# OPTICAL METHOD FOR PREDICTING THE NATURE OF FOOD

**M.Sc. Thesis**

By

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**DEPARTMENT OF BIOSCIENCES AND BIOMEDICAL ENGINEERING**

**INDIAN INSTITUTE OF TECHNOLOGY INDORE**

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# OPTICAL METHOD FOR PREDICTING THE NATURE OF FOOD

**A THESIS**

*Submitted in partial fulfillment of the*
*requirements for the award of the degree*
***of***
**Master of Science**

*by*
**ABHIJEET SINGH**


**DEPARTMENT OF BIOSCIENCES AND BIOMEDICAL ENGINEERING**
**INDIAN INSTITUTE OF TECHNOLOGY INDORE**
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# INDIAN INSTITUTE OF TECHNOLOGY INDORE

## CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"Optical method for predicting the nature of food"** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DEPARTMENT OF BIOSCIENCES AND BIOMEDICAL ENGINEERING, Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the period from Oct 2021 to May 2023. Thesis submission under the supervision of Dr. Sharad Gupta, Associate professor,BSBE, IIT Indore.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

**Signature of the student**

**(Abhijeet Singh)**

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This is to certify that the above statement made by the candidate is correct to the best of my/ourknowledge.

Signature of the Supervisor

**(Dr. Sharad Gupta)**

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**Abhijeet Singh** has successfully given his/her M.Sc. Oral Examination held on **May 9, 2023**.

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Convener, DPGC
Date:

Signature of PSPC Member
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Date:

Signature of PSPC Member
(Dr. Parimal Kar)
Date:

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**Abhijeet Singh**

# Abstract

The quality and safety of food is of critical importance to ensure health and long life. Traditional methods for assessing food quality and safety often require time-consuming and costly laboratory tests. In recent years, optical methods have emerged as a promising approach for non-destructive and rapid assessment of food quality attributes. Through this study, we are trying to devise an optical approach-based tool for predicting the nature of food which includes freshness and spoilage. Optical methods offer numerous advantages such as non-invasive, rapid, and cost-effective. They also have the potential for online and real-time monitoring of food quality during food processing and storage. We are making use of the principles and applications of two most fundamental spectroscopic techniques, such as UV-vis spectroscopy and fluorescence spectroscopy, which have shown great potential for assessing food quality attributes, including protein, fat, moisture, and sugar content. In this current study, we are studying the role of a coenzyme which has a role in maintaining the adequate nutrients and overall quality of food. Finally, we highlight the prospects and potential applications of optical methods in the field of food quality assessment by considering the coenzyme NADH (Nicotinamide Adenine Dinucleotide Hydrogen). The whole Electron Transport Chain and Reactive Oxygen Species formation revolves around this molecule. Advances in optical technologies, including miniaturization, automation, and machine learning, hold great promise for the development of robust and portable optical devices for food quality prediction.



**Keywords:** Spectral shift, optical tool, degradation profile, NADH activity.

# LIST OF PUBLICATIONS

- Effect of NADH concentration on the oxidative stress of Mung bean (*Manuscript under preparation*)

- Effect of different concentration of ICG on the NIR optical profile of Doxorubicin (*Manuscript under preparation*)

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| | |
|---|---|
| au | Arbitrary units |
| CPS | Count Per Second |
| D | Days |
| DI | Distilled water |
| ETC | Electron Transport Chain |
| H | Hour |
| HOMO | Highest Occupied Molecular Orbital |
| LUMO | Lowest Unoccupied Molecular Orbital |
| MB | Mung Bean |
| NAD+ | Nicotinamide Adenine Dinucleotide |
| NADH | Nicotinamide Adenine Dinucleotide Hydrogen |
| OD | Optical Density |
| ROS | Reactive Oxygen Species |
| RT | Room Temperature |
| Mt | Mitochondria |
| UV-Vis | Ultraviolet visible |
| TCA | Tricarboxylic Acid |
| SOD | Superoxide dismutase |
| GPX | Glutathione Peroxidase |
| HCA | Heterocyclic amines |

# Chapter 1

## Introduction

Food quality is a critical factor that directly impacts public health, safety, and well-being. Accurate and rapid assessment of food quality is essential for ensuring that only safe and high-quality food products reach the public interest. Traditional methods for evaluating food quality, such as sensory evaluation based on microbiological analysis and chemical analysis, are often time-consuming, labor-intensive, and subjective [1]. Therefore, there is a growing need for innovative and non-destructive methods that can accurately predict the nature of food in a fast and reliable manner.

Optical methods have emerged as a promising approach for food quality assessment due to their non-invasive, rapid, and cost-effective nature. Optical techniques are used to study the interaction between light and food components to obtain valuable information about the biochemical properties of food, such as color (it can be an indicator of specific flavors), texture, moisture content, fat content, freshness [2,3]. Optical methods for predicting the nature of food involve the use of various optical techniques to analyze and assess the characteristics, quality, and composition of food products. These methods utilize principles such as absorption, reflectance, transmittance, fluorescence, and scattering of light to extract quantitative and qualitative data about the nature of food [4]. For example, infrared spectroscopy can be used to determine the fat, protein, and carbohydrate content of food, while ultraviolet-visible spectroscopy can be used to assess the freshness and spoilage of fruits and vegetables [5]. The underlying idea behind this approach is to develop an innovative optical method for predicting the nature of food, with a focus on food quality.

This research will explore the use of advanced optical spectroscopy techniques, such as spectroscopy and fluorescence imaging, to obtain real-time and accurate information about food quality attributes [6]. Through this research, we want to demonstrate the potential of optical methods to contribute to the field of food science and technology by

providing a novel approach for predicting the nature of food using optical methods. The findings of this research could have practical applications in the food industry, including food safety, quality control, and product development. The development of an optical method for food quality assessment could lead to more efficient and reliable food quality evaluation, resulting in improved public health.

## 1.1 Food: a source of nutrition

Food is a complex mixture of various components that provide essential nutrients for human nutrition. Not only does it provide us with the energy needed to carry out our daily activities, but it is also a source of the vital nutrients that our bodies need to function properly. Food is not just about satisfying hunger or indulging in our desires of taste buds. It is about ensuring that our bodies are nourished and healthy [7]. Nutrition is something which can be defined as the process of providing or obtaining the necessary elements from food for health and growth. These essential elements are found in the foods we consume and are vital for our bodies to function correctly.

In addition to providing energy, food is a source of essential nutrients that our bodies need for various physiological functions. Proteins are crucial for the growth, repair, and maintenance of tissues in our bodies, such as muscles, bones, skin, and organs. They also play a vital role in the production of enzymes, hormones, and other molecules that are necessary for our bodies to function properly.

The nutritional content of foods can vary depending on factors such as soil quality, growing conditions, and storage methods. For example, vegetables grown in nutrient-rich soil can contain more vitamins and minerals than those grown in depleted soil. Similarly, improper storage of food can lead to nutrient loss over time. Choosing fresh, locally grown produce and storing food properly can help maximize its nutritional value.

