



# INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

## SPECTROSCOPY ANALYSIS OF FERMENTED BIOMEDICINE

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### ABSTRACT

Spectroscopy is branch of science dealing with the study of interaction of electromagnetic radiation with matter. Spectroscopy is the exact study of color as it applies to all bands of the electromagnetic spectrum, including visible light. Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure and is used in analysis of wide range of sample. Many type of spectroscopy analysis are used different types of fermentation process like UV Visible, Infrared, and Raman Spectroscopy. Dairy products that were fermented have a long history of manufacture. Ayurveda has a variety of medications, including fermented forms such as arishtas (fermented decoctions) and asavas (fermented powders). This study explains the fundamentals and recent advanced study in fermented biomedical spectroscopic analysis. The goal of this review is to describe and analyze commonly fermented foods (kefir, kombucha, arishta, asava, yoghurt, and sourdough bread), their modes of action (including the influence of spectroscopic analysis), and the evidence for impacts on human digestive health and diseases.

**Keyword:** Introduction, Methods of spectroscopy analysis, Fermented biomedicine, Application and example of spectroscopy analysis of fermented biomedicine etc.

### INTRODUCTION

Spectroscopy is the interaction of waves from the electromagnetic spectrum with molecules in the sample matrix under investigation. UV Visible, Infrared and Raman spectroscopic method are used to utilize fermented biomedicine but also Atomic and molecular spectroscopy are the two primary spectroscopic methods utilized in fermentation process. The development and use of these spectroscopic technologies in the field of food analysis is based on absorbance, fluorescence, and dispersion processes that occur when matter and light interact. These implementations in fermentation are based on a number of spectroscopic methods and techniques that take use of various wavelength ranges, such as UV-Visible, Near, Mid and Far Infrared, Raman and nuclear magnetic resonance. UV-Vis spectroscopy is a tool for detecting spectroscopic method that permits ultraviolet and visible light at a wavelength extending from 200 to 780 nm. When a molecule's

vibrational motion is followed by a change in its dipole moment, IR spectra are produced. These spectra occur in the spectral ranges of 500-4000  $\text{cm}^{-1}$ . Movements between the vibrational and rotational energy levels of a molecule in the applied magnetic field cause NMR spectra to appear<sup>[1, 2, 3]</sup>

Fermentation is a microorganism's anaerobic activity that is one of the oldest techniques of preserving food. Furthermore, it is a natural approach to improve the nutritional value as well as the appearance of food while eliminating the harmful ingredients. Fermented food has a strong tradition, and a wide variety of fermented biomedicine are enjoyed all over the world. Fermented foods made in a certain location based on the availability of raw materials and microorganisms in that region they're fermented with this method. For example Kefir, is a fermented drink produced from milk and berries. Kavas is a traditional British fermented drink made from rye flour and malt. Kumyss is made from sour and fermented milk in Turkey and Greece. India is often known for its fermented food consumption, with many varieties of fermented foods and drinks made in various parts of the country. Various manufacturing plants are being used to generate them. Changes in lifestyle and dynamic food habits have resulted in the growing prevalence of diseases such as type 2 diabetes, coronary heart disease, cancer, periodontal disease, and obesity in present society. As a result, in current history, economic growth for health-improving food products has increased. As a result, healthful meals are necessary to fulfil the public's demand for nutritious diets. These beverages have several medical benefits and are an excellent source of minerals and vitamins that contribute to our body's overall health. The goal of this review is to describe and analyze commonly fermented foods (kefir, kombucha, arista, asava, yoghurt, and sourdough bread), their modes of action (including the influence of spectroscopic analysis), and the evidence for impacts on human digestive health and diseases.<sup>[4, 5, 6]</sup>

### 1.1 METHODS OF SPECTROSCOPIC ANALYSIS

The study of the relationship of matter with electromagnetic waves as a function of the wavelength and intensity of the radiation is known as spectroscopy. The determination of electromagnetic intensity as a function of wavelength is called to as spectroscopy, and the word is frequently used to describe practical spectroscopic procedures.<sup>[1, 2]</sup>

Different types of spectroscopic method can be carried out of the following;

- a. Spectroscopy in the ultraviolet and visible ranges.
- b. Spectroscopy in the Infrared.
- c. Raman Spectroscopy is a technique for detecting molecules using light waves.
- d. Resonance Nuclear-Magnetic.

