to June 2025 under my supervision. I have read this dissertation, and, in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, I’m scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor in Medical Radiation (Honours).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or other institutions. I'm grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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Date: 31 July 2025

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LIST OF SYMBOLS AND ABBREVIATIONS

µL	Microliter
A	Absorbance
AF	Auto Focus
AF	Auto focus
CCD	Charge coupled device
CL	Chemiluminescence
CMOS	Complementary metal oxide semiconductor
D-glucose	Dextroglucose
dL	Decilitre
ECL	Electrochemiluminescence
EMA	Economically Motivated Adulteration
g	Gram
GI	Glycemic Index
Gox	Glucose Oxidase
H_2O	Water
HPLC	High-performance liquid chromatography
HRMS	High-Resolution Mass Spectrometry
HRP	Horseradish Peroxidase
IRMS	Isotope Ratio Mass Spectrometry

ISO	International Organization for Standardization
JPG	Joint photographic experts group
Kcal	Kilocalorie
L	Litre
LED	Light Emitting Diode
L-glucose	Levoglucose
MB	Megabyte
ml	Mililitre
MP	Megapixel
NIH	National institutes of health
NIR	Near-Infrared
NMR	Nuclear Magnetic Resonance
POC	Point-of-care
R	Correlation coefficient
R^2	Regression coefficient
RGB	Red-Green-Blue
ROI	Region of interest
T	Transmittance
UHPLC	Ultra-High Performance Liquid Chromatography
UV-VIS	Ultraviolet-Visible

WB	White Balance
Wi-Fi	Wireless Fidelity

PENGESANAN GLUKOSA INFRAMERAH DEKAT DALAM MADU TIRUAN MENGGUNAKAN PENGESANAN KOLORIMETRI BERASASKAN TELEFON PINTAR

ABSTRAK

Pemalsuan madu dengan glukosa menimbulkan masalah kesihatan dan ekonomi yang serius kerana nilai kesihatannya. Resonans magnet nuklear (NMR), Spektrometri Jisim Resolusi Tinggi (HRMS) dan Spektrometri Jisim Nisbah Isotop (IRMS) adalah contoh teknik analisis berasaskan makmal yang digunakan untuk membezakan antara madu tulen dan tiruan. Namun, ia tidak boleh diakses secara awam. Kajian ini meneroka kaedah yang cepat, mampu milik dan mudah digunakan untuk mengukur kepekatan glukosa dalam madu tiruan. Sampel larutan dengan kepekatan glukosa yang berbeza disediakan dan didedahkan kepada LED inframerah dekat (NIR) dengan panjang gelombang 940 nm. Kemudian, imej ditangkap dan dianalisis menggunakan ImageJ. Nilai merah-hijau-biru (RGB) purata untuk setiap sampel direkodkan. Selepas itu, nilai penyerapan dan penghantaran dikira menggunakan formula Hukum Beer Lambert. Hasil dapatan yang diperoleh untuk nilai R^2 bagi nilai penyerapan glukosa ialah 0.5562, 0.5435 dan 0.5414 untuk komponen merah, hijau dan biru. Ini menunjukkan hubungan yang lebih tinggi dan kuat antara nilai penyerapan dan kepekatan glukosa yang menunjukkan kepekaan tinggi. Selain itu, perbezaan ketara dapat diperhatikan antara keamatan piksel purata RGB dalam kategori madu tulen dan madu tulen yang ditambah dengan 50% air dan glukosa (100 g/dL). Oleh itu, kajian ini dapat membezakan antara madu tiruan dan tulen. Ini menunjukkan bagaimana pengesanan kolorimetri inframerah dekat berasaskan telefon pintar berpotensi sebagai alat yang berguna untuk saringan keaslian madu di lapangan, meningkatkan kebolehcapaian pengguna dan membantu dalam memerangi pemalsuan madu.

NEAR- INFRARED GLUCOSE SENSING IN ADULTERATED HONEY USING SMARTPHONE-BASED COLORIMETRIC DETECTION

ABSTRACT

Honey adulteration particularly with glucose raises serious health and economic problems, especially in areas where honey is highly valued for its health values. Nuclear magnetic resonance (NMR), High-Resolution Mass Spectrometry (HRMS) and Isotope Ratio Mass Spectrometry (IRMS) are the examples of laboratory based analytical technique applied to differentiate between pure and adulterated honey. However, they are not publicly accessible. This study explores a quick, affordable, and easy to-use method for measuring the concentration of glucose in adulterated honey. Sample solutions with different glucose concentrations were prepared and exposed with near-infrared (NIR) LED with 940nm wavelength. Then the image is captured and analysed using ImageJ. The mean red-green-blue (RGB) values for each sample are recorded. After that, absorbance and transmittance values are calculated using the Beer Lambert Law formula. The obtained findings for R^2 value of the glucose-absorbance value are 0.5562, 0.5435 and 0.5414 respectively for red, green and blue component. This shows a strong higher correlation between the absorbance value and glucose concentration that indicates strong sensitivity. In addition, there is significant difference can be observed between RGB mean pixel intensity in category of pure honey and added pure honey with 50% water and glucose (100 g/dL). Therefore, this study can be used to differentiate between adulterated and pure honey. This illustrates that this study has potential as a useful tool for on-site honey authenticity screening, improving consumer accessibility and helping in combating against honey adulteration.

CHAPTER 1 INTRODUCTION

1.1. BACKGROUND OF STUDY

1.1.1. GLUCOSE SENSING

Simple carbohydrates, classified into monosaccharides and disaccharides, are essential for nutrition, with glucose, fructose, and galactose being the three most significant monosaccharides (Nutrient and Health - Carbohydrates: Sugars, 2018). Blood glucose, often known as blood sugar, is the body's principal energy source obtained from meals. It is broken down into glucose and transported into the bloodstream (Blood Glucose, 2024). Blood glucose levels are critical for keeping a healthy body since low levels impair normal thinking and function, while high levels cause harm or difficulties over time. Blood glucose monitoring is the key method for determining whether blood glucose levels are within the target range and provides real-time data (American Diabetes Association, 2025).