The components of food can be broadly categorized into macronutrients and micronutrients.

**1. Macronutrients:** These are the nutrients that are required in large quantities and provide energy to the body. The three main macronutrients are:

- **Carbohydrates:** Carbohydrates are the main source of energy for the body. They are found in foods like grains (such as rice, wheat), fruits, vegetables, and dairy products, etc.
- **Proteins:** Proteins are essential for building and repairing tissues and are important for various bodily functions. They are found in foods like meat, fish, eggs, dairy products, legumes, and nuts.
- **Fats:** Fats are a concentrated source of energy and play a crucial role in many physiological processes. They are found in foods like oils, butter, margarine, fatty meats, nuts, and seeds. Fats can be categorized as saturated (e.g., found in animal fats) or unsaturated (e.g., found in plant-based oils).

**2. Micronutrients:** These are the nutrients that are required in smaller quantities but are essential for overall health and well-being. The main micronutrients are:

- **Vitamins:** Vitamins are organic compounds that are necessary for various biological functions, such as metabolism, immunity, and growth. They are found in a wide range of foods, including fruits, vegetables, dairy products, meat, and fish.
- **Minerals:** Minerals are inorganic elements that are essential for many processes, such as bone formation, nerve function, and fluid balance. They are found in foods like dairy products, meat, fish, grains, fruits, and vegetables.
- **Dietary fiber:** Dietary fiber, also known as roughage, is a type of carbohydrate that is not digested by the body. It plays a crucial role in maintaining digestive health and is found in foods like whole grains, fruits, vegetables, legumes, and nuts.

A well-balanced diet that includes an appropriate balance of macronutrients and micronutrients is important for overall health and well-being. Different treatment of food can affect the nutrient content of foods. For example, boiling vegetables can result in some of the water-

soluble vitamins being lost, while grilling or broiling meat can lead to the formation of harmful compounds called heterocyclic amines (HCAs) [8]. It is important to choose cooking methods that preserve as many nutrients as much as possible while minimizing exposure to harmful substances. Therefore, these days raw or sprouted food is becoming famous as it maintains nutrition. Many foods contain compounds that have health- promoting properties beyond their nutrient content. For example, garlic contains allicin, a compound with antibacterial and antifungal properties, while turmeric contains curcumin, a compound with anti- inflammatory properties. These compounds are known as phytochemicals, and consuming a variety of plant-based foods can help provide a range of health benefits [9].

Some nutrients can interact with each other, affecting their absorption and utilization in the body. For example, consuming vitamin C with iron-rich foods can enhance the absorption of iron, while consuming calcium and iron together can inhibit the absorption of both. Understanding these interactions can help ensure that we are getting the most out of the nutrients we consume.

## 1.2 Optical methods for predicting the nature of food

Optical methods such as hyperspectral imaging, polarimetry, light scattering techniques, reflectance, and transmittance measurements are widely used in the food industry for predicting the nature of food. [10] These methods utilize various principles of light interaction with food components, such as absorption, reflection, scattering, fluorescence, and polarization, to obtain information about the physical, chemical, and biological properties of food. The most common approach is spectroscopy which is described as follow:

- **Optical spectroscopy:** It is a technique that measures the interaction of light with matter. Different types of spectroscopies, such as UV-vis spectroscopy, near-infrared (NIR) spectroscopy, infrared (IR) spectroscopy, and Raman spectroscopy, can be used to predict the nature of food. UV-vis spectroscopy measures the absorption and reflection of ultraviolet and visible light, which can provide information about the

color, pigments, and other chromophores in food [11]. NIR spectroscopy measures the absorption of near-infrared light, which can be used to determine the moisture content, fat content, protein content, and other constituents in food. IR spectroscopy measures the absorption and reflection of infrared light, which can provide information about the molecular structure, functional groups, and chemical composition of food components. Raman spectroscopy measures the scattering of light, which can provide information about the molecular vibrations and chemical structure of food components. Fluorescence spectroscopy measures the emission of light from food components that are excited by ultraviolet or visible light. This method can provide information about the presence of certain compounds, such as vitamins, pigmen and contaminants, in food. Fluorescence spectroscopy can also be used to monitor food quality, freshness, and shelf-life, as well as to detect foodborne pathogens and contaminants [12].

The food contains different components which will contribute to the overall properties. These components will add value to the nutritional profile of food which changes according to the prevailing conditions as well as with the different treatment. These two conditions can create an influence on the nutritional value by different means such as oxidation reduction reactions, antioxidant enzymes production and ROS (Reactive Oxygen Species) generation.

Here we want to study the effect of treatment of food on the quality and chemical composition using optical spectroscopy techniques. For this purpose, we have primarily used absorption and fluorescence emission spectroscopy. The food of choice was Mung bean because it is consumed in various ways such as sprouted, cooked etc.

We want to trace down the biochemical changes by making use of the simplest optical tool. We have observed significant change in absorption and emission profile from differently treated Mung bean. Specifically, we observed that the concentration of NADH was varying as per the treatment of Mung bean. This was studied using absorption and emission profile of differently treated Mung bean sample. We observed that the sprouted version of Mung bean had the highest

concentration of NADH in comparison with unsoaked and 12 H soaked Mung bean sample. The emission profiling also gives up the idea about the degradation of food which might be occurring due to the release of bound NADH from the cellular compartment. The release of NADH might be leading to the other biochemical changes after a period of time as the half-life of NADH is 5-6 hours only.

# Chapter 2
# Materials and Methods

## 2.1 Materials

Whole Mung bean (Vigna radiata) ordered from MS Global Foods, Ahmedabad, Gujarat, India for this thesis work. The chemicals used in the different stages of experimentation are mentioned in table 2.1.

Table 2.1: List of chemicals used for experimentations.

| **Name of chemicals** | **Makers** |
|---|---|
| Nicotinamide Adenine Dinucleotide Hydrogen | Loba Chemicals |
| Perchloric acid | Thermo Scientific |
| Sodium suplhate | Thermo Scientific |
| Potassium hydroxide | HiMedia |
| Tris (hydroxymethyl aminomethane (tromethamine THAM) hydrochloride | HiMedia |
| Hydrochloric acid | Thermo Scientific |
| Phenol | Sigma Aldrich |
| Isopropanol | Thermo Scientific |
| Chromic acid | Sigma Aldrich |

## 2.2 Methods

### 2.2.1 Different conditions of Mung bean

Three different conditions of interest of Mung bean such as unsoaked, soaked, and sprouted are taken into consideration for designing the experiments. The conditions are shown in Fig. 2.1 and described in detail below:

- **Unsoaked:** The beans of Mung are taken in a fixed amount which is followed by soaking in water and later crushing them with the help of a grinder or mortar pestle to obtain a slurry. Filter it out or centrifuge it. The sample extracted is ready for the experiment [13].
- **Soaked: (12 H soaking):** The beans of Mung are taken in a fixed amount which is followed by soaking them in water for 12 H which is

followed by crushing them with the help of a grinder or mortar pestle. After obtaining the slurry, filter it out or centrifuge it. The sample extracted is ready for the experiment.