Excess sugar consumption raises the risk of obesity and chronic diseases including diabetes, hypertension, and heart disease. The World Health Organization recommends that sugars contribute less than 10% of total energy in food production and consumption, resulting in a 2000-kcal diet containing fewer than 50g of sugar (Centre for Food Safety, 2018). Although food is necessary for humanity to live, it may also be a source of fake goods. Adulteration, which involves purposely lowering food quality by adding or substituting inferior chemicals or removing valuable ones, is a major concern in food science (Haji et al., 2023). Sucrose, glucose, fructose, maltose, beet sugar, corn sugar, and cane sugar are the examples of adulterants used in food industry (Mysha Momtaz et al., 2023).

1.1.2. HONEY ADULTERATION

Economically motivated adulteration (EMA) happens when someone purposefully removes, substitutes, or replaces a valuable ingredient or component of a food, or adds a substance to make it appear better or of greater value (Human Foods Program, 2024). In Malaysia, honey is undeniably popular due to its health benefits. However, the increased request for honey causes an alternative way to produce more honey which is adulteration. Adulteration is the direct or indirect introduction of external substances into food products. Direct adulteration occurs when a chemical substance is added to honey, while indirect adulteration occurs when bees are fed with adulterated chemical affluences (Vikas et al., 2020).

Most commonly, adulterated honeys or diabetic honeys are produced by adding adulterants such as sugar syrups which can be extracted from corn and cane. Other common adulterants, namely glucose syrup, fructose syrup and floral honey are also used for adulteration. The adulterants are not as good as pure honeys due to less nutritious which makes them less expensive. Thus, adulterated honeys are bad for health especially for those who suffered from diabetes (Yong et al., 2022).

1.1.3. OPTICAL METHODS AND SMARTPHONE APPLICATIONS

Traditional glucose monitoring procedures, such as finger prick or finger stick testing, are uncomfortable and painful because they can cause skin irritation and infection. Optical glucose sensors monitor glucose concentrations using light, providing a non-invasive alternative. These sensors identify glucose-specific changes in light characteristics, which increases patient compliance and reduces pain. Researchers are working to increase the effectiveness of these sensors by developing optical detecting technologies such as near-infrared, photoacoustic, fluorescence and Raman spectroscopy. For glucose sensor applications, NIR spectroscopy is the most advantageous of these four optical sensing technologies because of its quick detection, simplicity, and deeper skin penetration (Yang et al., 2024).

Healthcare tests are increasingly being carried out using powerful image sensors and the computer capability of smartphones. This trend is transforming the way we manage our social lives, with smartphones serving as potent platforms for low-cost, portable, and easily available alternatives to advanced biomedical imaging and measurement devices. In the future, standard medical testing including blood analysis, estimating cholesterol levels, diagnosis of infectious diseases, monitoring chronic allergies and many more, these testing could be performed in labs and results forwarded to family doctors (Nature Photonics, 2014).

1.2. PROBLEM STATEMENT

Adulterated honey, generally produced with maple, corn, or grape syrups, is frequently added to boost its volume. Long-term consumption in animal studies is associated with abnormal hepatic, renal function, elevated weight, elevated glucose and triglyceride levels and increased fat deposition, whereas short-term consumption had no negative consequences. Long-term intake in human can cause obesity, diabetes, and organ damage (Boateng et al., 2022). This is proven that fake honey can cause diabetes among customers. The global honey industry is in peril as a result of fraud, a complicated and changing method of adulteration. Honey fraud has caused a drop in raw honey prices and increased production expenses (Honey Profiling | NMR | Nuclear Magnetic Resonance, 2023). Thus, honey adulteration can greatly impact economy.

The unique composition of honey presents a challenge when evaluating its authenticity. This is because honey can be effectively adulterated, making it difficult to distinguish between genuine and altered honey. To address this issue, several laboratory-based analytical techniques are employed. These include Nuclear Magnetic Resonance

(NMR), Isotope Ratio Mass Spectrometry (IRMS), and High-Resolution Mass Spectrometry (HRMS) (Walker et al., 2022). The principal application of IRMS has been to identify the presence of C4 sugar additions. Conversely, a reliable ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry, UHPLC-HRMS technique is frequently employed for polyphenol measurement. Alternatively, the proton NMR spectrum, together with many chemometric approaches, is applied to differentiate between honeys of different botanical sources (Labsvards et al., 2021).

To tackle this, beekeepers and honey packers are implementing the NMR-based Honey-Pro packing technology to improve their premium brand image. This technique protects stakeholders, fosters trust between suppliers and wholesalers, and sustains consumer trust in brands. Government agencies recognize NMR as an effective tool for combating food fraud and unfair competition. This shift in focus is critical to global food security (Honey Profiling | NMR | Nuclear Magnetic Resonance, 2023).

In the future, the detection of honey authenticity is expected to become more streamlined and accessible. Simplified and portable techniques will be implemented to determine whether honey is genuine or adulterated. These advancements will enable quick on-site detection, reducing the reliance on laboratory settings. By transferring detection technologies from laboratories to industrial environments, the process will become more efficient and widespread (Zhang et al., 2023). This shift is crucial for ensuring that consumers receive high-quality, authentic honey products. The development of portable detectors will also facilitate the monitoring of honey quality throughout the supply chain. As a result, there is a growing need for a publicly accessible detector that can be used by various stakeholders, including consumers and small-scale producers. By making these detection tools more accessible, the industry can better combat honey adulteration and maintain consumer trust.

The research, which was part of a final year project for 2023/2024, explored the use of smartphone-based near-infrared colorimetric detection for measuring glucose concentration. This method was specifically effective in water mediums, particularly at higher concentration ranges. The study achieved a high correlation coefficient (R^2), indicating a strong linear relationship between the measured and actual glucose concentrations. Hence, the research has shown that the high potential of smartphone-based near-infrared colorimetric detection for glucose analysis in water mediums (Nurul Azlan, 2024).

The system demonstrates significant potential for application in glucose sensing within honey mediums. However, before it can be effectively used, a feasibility study is necessary. This study would focus on evaluating the system's sensitivity, and overall applicability for glucose detection in honey. Assessing sensitivity is important, as it determines how accurately the system can detect glucose levels. The feasibility study will help identify any limitations or challenges specific to honey, which might differ from other mediums like water.

1.3. OBJECTIVES

1.3.1. GENERAL OBJECTIVES

To analyse the feasibility of applying smartphone-based colorimetric system for near-infrared glucose sensing in adulterated honey.

1.3.2. SPECIFICS OBJECTIVES

1. To construct smartphone-based colorimetric detection system.
2. To prepare adulterated honey sample which are added with different glucose concentrations.

3. To capture sample images, measure RGB pixel intensities using ImageJ software, and calculate absorbance and transmittance based on Beer-Lambert law.
4. To analyse sensitivity and applicability of the colorimetric detection system for near-infrared glucose sensing in honey medium.

1.4. STUDY HYPOTHESIS

1.4.1. NULL HYPOTHESIS

1. There is no significant difference in glucose detection between pure honey and adulterated honey using smartphone-based colorimetric detection.
2. Smartphone-based colorimetric detection of glucose levels in honey does not accurately detect adulteration, leading to no better than chance-level accuracy.

1.4.2. ALTERNATIVE HYPOTHESIS

1. There is a significant difference in glucose detection between pure honey and adulterated honey using smartphone-based colorimetric detection.
2. Smartphone-based colorimetric detection of glucose levels in honey accurately detects adulteration, with accuracy significantly better than chance.

1.5. RESEARCH QUESTION

1. Can smartphone-based colorimetric detection effectively distinguish between pure and adulterated honey samples based on glucose levels?
2. How accurate is smartphone-based colorimetric detection in quantifying glucose levels in honey compared to traditional methods like near-infrared spectroscopy?
3. What are the optimal wavelengths and conditions for smartphone-based colorimetric detection to accurately measure glucose in honey samples?
4. Can machine learning algorithms enhance the predictive power of smartphone-based colorimetric detection for detecting honey adulteration?

5. How does the smartphone-based colorimetric detection method compare in terms of cost, speed, and user-friendliness to other spectroscopic methods for detecting honey adulteration?

1.6. SIGNIFICANCE OF STUDY

Smartphones are widely available worldwide, providing accessibility in various environments, including remote or underdeveloped rural areas. Equipped with fast multicore processors, digital cameras, batteries, and user interfaces, they offer measurement and detection capabilities. Wireless data transfer modalities like Wi-Fi and Bluetooth allow immediate display of test results. Smartphone spectroscopy is a non-invasive, rapid, and non-destructive technique used in research, industrial applications, diagnostics, food quality assessment, environmental sensing, and drug analysis testing (Rateni et al., 2017).

The rising consumption of honey, a natural sweetener, has resulted in a decline in trust due to increased adulteration. One of reason is adulterated honey is often sold as pure and charged similarly to pure honey, which is dishonest and unfair to consumers (Teferi Damto et al., 2024). In addition, honey safety is not frequently checked, impacting customer interest and trust in this valued product. Adulterated honey raises blood sugar levels, which leads to high blood pressure, blood lipid levels, diabetes, obesity, and abdominal weight gain. The frequently damaged organs are the liver, kidneys, heart, and brain (Rafieh Fakhlaei et al., 2020).

Smartphones offer practical and efficient portable healthcare monitoring due to their built-in sensors and fast-rising performance of CMOS-based photo cameras, making them ideal for portable biosensors and advanced analytics devices. The advantage of using colorimetric biosensors is the sensors in smartphones are cost-effective (Upadhyay et al., 2024). Besides that, smartphone offers an access to laboratory-based work. This will help the public especially from the low-income family to receive molecular diagnostics and imaging technologies. Apart from that, students and researchers are benefited from inexpensive smartphones camera to conduct any research in the future (Nature Photonics, 2014).

CHAPTER 2 LITERATURE REVIEW

2.1. GLUCOSE

2.1.1. PHYSICAL PROPERTIES OF GLUCOSE

Glucose, a common monosaccharide in the body, is found in higher animals and in the liver and muscle. It is produced through photosynthesis in green plants by turning carbon dioxide and water into glucose. Known as Dextrose, it is an optically dextrorotatory isomer in nature. Glucose is a colorless sweet crystalline compound with a melting point of 419 K, easily dissolves in water but not in alcohol or ether. It turns into a monohydrate with a melting point of 391 K, and its solution is dextrorotatory for better visibility. It is about three-quarters as sweet as sugarcane or sucrose. Glucose exists in two forms which are D and L configurations, with Dextrose having the D shape (Physical Properties of Glucose, 2022). L-glucose has three hydroxyl groups and one hydrogen group on the left side, whereas D-glucose has three hydroxyl groups and one hydrogen group on the right. The letters "D" and "L" represent structural variations, indicating that they are enantiomers since their molecular structures are mirror reflections of each other (S.D., 2016).