- **Sprouted: (24 H soaking):** The beans of Mung are taken in a fixed amount which is followed by soaking them in water for 24 H which is followed by crushing them with the help of a grinder or mortar pestle. After obtaining the slurry, filter it out or centrifuge it.


**Fig.2.1:** Conditions of the Mung bean (Vigna radiata)

### 2.2.2 Extract Isolation

Figure 2.2 shows the extraction protocol as methodology for liquid extraction from Mung bean. The different stages of methodology are described below.

- **Preparation:** Collect fresh mung beans and remove any debris or impurities. Rinse the beans thoroughly with water to remove dirt or dust.
- **Soaking in water:** Place the mung beans in a clean glass container or tube and cover them with water. The ratio of mung beans to water will depend on the specific extraction you are trying to achieve, but a general guideline is to use about 1:10 (w/v) ratio of mung beans to water. Allow the mung beans to soak in the water for a certain period, typically ranging from several hours to overnight, depending on the conditions we had considered for the experimental purpose [14].
- **Blending or grinding:** After soaking, we used a mortar and pestle to grind the mung beans into a slurry. This will help to release the compounds from the beans and facilitate their extraction into the water. Grind the mung beans until a smooth and uniform slurry is

obtained.

- **Centrifugation:** Once you get the uniform slurry, put it into the falcon tube. Centrifuge it on 5000 rpm for 5 min. There would be a clean-cut distinctive difference between upper aqueous layer and lower non aqueous or solid layer.
- **Filtration:** Set up a filtration setup using a filter paper and funnel. Pour the upper aqueous layer through the filter paper to separate the liquid (filtrate) from the solid fine residue of (mung bean pulp). The filtrate contains the extracted liquid dissolved in water, while the fine mung bean pulp contains the solid residue.
- **Collection of the extract:** Collect the filtrate in a clean glass container or tube. Label the container with appropriate information, such as the date, type of extract, and any additional notes.
- **Storage:** Store the extract according to the requirements of your suitable desired condition of the experiment. Some extracts may need to be stored in the refrigerator or freezer to prevent degradation, while others may be stable at room temperature.

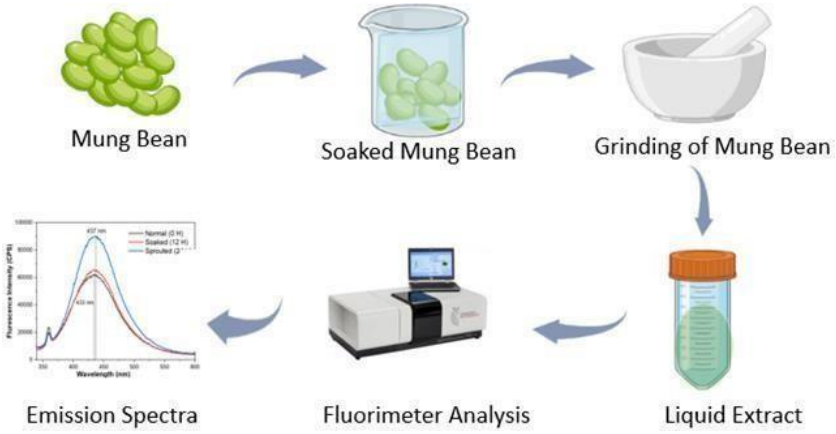



**Fig. 2.2:** Methodology for liquid extraction from Mung bean

### 2.2.3 Change in weight of Mung bean during sprouting as a function of time

The change in weight of Mung bean during the sprouting as a linear curve with the passage of time is shown in Fig. 2.3. The weight has been noted down for 5 consecutive days which shows its uniformity. The soaking of Mung bean in water induces some biochemical changes which leads to an increase in the weight of time.

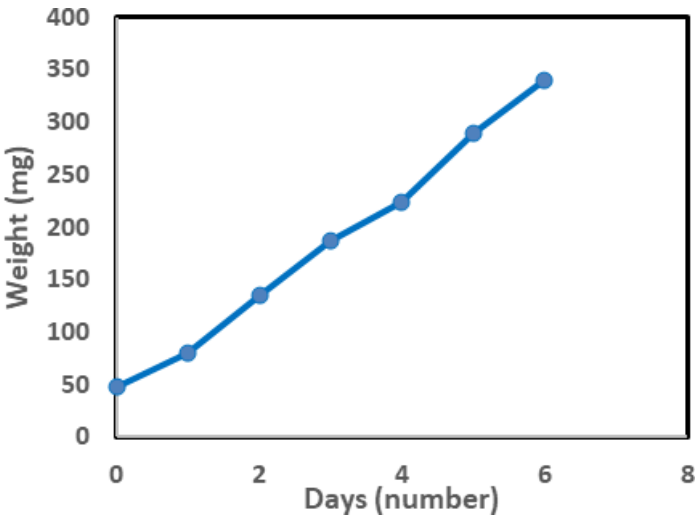



**Fig. 2.3:** Change in weight of Mung bean during sprouting

### 2.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR stands for Fourier Transform Infrared Spectroscopy. This technique is used in analytical chemistry to study the interaction of infrared (IR) radiation with matter. Figure 2.4 depicts a schematic diagram of FTIR instrument. It involves measuring the absorption, transmission, or reflection of IR radiation by a sample, and then obtaining a spectrum that provides information about the molecular composition and structure of the sample [15].

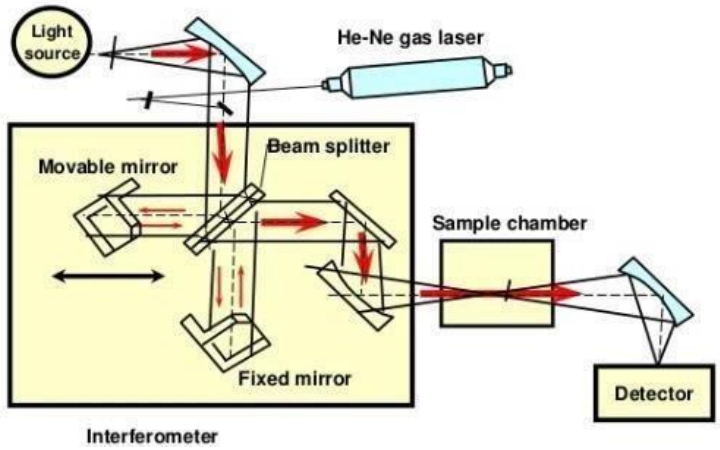



**Fig. 2.4:** Schematic of FTIR Instrument [16]

The FTIR analysis through the pellet method involves preparing a solid sample as a pellet or disk for analysis using an FTIR spectrometer. Below section describes a procedure for FTIR analysis through the pellet method:

- **Sample preparation:** Start by selecting a representative sample of the material you want to analyze. Grind or crush the sample into a fine

powder using a mortar and pestle or any other suitable method. Ensure that the sample is homogeneous and representative.