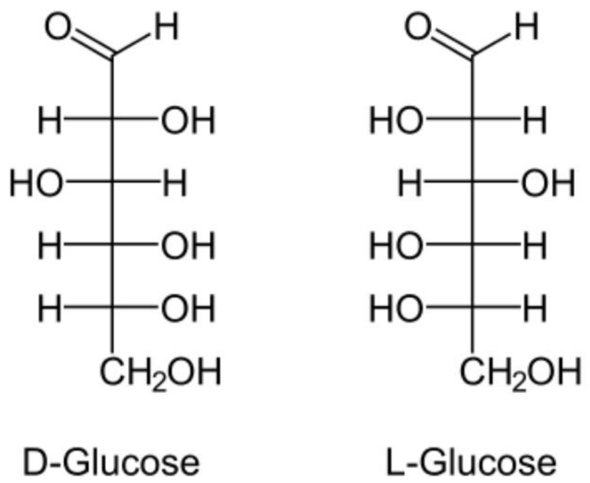

Figure 2.1 shows the structure of D-glucose and L-glucose (S.D., 2016).

2.1.2. GLUCOSE IN HONEY

Honey is a complex natural mixture mainly comprised of carbohydrates, with the sugar content varied depending on the type of flower used by bees. It includes approximately 95% honey dry weight, with 70% monosaccharides, 10-15% disaccharides, and oligosaccharides composed of glucose and fructose. Nectars are primarily composed of fructose, glucose, and sucrose, with relative proportions that vary but are similar among botanical groups. Glucose is the second most common sugar in honey, ranging from 25 to 42g per 100g. The amount of glucose and fructose in honey varies depending on the source of nectar. Furthermore, the main disaccharides in honey include maltose, isomaltose, kojibiose, turanose, trehalose, nigerose, melibiose, maltulose, gentiobiose, palatinose, nigerose, and laminaribiose, which are found in smaller concentrations. Oligopolysaccharides include melesitose, isomaltotriose, theanderose, isopanose, erlose, panose, maltotriose, kestose, and cellobiose, which are commonly present in honeydew honeys (Sevgi Kolayli et al., 2012).

Monosaccharides	Disaccharides	Trisaccharides
Glucose	Sucrose (max. %5)	Melezitose
Fructose	Maltose (0.5–3.5)	Isomaltotriose
Arabinose	Isomaltose	Raffinose
	Turanose	Theanderose
	Trehalose	Isopanose
	Neotrehalose	Erlose
	Melibiose	Panose
	Maltulose	Maltotriose
	Kojibiose	Laminaritriose
	Gentiobiose	Kestose
	Palatinose	Neokestose (Fructose Trisaccharide)
	Nigerose	Cellobiose
	Laminaribiose	
	Difructose Anhydride	

Figure 2.2 shows the sugar types present in honey (Sevgi Kolayli et al., 2012).

2.1.3. GLUCOSE IN FOOD

Carbohydrates such as rice, bread, and other high-carb foods are digested by the stomach and small intestine before being absorbed into the bloodstream as glucose. The pancreas then secretes insulin to counteract the increase in glucose levels. This mechanism in Figure 2.3 causes blood sugar levels to decrease to pre-eating levels, as seen in healthy people (Otsuka Pharmaceutical Co., Ltd., 2025). The glycemic index (GI) is a scale that classifies carbohydrate-containing foods and beverages based on how much they elevate blood sugar levels after eating or drinking. Foods with a high glycemic index raise blood sugar levels more quickly than foods with a low GI. There are three GI classifications which are low GI (55 or less), medium GI (56 to 69), and high GI (70 or more). Examples of low GI, medium GI and high GI foods are oat bran, brown rice, and soda crackers. Diabetes Canada recommends eating and drinking lower GI meals and beverages more frequently to help control blood sugar (Diabetes Canada, 2013). Adulteration with glucose and other sugars in the food business can destroy honey's nutritional value, alter its chemical and biological properties, alter flavor, and potentially impact consumer health (Mayeli Anais Pérez-Rosas et al., 2024). Therefore, consumer should be aware of this issue in order to maintain blood sugar level and stay healthy.

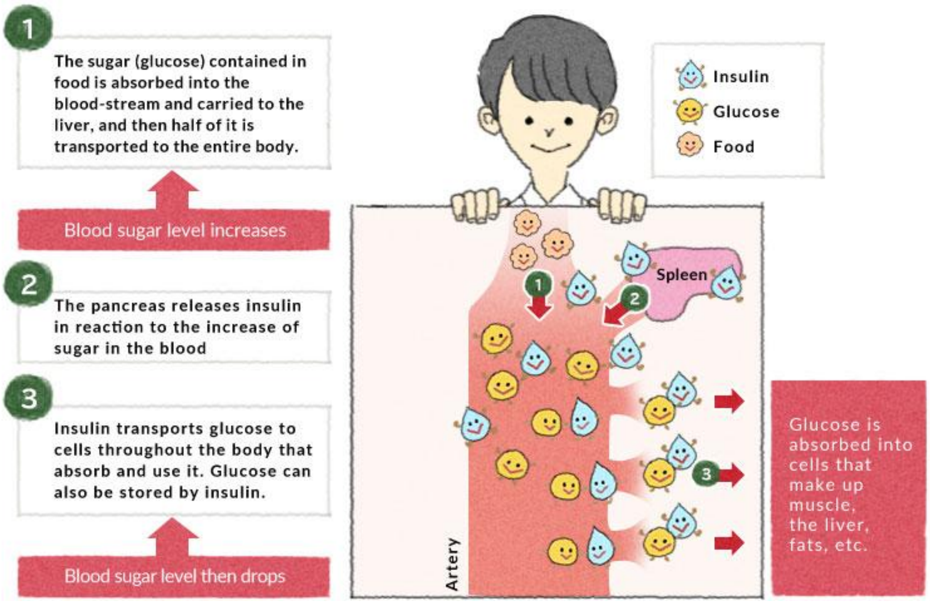

Figure 2.3 shows the glucose absorption process (Otsuka Pharmaceutical Co., Ltd., 2025).

2.2. BIOPHOTONICS

2.2.1. DEFINITION

Biophotonics is an interdisciplinary research area that applies light-based technology to life sciences and health. Photonics refers to approaches and technologies that use light over all parts of spectrum, from ultraviolet to the terahertz range. Because of its multiple advantages, light is an excellent instrument in health and life sciences (Jürgens et al., 2013). The optical microscope which invented in the sixteenth century has made major advances in biophotonic applications since its creation. Known as "the age of photonic," photonic technology has taken over electronics in a number of industries in the 21st century due to its greater bandwidth, faster speed, and no mass. Biophotonic applications are used in photomedicine, a crucial field of medicine, for both therapeutic and diagnostic purposes. Photons are suitable for therapeutic and diagnostic applications because of their great resolution, extensive information, and noncontact sterile interaction. Nonlinear optical imaging, for instance, has been transformed by short-pulse high-power laser technology, which makes it possible to visualize structures at various levels without causing any harm. This development of photonic technology has expanded the range of diagnostic applications in healthcare (Liu & Cheng, 2021). Medical and biological research is predicted to improve precision and effectiveness as optical approaches such as photometric, spectrometric, colorimetric, and fluorometric techniques evolve.

2.2.2. OPTICAL METHODS

a) PHOTOMETRIC

Photometry is the scientific study of light measurement in optics (Admin, 2017). Photometry is a key tool to measure light sources and objects used in signalling, displays, lighting, and other applications where light is meant to be viewed by humans since it takes into consideration the sensitivity of the human visual system (Photometry | NIST, 2025). By measuring light intensity using wavelengths such as ultraviolet and infrared light, photometry quantifies absorption or transmission through a substance. Lambert-Beer's law is used for photometric concentration determination (Hoffmann, 2025). According to Beer Lambert's law as shown in Figure 2.4, when a light beam passes through an absorbing substance, the intensity of monochromatic light reduces with thickness, which is directly proportional to the intensity and concentration of the solution (Bashyal, 2023). Nowadays, modern science uses Beer-Lambert Law for laboratory quantification, organic chemistry, and medication testing (Admin, 2017).

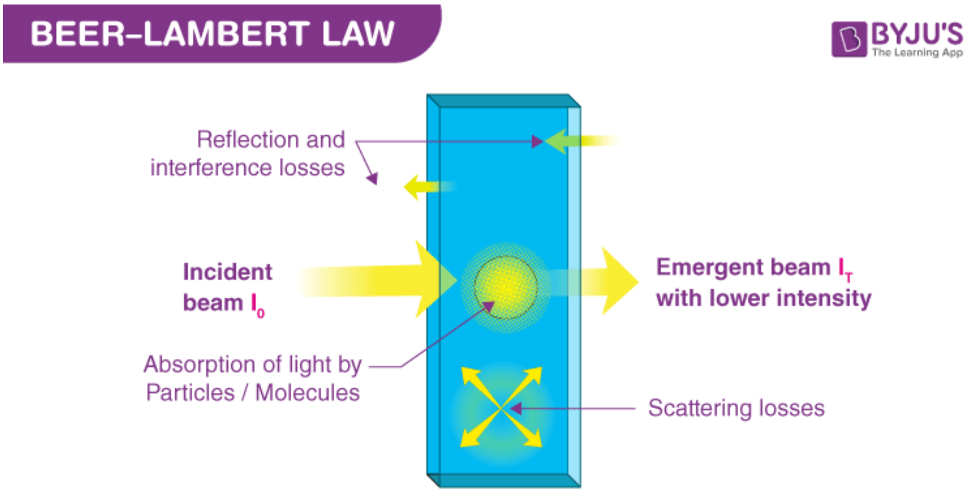

Figure 2.4 shows the Beer-Lambert Law explanation (Admin, 2017).

b) SPECTROMETRIC

Spectroscopy is a theoretical science that investigates the interaction of matter and radiated energy, with a focus on absorption properties and behavior under electromagnetic radiation. It does not produce outcomes, but rather measures absorbance, optical density, or transmittance. In contrast, spectrometry offers a realistic approach for quantifying these characteristics, assisting in the balancing of matter at the atomic and molecular levels. Both areas are required for comprehending the nature of matter (Atascientific, 2020). There are 5 types of spectroscopy which are infrared (IR), ultraviolet-visible (UV/Vis), nuclear magnetic resonanace (NMR), fluorescence and Raman spectroscopy. IR spectrometers analyze the absorption of various frequencies in a sample, which can be applied to determine the molecular bonds and structures exist. UV/Vis spectroscopy is a technique for studying the electronic structure of molecules in a sample, which allows compounds to be identified based on electron energy level transitions. Nuclear magnetic resonance spectroscopy is a method for measuring the magnetic fields surrounding atomic nuclei (Bedolla, 2021). Fluorescence spectroscopy is mostly used to determine the electronic structure and energy levels of molecules. It is great for determining fluorescent chemicals or compounds. Raman spectroscopy is a molecular fingerprinting method. It offers information on the structure, chemistry, and molecular bonds (Kevin, 2023).

c) COLORIMETRIC

Colorimetry is an example of photometry that detects light and measures changes in its intensity. The root word "photo" signifies light. If the measured light falls within the visible spectrum of electromagnetic radiation, which is between 320 and 700 nm, colorimetry is utilized. A colorful solution absorbs some of the light and transmits the other part when a monochromatic light beam passes through it. The intensity of color is correlated with light absorption. The color intensity will be proportional to the concentration of the chemical (analyte) that produces the color based on Beer-Lambert law (Shrestha & Shrestha, 2023). For better understanding, the concentration of the solution determines how much light a colored solution may absorb. A solution absorbs more light as its concentration increases (Colorimetry (A-Level) | ChemistryStudent, 2024). Colorimetry is a low-cost and effective method for determining the quantity of colored compounds. It is easy to carry and simple to use because it requires only a few manual operations (Shrestha & Shrestha, 2023).