- **Select a suitable binder:** Choose a binder material that is compatible with your sample and will help form a solid pellet. Common binder materials include KBr (potassium bromide) or polyethylene. The binder should be transparent in the IR range and should not interfere with the spectral analysis.
- **Mixing:** Mix the ground sample with the binder material in the appropriate ratio. The ratio will depend on the nature of the sample and the desired thickness of the resulting pellet. Typically, a small amount of sample (e.g., a few milligrams) is mixed with a larger amount of binder (e.g., a few hundred milligrams).
- **Pellet formation:** Place the mixture of sample and binder into a pellet die or pellet mold. Apply pressure using a hydraulic press or a manual press to compress the mixture into a solid pellet. The pressure applied should be sufficient to form a pellet with good structural integrity.
- **Pellet handling:** Carefully remove the pellet from the pellet die or mold. Handle the pellet with care to avoid any damage or contamination. Place the pellet in a suitable container or sample holder for FTIR analysis.
- **FTIR analysis:** Insert the pellet into the sample compartment of the FTIR spectrometer. Ensure that the sample compartment is purged with dry air or an inert gas to minimize any interference from water vapor or atmospheric gases. Acquire the FTIR spectrum of the pellet by scanning the appropriate spectral range.

### 2.2.5 UV-vis Spectroscopy

Figure 2.5 shows the UV-vis spectrophotometer which works on the same technique used in analytical chemistry and molecular spectroscopy to study the interaction of ultraviolet (UV) and visible (vis) radiation with matter. It involves measuring the absorption or reflection of UV and visible light by a sample, and then obtaining a spectrum that provides information about the electronic structure and properties of the

sample. UV-vis spectroscopy (Shimadzu UV 1900i) is used for experimentation. It typically consists of a light source that emits UV and visible light, a monochromator that selects a specific wavelength of light, a sample holder for holding the sample, and a detector that measures the intensity of transmitted or reflected light. The basic principle of UV-vis spectroscopy is based on the Beer-Lambert law, which describes the relationship between the absorbance of a sample and the concentration of the absorbing species in the sample in the sample [17].

$$\mathbf{A = \varepsilon cL}$$

where, '**A**' is the amount of light absorbed for a particular wavelength by the sample, '**ε**' is the molar extinction coefficient, '**L**' is the distance covered by the light through the solution, and '**c**' is the concentration of the absorbing species.


**Fig. 2.5:** UV vis spectrophotometer

### 2.2.5.1 Absorption Spectral profiling of NADH

The absorption spectra of NADH (Nicotinamide adenine dinucleotide, reduced form) shown in Figure 2.6 typically exhibit two main peaks in the UV-visible range, as shown below:

- **Peak around 260-270 nm:** NADH has a strong absorbance peak in the UV region, typically around 260-270 nm. This peak is associated with the π→π* transition of the adenine ring in NADH.[18]
- **Peak around 340-350 nm:** NADH also exhibits a broad absorbance peak in the visible region, typically around 340-350 nm. This peak is related to the n→π* transition of the nicotinamide ring in NADH [19].

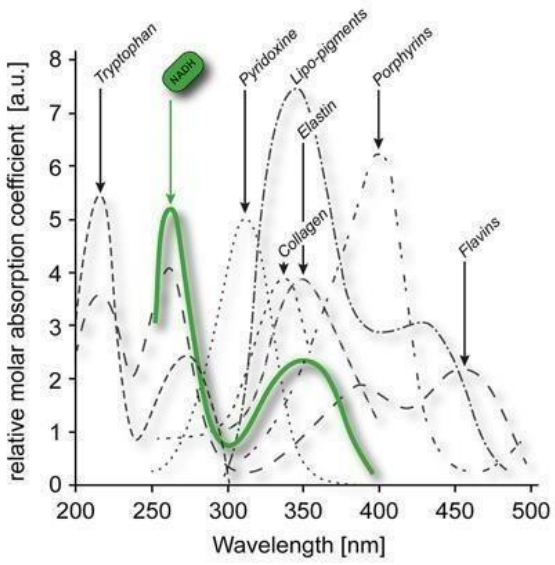



**Fig. 2.6:** Absorption Spectra of NADH [19]

### 2.2.6 Fluorescence Spectroscopy

Fluorescence spectroscopy is a technique used to study the interaction of light with matter, particularly the emission of light by molecules called fluorophores after they absorb light energy. Fluorescence by Horiba shown in Figure 2.7 is a type of photoluminescence, where a molecule absorbs light at a specific wavelength (excitation wavelength) and then re-emits light at a longer wavelength (emission wavelength) after a short-lived excited state. The basic components of a fluorescence spectroscopy setup include a light source, monochromators for selecting specific excitation and emission wavelengths, a sample holder for holding the sample containing fluorophores, and a detector for measuring the emitted light. The excitation wavelength is selected and focused onto the sample, and the emitted light is collected and measured to obtain a fluorescence spectrum.

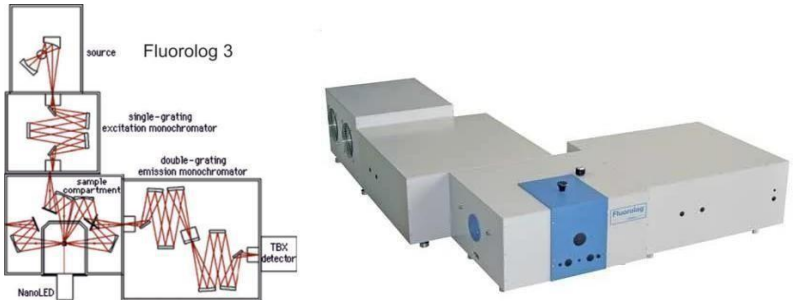



**Fig. 2.7:** Schematic of Fluorolog 3

### 2.2.6.1 Emission Spectral profiling of NADH

The emission spectra of NADH (Nicotinamide adenine dinucleotide, reduced form) shown in the Figure 2.8 typically exhibit fluorescence emission peaks in the visible range, as shown below:

Emission peak around 435-440 nm: NADH exhibits a strong fluorescence emission peak in the visible range, typically around 435-440 nm. This peak is associated with the excited state relaxation of NADH after absorption of UV light [19]