d) FLUOROMETRIC

Fluorometry is one of the analytical technique that measures the intensity of emitted fluorescent light, which is the electromagnetic radiation produced when a species absorbs exciting radiation from an external source. Electrons move from the singlet ground state to the singlet excited state as a result of the absorption of ultraviolet, UV or visible light. This new excited state is unstable and releases energy in the form of UV or visible radiation, which causes it to fall back to a singlet ground state. The concentration of the excited species will directly correlate with the intensity of light emitted. Fluorescence is released when molecules or atoms that fluoresce return from singlet electronic excited states to permissible vibrational levels in the electronic ground state. The vibrational level structures in the ground and excited states are reflected in the fluorescence excitation and emission spectra (Labtests, 2022). A fluorometer has many advantages, it is an inexpensive, quick diagnostic equipment for assessing luminous species, providing high-precision procedures, and determining decay time and particle concentration in samples (Paudel, 2024).

2.3. SMARTPHONE-BASED BIOPHOTONIC INSTRUMENTATION USING COLORIMTERIC METHOD

2.3.1. NEAR-INFRARED LED

Infrared radiation (IR) is a range in the electromagnetic radiation spectrum having wavelengths more than red visible light ranging from 780 nm to 1 mm. IR is divided into three types which are IR-A, IR-B, and IR-C. IR-A or near-infrared has wavelengths from 780 nm to 1.4 µm (International Commission on Non-Ionizing Radiation Protection (ICNIRP), 2024). Infrared having 940nm wavelength is chosen since it has best absorption correlation to glucose concentration. This infrared is widely implemented by applying light absorption to identify glucose in non-invasive device. (Owoeye et al, n.d.).

Nowadays, spectroscopic method known as near-infrared (NIR) spectroscopy has increasingly developed for using in content quantification and sample characterization. It is critically important in areas including food product characterization, medical applications, and biophotonics (Beć & Huck, 2019). Near-infrared (NIR) spectroscopy can penetrate tissue with low energy radiation, making it an efficient optical sensing method (Attia et al., 2024). The invention of a non-invasive blood glucose measuring system that uses a painless near infrared-based optical technology signifies a major advancement in medical practice. This approach removes the need for invasive methods such as finger puncture, which can be costly and painful, as well as increasing the danger of infectious disease transmission. Moreover, this technology uses 940 nm wavelength signals to provide a safer and more precise method for diabetic patients diagnosis (Narkhede et al., 2016)

The molecular vibrations of chemical bonds in molecules, especially glucose molecules, influence the absorption and reflection of near-infrared (NIR) radiation. Carbon, hydrogen, and oxygen atoms combine to create C-H, C-O, and O-H bonds to form glucose molecules. NIR waves interact with glucose molecules, absorbing or reflecting them depending on their concentration, allowing them to penetrate the skin and blood vessels (M Naresh et al., 2024). Lower glucose levels cause more scattering and longer optical paths, which reduces absorption. On the other hand, higher glucose levels cause shorter optical paths and less scattering, which increases absorption. As a result, reflected light intensity is lower in high glucose tissue than in tissue with lower glucose concentration because of increased absorption (Ramachandran, 2022).

There are two main ways to measure light in biological tissues which are transmittance and reflectance. Light that travels through a tissue and exits on the other side is measured by transmittance, whereas light that is reflected back from the tissue's surface is measured by reflectance. Transmittance gives information about the absorption and scattering properties of tissues and is frequently utilized in spectroscopic and imaging methods (Ramachandran, 2022).

$$A = -\log_{10} T \qquad \text{Equation 3.1}$$

Equation 3.1 shows the formula between absorbance, A and transmittance, T (Edinburgh Instruments, 2021).

The absorbance, A, and the transmittance, T, have logarithmic relationship, where absorbance of 0 indicates a transmittance of 100%, while an absorbance of 1 indicates a transmittance of 10% (Edinburgh Instruments, 2021). In other words, lower light absorption produces more light is transmitted and vice versa. More examples are shown in Figure 2.5.

Absorbance	Transmittance
0	100%
1	10%
2	1%
3	0.1%
4	0.01%
5	0.001%

Figure 2.5 shows the relationship between absorbance and transmittance (Edinburgh Instruments, 2021).

2.3.2. CMOS CAMERA

Smartphones have impacted several parts of healthcare, including diagnosis, prognosis, detection, quantification, monitoring, control, and the creation of mobile apps. Smartphone sensors also known as photodetectors, it has the potential to perform as portable biofeedback devices for a variety of scientific applications. Its processor allows for the embedded collection, analysis, and processing of pictures or signals (Hernández et al., 2017). There are two types of photodetectors which are complementary metal oxide semiconductor (CMOS) and charge coupled device (CCD). They both function as electronic eyes. CCD technology was popular at first because to higher image quality and sensitivity, but successive developments in CMOS sensors led to them surpassing CCD sensors in number of shipments beginning in 2004 (Tokyo Electron Ltd, 2025). CMOS sensors are beneficial due to its high performance and low power usage (Sinoseen, 2024).

CMOS sensors are currently the most common image sensor technology found in digital cameras and phones. It converts light into an electric charge using a photodiode array. Every pixel in a CMOS sensor has a photodetector, a semiconductor device that transforms incident radiation into electric power to generate an electric current. The intensity of the light is proportional to the amount of the electric charge. In addition, every pixel of a CMOS sensor is surrounded by transitors, which are electronic components that collect low electrical impulses and amplify them, then send them to a processor. It converts these charges into a digital signal, which is then processed by the camera's image processor to ensure image harmony. In short, photons of light are converted by CMOS sensors into electric voltage values that can be processed and converted into a digital image (Sinoseen, 2024).