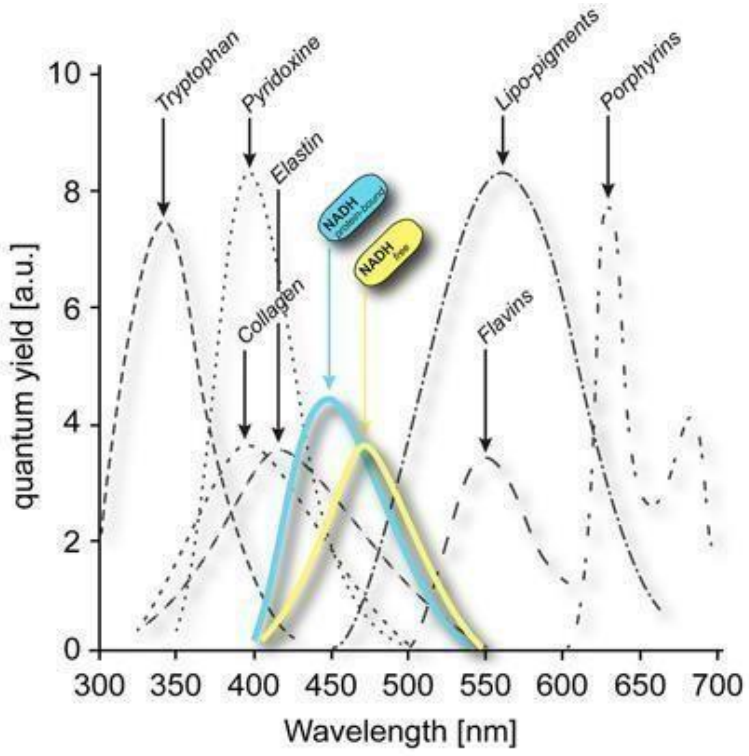



**Fig. 2.8:** Emission spectral profile of NADH [19]

## 2.2.7 Preparation of Standard Calibration curve of NADH

Measure the absorbance of each NADH dilution at 340 nm using the spectrophotometer, recording the absorbance values for each concentration. Plot a calibration curve using the known concentrations of NADH on the x-axis and the corresponding absorbance values on the y-axis. We have used Excel graphing tool to create a linear regression of the data points. Determine the equation of the calibration curve, typically in the form of y = mx + b, where y is the absorbance, m is the slope, x is the concentration of NADH, and b is the y-intercept. The slope (m) of the calibration curve represents the molar

absorptivity or extinction coefficient of NADH at 340 nm, and the y-intercept (b) represents the background absorbance or noise level.

Use the calibration curve shown in Figure 2.9 to quantify the concentration of NADH in unknown samples by measuring their absorbance at 340 nm and using the equation of the calibration curve to calculate the concentration.

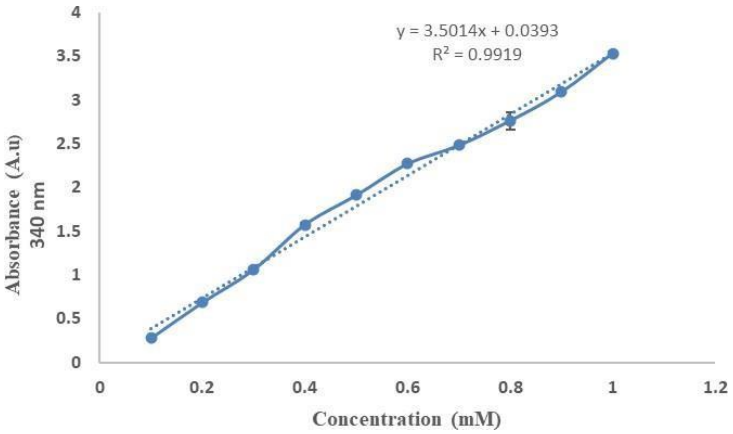



**Fig. 2.9:** Standard calibration curve for NADH

### 2.2.8 NADH Extraction Protocol

To extract the NADH from Mung bean, a standard protocol was followed according to the reference [18]. The details are given below:

- Collect the extract of Mung bean from our three chosen conditions and handle it appropriately to preserve the integrity of NADH. Snap freezes the sample in liquid nitrogen or dry ice if needed to prevent NADH degradation. After snap freezing, we will have to do the vortex to let the mixing be uniform.
- Prepare the 0.1 M KOH and add it to the fixed amount of Mung sample. Keep the sample on ice or in a cold room to maintain sample integrity.
- Add 0.1 M HClO4 to the mung bean sample, which is followed by the addition of TRIS HCl (pH 8.4)
- Disrupt the sample using a homogenizer or sonicator for the uniformity of fine particle and release NADH [21]. Use caution to avoid sample heating during disruption, as NADH is sensitive to temperature.
- Centrifuge the sample at a low temperature (e.g., 4 °C) and high

speed to separate the insoluble debris (e.g., cell debris, tissue fragments) from the soluble supernatant containing NADH. Transfer the supernatant to a fresh tube.

- Optionally, quantitate the extracted NADH using a NADH standard solution and appropriate spectroscopic or enzymatic assays, such as UV-vis spectroscopy or enzymatic cycling assays.
- Store the extracted NADH samples at -80 °C for using it for the future experiment.

# Chapter 3

## Result and discussion

### 3.1 Spectroscopic Analysis of Mung bean Extract

It is believed that sprouted beans are good for maintaining health. So, in order to understand this, we treated Mung beans differently and studied them using the optical spectroscopy techniques. For this purpose, we used unsoaked Mung beans, 12 H soaked Mung beans and sprouted Mung beans.

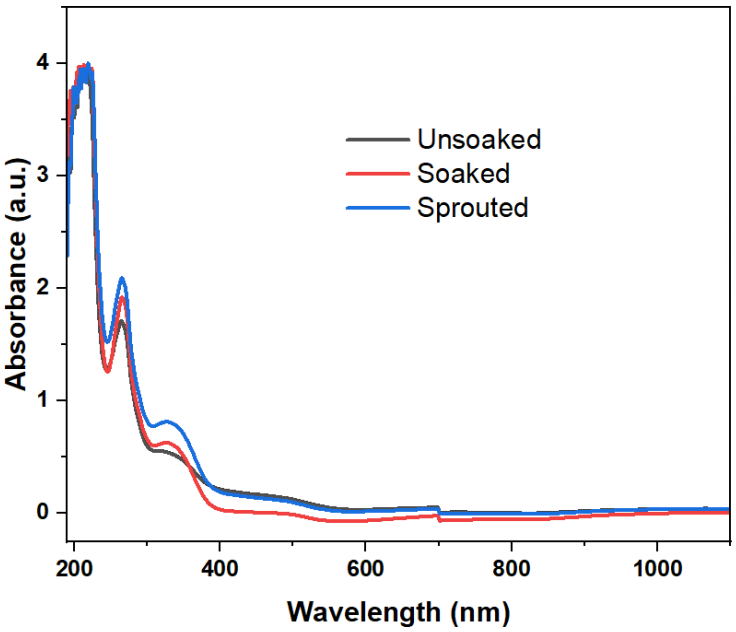



**Fig. 3.1:** Absorption spectra of Experimental Mung bean extract

Figure 3.1 shows the absorption spectra of the Mung bean extract in all the three chosen experimental conditions with 2 characteristic peaks at 340 nm and 260 nm wavelength. These absorbance peaks go on increasing as we move from Unsoaked to Soaked and Soaked to Sprouted conditions. The absorption spectrum matches with the absorbance of NADH [19]. Therefore, this continuous increase in absorption might be due to the varying NADH concentration in all the three chosen experimental conditions.

Figure 3.2 shows the fluorescence emission spectra of Mung bean extract in all the three chosen experimental conditions with one characteristic emission peak 440 nm. This peak shows a red shift with significant increase in the Fluorescence Intensity count as we move from

Unsoaked to Soaked and Soaked to Sprouted conditions. The fluorescence spectra of the extract are similar to the fluorescence spectrum of NADH as found in the literature [19]. This continuous increase in emission intensity might be due to the varying NADH concentration in all the three chosen experimental conditions which are giving different emission profile. Therefore, it might be assumed that the observed fluorescence emission might be due to NADH present in the extract.

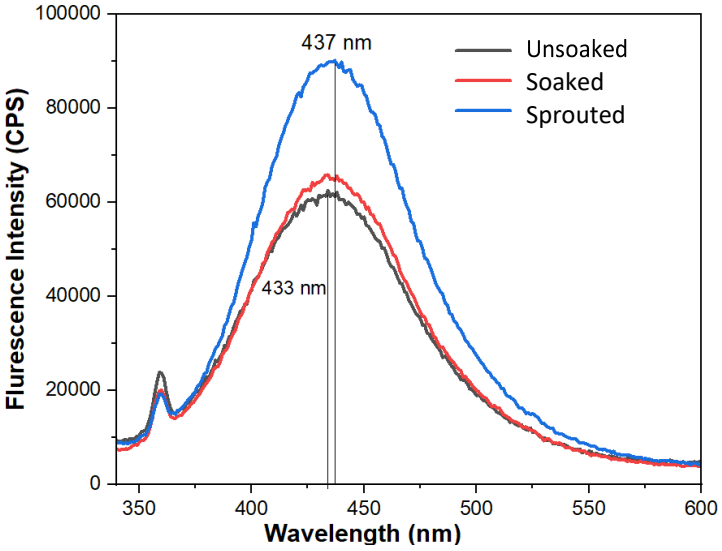



**Fig. 3.2:** Emission spectrum of Mung bean extract

## 3.2 FTIR of Mung bean extract

For further confirmation of the above results of absorption and emission, we took help of the FTIR spectra-based analysis. Figure 3.3 shows the FTIR spectra of the extract of Mung bean. The characteristics of FTIR obtained are as follows.

- 3200-3600 cm-1 corresponds to the stretching vibrations of the **N-H bonds** in the adenine and nicotinamide rings. (3290 peak)
- 2925 cm-1, which corresponds to the stretching vibrations of the **C-H bonds** in the molecule.
- 1540 cm-1, which corresponds to the stretching vibrations of the **C=C bonds** in the adenine ring.
- 1380 cm-1, which corresponds to the bending vibrations of the **C-H bonds** in the molecule.

These peaks also indicate that NADH might be an integral part of

Mung bean extract. So, from all these measurements, it seems that optical spectroscopy methods might give us quantitative and biochemical information of NADH present in the extract. As NADH works as a coenzyme that plays a key role in Electron Transport Chain to produce ATP via cyclic transport of electron passing through five series of complexes.

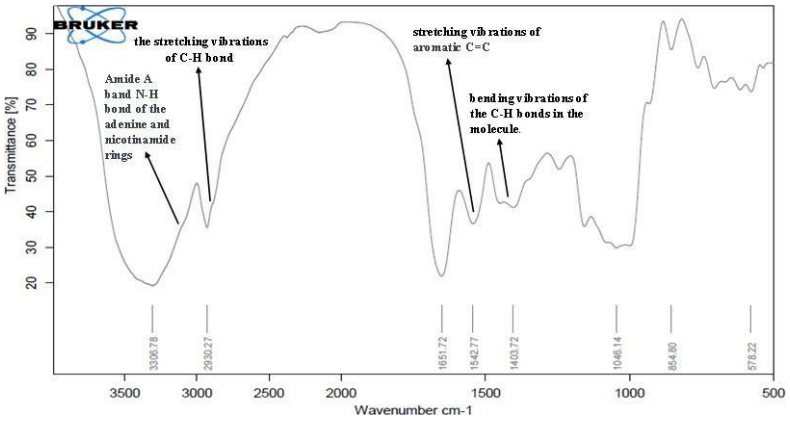



**Fig. 3.3:** FTIR Spectra of Mung bean extract

## 3.3 Effect of soaking on the concentration of NADH in different sample

To understand the quantitative change in NADH conc. in three different samples, we used a protocol to isolate NADH from Mung bean extract. The protocol has been explained in Chapter 2.

For quantifying the NADH concentration from the extract, we prepared a calibration curve as shown in Chapter 2. For this absorption of purified NADH was obtained and quantified using the calibration curve. Figure 3.4 shows the change in the NADH conc. for three samples. It can be observed that sprouted Mung bean sample had the highest amount of NADH and unsoaked sample had the least amount. High NADH concentration is associated with more functional activity in terms of ATP generation cycle and ability to neutralize ROS activity. The lesser the NADH concentration, the lesser would be the neutralization of ROS and more damage to the components of Mung bean and less functionally active Electron Transport would function. [22].

These results suggest that sprouted Mung bean samples have highest

NADH content, which might be good for health. Therefore, we can say that the higher NADH conc, more functional would-be Electron Transport Chain in terms of ATP generation.

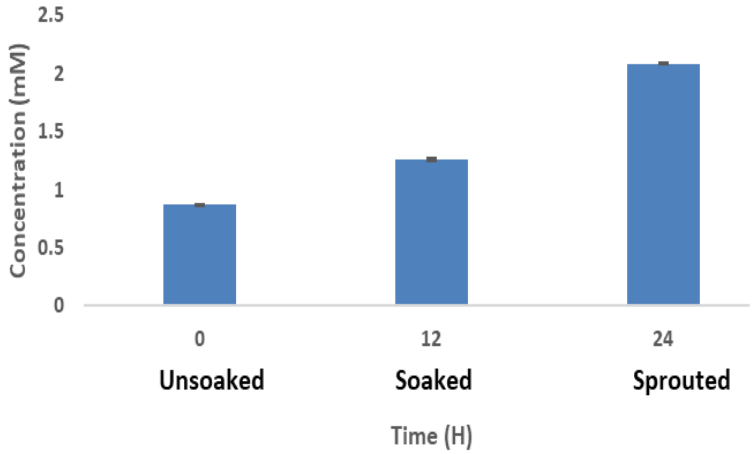
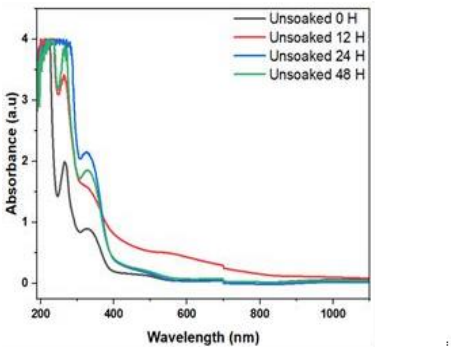