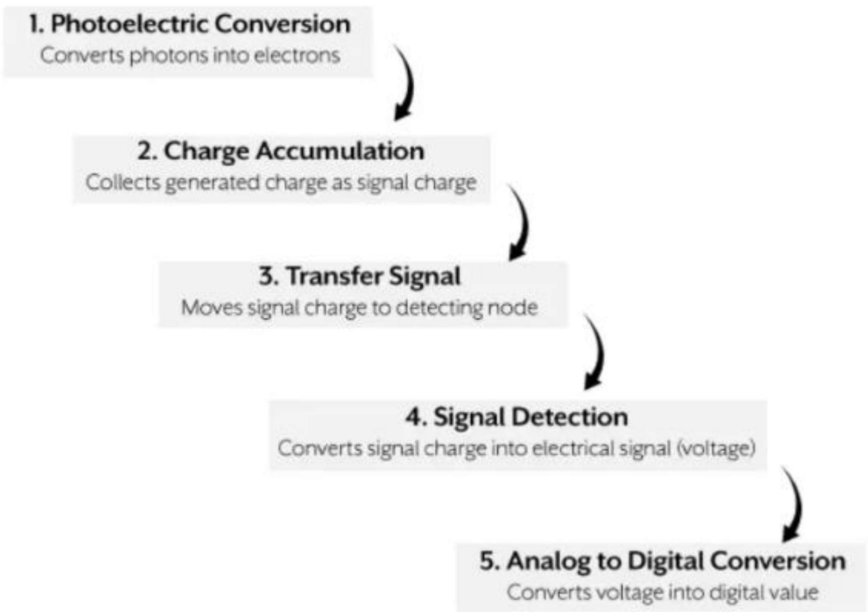

Figure 2.6 shows how photons are processed into digital signal (Sinoseen, 2024).

Furthermore, a suitable way of generating pictures from smartphones is by using the red-green-blue color space since the pictures are stored as color channels or RGB pixel intensities (Coleman et al., 2019). In CMOS image sensors, a Bayer Color Filter Array (CFA) which is a Camera Filter that cover each pixel with a particular wavelength range. The CFA configuration consists of R, G, and B filters that cover a wide color range. A demosaicing interpolation algorithm is used to estimate the missing two color values, with each pixel capturing one color from R, G, or B (Park et al., 2022).

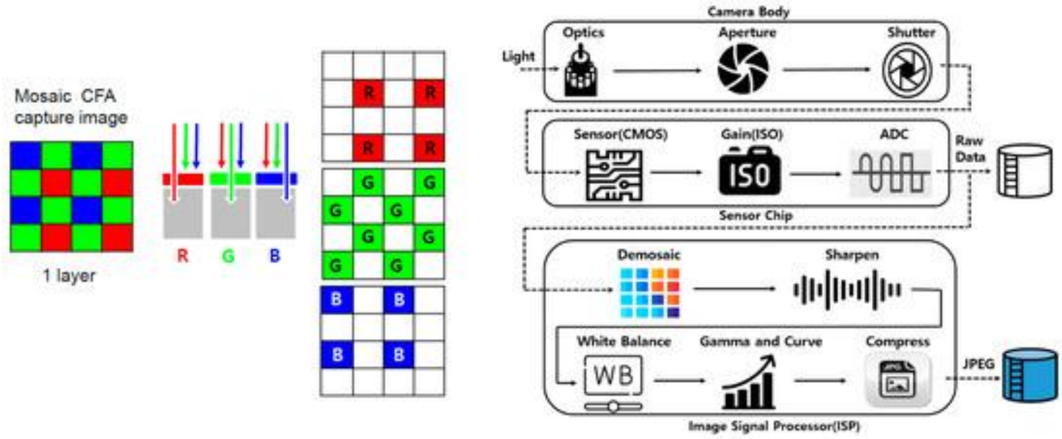

Figure 2.7 shows the color filter array capture images in RGB pixel intensities and process of making jpeg image (Park et al., 2022).

2.3.3. IMAGEJ SOFTWARE

An essential analytical tool in the scientific community since its first use in 1987 is ImageJ, a Java-based image processing application created by the National Institutes of Health (NIH) and published in 1997. Since it was made available to the public, it has grown to be a popular research tool (Schroeder et al., 2020). In this approach, raw RGB photos are captured for image segmentation using inexpensive imaging devices, such as cellphones. The raw color response data is exported for statistical analysis and visualization, and it is preserved for further analysis. This approach is easy, allowing users with limited image processing or computer programming knowledge to obtain and change objective, colorimetric data. Red, green, and blue are the three components of RGB models, which are additive tristimulus systems that represent every single color as a tuple.

These models provide each color component an 8-bit value between 0 and 255, resulting in numerical representations of more than 16 million colors that can be reproduced (Woolf et al., 2020). When each of the components are at minimum (0,0,0), the output is black whereas when each of the components are at maximum (255,255,255), the result is the brightest white that can be represented (Texas Wesleyan University, 2024). Open-source software generates an ecosystem of tools, making image-analysis methods available to scientists through a simple user interface and low-cost desktop computers (Johannes Schindelin et al., 2015).