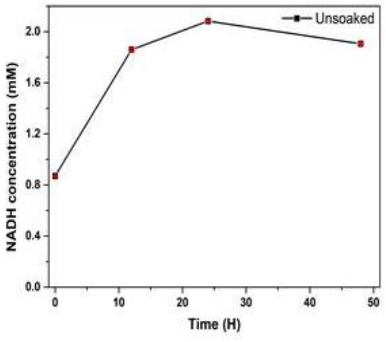

**Fig. 3.4:** NADH concentration in Mung bean post extraction

## 3.4 Effect of ageing time on Absorption Spectra of NADH after extraction

We were also interested to know the effect of aging time on the Mung bean extract as it might tell us about the change in NADH concentration in the extract at different time points after extraction. For this, we purified NADH from the bean extract at different ageing time and measured its quantity. Figure 3.5 shows the absorption spectral profile of NADH after extracting it from our three chosen experimental conditions i.e., Unsoaked Mung bean (Fig. 3.5$a_1$ and 3.5$a_2$), Soaked Mung bean (Fig. 3.5$b_1$ and 3.5$b_2$), and Sprouted Mung bean (Fig. 3.5$c_1$ and 3.5$c_2$) taken in a time dependent fashion.




**($a_1$)**




**($a_2$)**

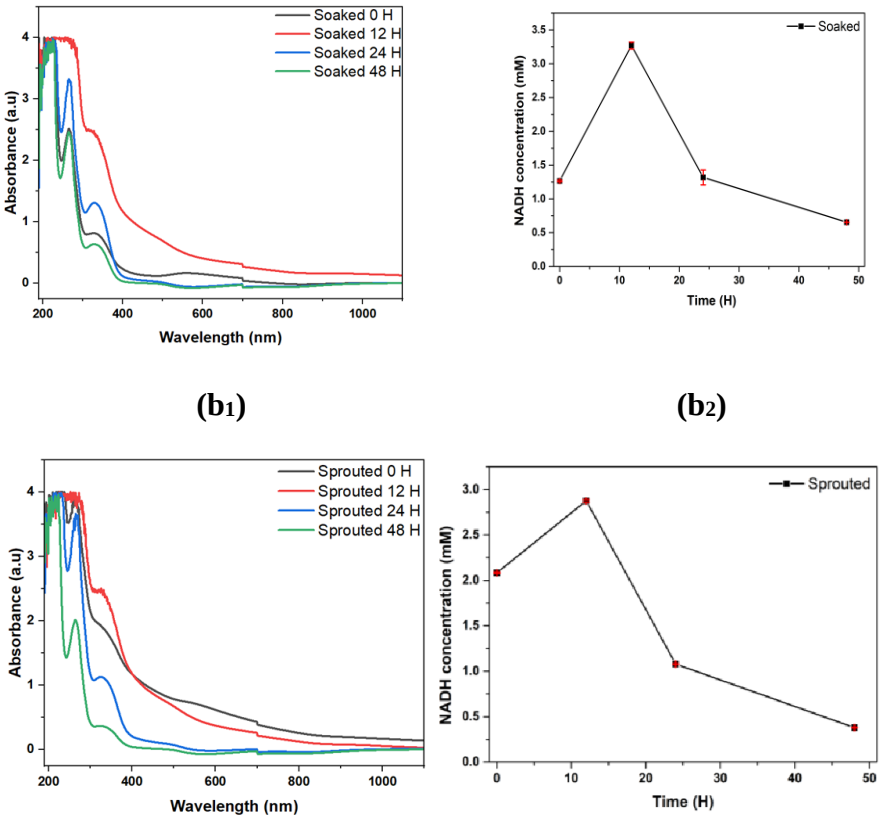
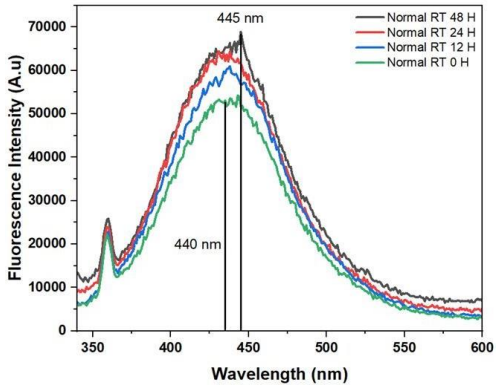



**Fig. 3.5:** Absorption Spectra of NADH extracted from Unsoaked Mung bean ($a_1$ and $a_2$), Soaked Mung bean ($b_1$ and $b_2$), and Sprouted Mung bean ($c_1$ and $c_2$).

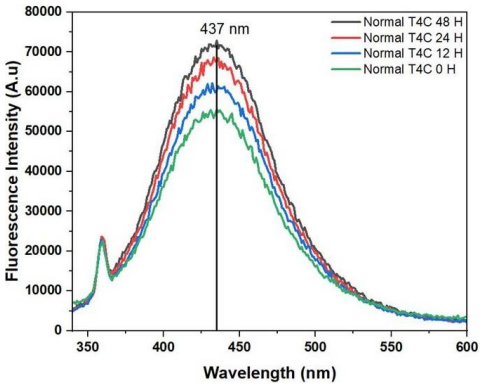
It is evident from Fig. 3.5$a_1$-3.5$c_1$ that there is a prominent change in absorption profiling of NADH taken in a time dependent manner. Figure 3.5$a_1$ and 3.5 $a_2$ depicts the NADH profile after extraction from unsoaked Mung bean, there is continuous increase in NADH conc. up to 24 H beyond which it starts to get decrease. The reason behind the initial increase might be due to the enzymatic reactions responsible for the release of bound NADH.

Figure 3.5$b_1$, 3.5$b_2$, and figure 3.5$c_1$,3.5$c_2$ shows the NADH profile after extracted it from Soaked Mung bean, there is continuous increase in the absorption profile of NADH over a period of time right from the 0 H to 12 H beyond which it starts to get decrease. The reason behind that initial increase and very significant decrease might be due to the fact that NADH which was initially present in the bound form starts to release.

To understand the aging process of Mung bean extract, we measured the fluorescence emission of Mung bean extract at different time point at two temperature, 25°C and 4°C. Figure 3.7a and 3.7b show the fluorescence spectra of the extract of Mung bean taken in a time dependent manner at two different temperature in order to study the degradation profile of food by making an approach through the emission spectral.




**(a)**

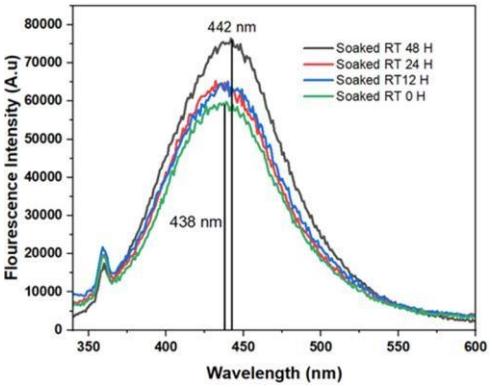



**(b)**

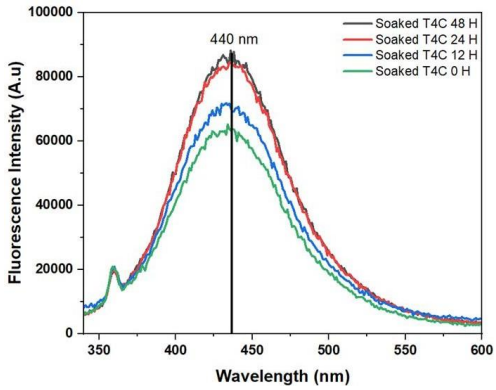
**Fig. 3.6:** Emission spectra of extract of Unsoaked Mung bean kept at (a) room temperature and (b) 4°C

Figure 3.7a and 3.7b depict the fluorescence emission spectral profile of the extract of soaked Mung bean at two temperature 25°C and 4°C respectively. It can be observed from Fig. 3.7a, that there is continuous increase in the fluorescence intensity count with a red shift of 5 nm at 25°C.This might be due to the release of NADH in mung

beans from its bound form relatively quickly due to the activity of enzymes which is reflected by a 5 nm red shift from 0 to 48 H. [19] However no spectral shift was observed at 4°C sample as can be seen in fig 3.7b. This might be due to slow release of bound NADH and slower enzymatic activity.




**(a)**

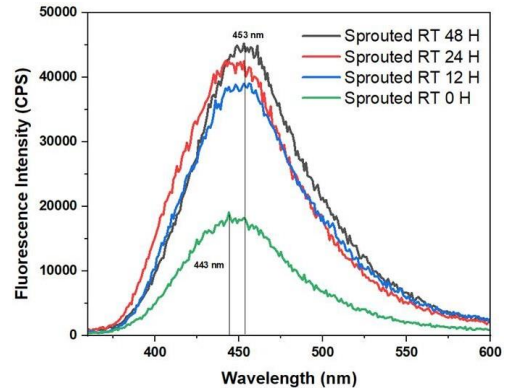



**(b)**

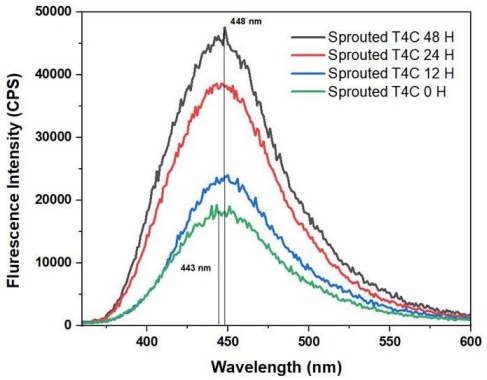
**Fig. 3.7:** Emission spectra of extract of Soaked Mung bean kept at (a) room temperature and (b) 4°C

A red shift in the fluorescence peak can be observed in Fig. 3.7a at room temperature (approximately 25°C). This might be due to enzymatic activity and other chemical reactions that may lead to the release of NADH from its bound state. Whereas fluorescence spectra at 4°C in fig. 3.7b shows no red shift. This might be due to the slower enzymatic activity leading to slow or no release of NADH from its bound state.

Figure 3.8a and 3.8b show the fluorescence emission spectral profile of the extract of sprouted Mung bean taken in a time dependent manner at two different temperatures in order to study the aging of food.




**(a)**




(b)

**Fig. 3.8:** Emission spectra of extract of Sprouted Mung bean kept at (a) room temperature and (b) 4°C

Figure 3.8a illustrates the fluorescence emission spectral profile of sprouted Mung bean. The spectra profile of sample kept at room temperature shows as red shift of 10 nm from 0 to 48 H but shows no shift from 0 to 24 H. This red shift of 10 nm might be linked to the higher release of NADH from bound form due to more enzymatic activity. [23] Interestingly, the spectral profile of sample kept at 4°C (Fig. 3.9b) also shows a significant shift of 5 nm with the continuous increase in intensity which indicates that the NADH is getting released from its bound state at a slow pace as compared to the same sample kept at room temperature but still there might be some biochemical reactions taking place which is responsible for increase in intensity count.

These results suggest that the sprouted Mung bean sample has biochemical activity even at the low temperature which was not observed in case of unsoaked and 12 H soaked Mung bean extract. This might be due to functionally more active electron transport chain in case

of sprouted sample. Therefore, it might be more beneficial for health to consume the sprouted Mung bean as they might be containing more biochemically active enzymes that provides more nourishment to the body.

## 3.7 List of Enzymes becoming dominant after 24 Hours

Table 3.1 presents the tentative list of enzymes which might be dominant post 12 H in extract of Mung bean. These enzymes might be responsible for an increase in the emission spectral profile over a period of time [24].

**Table 3.1:** Tentative list of enzymes post extraction from Mung bean

| **Enzyme** | **Time-dependent activity** |
|---|---|
| Alpha-amylase | Increases during the first few days of sprouting, peaks around day 3, and then gradually declines. |
| Beta-amylase | Increases during the first few days of sprouting, peaks around day 3, and then gradually declines. |
| Invertase | Increases during the first few days of sprouting, peaks around day 3, and then gradually declines. |
| Proteases | Shows a more gradual increase during sprouting and may continue to increase throughout the sprouting process. |
| Lipases | Shows a gradual increase during sprouting, peaks at day 3, then declines. |

# Chapter 4

## Conclusion and Future Aspects

In this thesis, we used absorption and fluorescence spectroscopy to understand the behavior of Mung beans treated differently. Firstly, sprouted Mung beans exhibit higher NADH concentration among three chosen experimental conditions (unsoaked, 12 H soaked and sprouted). This might be due to the prevailing biochemical reactions taking place inside the bean. As the biochemical active reactions are more active in Sprouted sample, it might be the reason for the increases NADH concentration as compared to soaked and unsoaked beans, suggesting increased functional activity in ATP generation.

Thus, more ATP generation activity suggests which follows the order as sprouted > soaked > unsoaked. Sprouted and Soaked beans might also possess more antioxidant enzymes than unsoaked beans which are crucial for neutralizing ROS and preventing oxidative stress which can degrade protein, carbohydrates, and fats, rendering the beans unfit for consumption. The project's aim is to develop an optical spectroscopy-based tool that detects biochemical changes in food using various parameters. This tool might empower individuals to make informed decisions about their food choices and fosters food consciousness.

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