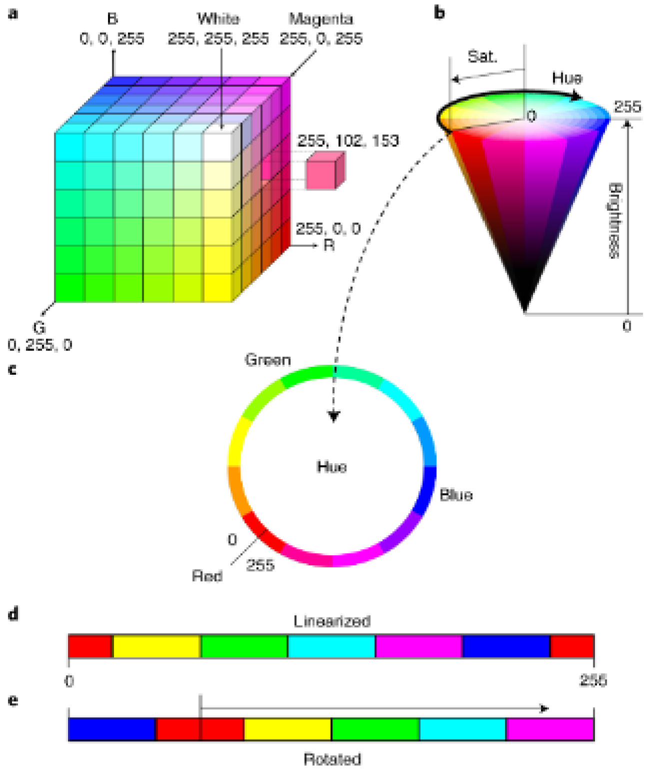

Figure 2.8 shows the color spaces and linearization of the hue (Woolf et al., 2020).

2.4. REVIEW OF PREVIOUS STUDIES RELATED TO OPTICAL SENSING OF GLUCOSE

2.4.1. OPTICAL SENSING OF GLUCOSE IN FOOD USING RGB COLORIMETRIC METHOD AND SMARTPHONE AS THE DETECTOR

There are many analytical techniques to detect glucose such as spectrophotometry, fluorescence, high-performance liquid chromatography (HPLC), electrochemical methods, chemiluminescence (CL), and electrochemiluminescence (ECL). Spectrophotometry, particularly based on horseradish peroxidase (HRP) and glucose oxidase (Gox) is popular due to low-priced, high sensitivity, simplicity and fast response. Plus, colorimetry findings can be recorded and viewed with smartphone or naked eyes (Su et al., 2023). Digital image colorimetry (DIC) on smartphones is an easy and inexpensive way to determine target analytes using color changes in the built-in camera. However, illumination, camera design, settings, and distance all have an impact on image quality. Using a portable closed chamber for samples is one of the strategies that researchers have suggested to lessen the influence of outside variables on colorimetric detection. This chamber offers uniform lighting and maintains a stable distance between the camera and the analyte, however it limits the device's application possibilities. The second suggestion is to use algorithms to map sample images' colors under different shooting conditions to a consistent standard. This is because there is a linear relationship between color intensities under different lighting conditions. Linear operations can be performed on RGB channel parameters to achieve color uniformity (Meng et al., 2024).

2.4.2. APPLICATION OF SMARTPHONE-BASED BIOPHOTONIC INSTRUMENTATION FOR GLUCOSE SENSING USING COLORIMETRIC METHOD IN WATER

A 940 nm NIR light emitting diode (LED), CMOS smartphone camera, and a customized black enclosed chamber to prevent ambient light interference were used to create an easy, portable glucose measuring system. Microsoft Excel was used for calculations and correlation analysis, and ImageJ software was used for RGB picture analysis. According to the Beer-Lambert Law, the procedure validated the system's capability for quantitative glucose analysis through the use of optical absorbance and transmittance measurements. In contrast to conventional colorimetric techniques that call for reagents such as nanoparticles or enzymes (like GOx, HRP), this study relied entirely on digital RGB analysis and did not utilize any costly compounds or chemical reactions. In terms of cost-effectiveness and accessibility, the implementation of a commercial smartphone with free software (ImageJ) causes this system reasonably priced, readily available, and appropriate for point-of-care (POC) or home-based glucose monitoring, particularly in low-resource settings.

In terms of sensitivity, two glucose concentration ranges were tested which are high range (0% to 100%, g/dL) and low range (0% to 0.3%, mg/dL). A strong linear correlation was found between glucose levels and RGB pixel intensity, absorbance and transmittance in high concentration range. The R^2 value for red, green and blue intensity are higher which are 0.8848, 0.9018, and 0.8943 respectively. Next, the R^2 value for absorbance value for red, green and blue components are higher which are 0.892, 0.908, and 0.907 respectively. Moreover, the R^2 value for transmittance value for red, green and blue components are higher which are 0.8848, 0.9018, and 0.8943 respectively. These shows that glucose detection has higher sensitivity. In the low concentration range, the correlation was weaker and shows lower R^2 value (Nurul Azlan, 2024).

CHAPTER 3 METHODOLOGY

3.1. RESEARCH TOOLS AND DESIGN SETUP

3.1.1. MATERIAL AND REAGENTS

One of the materials used is laboratory and scientific grade distilled water (1L) which has the chemical formula of H_2O, molar mass of 18.02 g/mol, Bendosen Laboratory Chemicals product (C1413) and provided by Progressive Scientific Sdn. Bhd, Malaysia. Apart from that, the second material used is laboratory and scientific grade D (+)- Glucose Monohydrate (Dextrose) (500g) powder wich has chemical formula of $C_6H_{12}O_6$, molar mass of 198.17 g/mol, Bendosen Laboratory Chemicals product (C1413) and provided by Progressive Scientific Sdn. Bhd, Malaysia. Plus, the last material used is pure acacia honey (1 kg) which is a monofloral honey produced by bees which contain ingredients such as Borneo Acacia Mangium Honey.

3.1.2. HARDWARE AND EQUIPMENT

Realme C2 smartphone manufactured in BBK Electronics, China with a 13MP+2MP rear dual camera (CMOS sensor) was utilized for colorimetric method. A 12 mm diameter macro lens with 10x magnification is used to capture high quality images was placed horizontally on the smartphone camera lens by using a tripod. This setup gives an advantage for high quality images captured from a fixed distance of 2.5cm from CMOS camera to the Eppendorf tube. On the other hand, the standard smartphone camera just gives high quality images from a distance of greater than 6 cm (Nurul Azlan, 2024).