**a. Spectroscopy in the ultraviolet and visible ranges:** Several researchers have reported on the use of UV-Vis spectroscopy in diagnostic biomedicine to measure various chemical components. Only in a uniform liquid solution can UV absorption analysis of solutes can be performed. The color of the substances involved is directly affected by their absorption or reflection in the electromagnetic frequencies. UV and Vis light are only absorbed by chromophores, which are molecular functional groups in which electrons are activated at various wavelengths.<sup>[2]</sup>

**b. Spectroscopy in the Infrared:** The study of infrared light interactions with molecules is known as infrared spectroscopy. Measurements of absorption, emission, and reflection can all be used to examine this method. Using mid-infrared spectroscopy, a new approach including on observation of fermentations has been created. The technique has been used to determine sucrose and alcoholic concentrations during berry's yeast fermentations. For years, infrared spectroscopy has been used as a substitute for traditional fermentation monitoring methods.<sup>[1]</sup>

**c. Raman Spectroscopy:** Raman spectroscopy is a spectroscopic method that is commonly used to identify the vibrational modes of compounds, however it can also be used to detect rotational and other less-frequency modes of systems. Raman spectroscopy is widely used in science to produce a structural fingerprint that may be used to identify compounds. When compared to standard IR techniques, Raman spectroscopy has the benefit of having very little water interference when detecting aqueous samples, making it potentially helpful for in vitro evaluation of critical industrial bioprocesses.

**d. Resonance Nuclear-Magnetic:** The spectroscopic method of nuclear magnetic resonance spectroscopy, often known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is used to examine local magnetic fields surrounding atomic nuclei. The NMR signal is created by excitement of the nucleus samples with radio signals into nuclear magnetic resonance, which is detected using sophisticated radio receivers, and the sample is put in a magnetic field. For the first time, employing Nuclear Magnetic Resonance spectroscopy, bioactive chemicals in apple juice and intermediate goods of the cider making process were characterized together.

## 2.1 FERMENTED BIOMEDICINE

Fermentation is a microorganism's anaerobic activity that is one of the oldest techniques of preserving food. Fermented product are described as dietary supplements prepared through the controlled growth of microorganisms and the enzymatic conversion of dietary components.<sup>[4]</sup> The goal of this review is to describe and analyze commonly fermented foods (kefir, kombucha, aristha, asava, yoghurt, and sourdough bread), their modes of action (including the influence of spectroscopic analysis), and the evidence for impacts on human digestive health and diseases. Fish and meat, dairy, green vegetables, soybeans, other legumes, grains, and fruits are among the things that have traditionally been fermented.<sup>[5]</sup> The microbes, nutritional elements, and climatic circumstances all have a role in the fermentation process, resulting in hundreds of different types of fermented foods. Fermentation of foods can be accomplished in two ways. For starters, foods can naturally ferment. Sauerkraut, kimchi, and various fermented soy products are examples. Second, foods can be fermented by adding starting cultures to them. For example kefir, kombucha and natto.<sup>[6]</sup> These beverages have several medical benefits and are an excellent source of minerals and vitamins that contribute to our body's overall health. Various example of fermented biomedicine are as follow;

- ✓ Aristha
- ✓ Asavas
- ✓ Yoghurt
- ✓ Kefir
- ✓ Kombucha

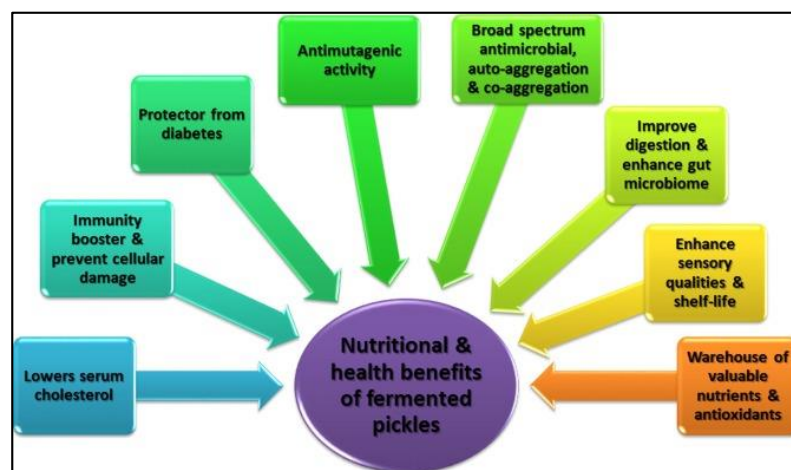


Figure no 1

### 3.1 APPLICATION AND EXAMPLE OF SPECTROSCOPY ANALYSIS OF FERMENTED BIOMEDICINE

These implementations in fermentation are based on a number of spectroscopic methods and techniques that take use of various wavelength ranges, such as UV-Visible, Near, Mid and Far Infrared, Raman and nuclear magnetic resonance.

Different application and example of spectroscopy analysis of fermented biomedicine are as follow;

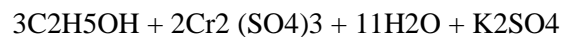
- 1) Yogurt Fermentation Process by **Near-Infrared Spectroscopy**.
- 2) Arishta fermentation process by **UV Spectroscopy**.
- 1) Yogurt Fermentation Process by **Near-Infrared Spectroscopy:-**

The manufacturing of fermented dairy products has a long tradition. They have long been known to have numerous health benefits, so they are a perfect match for the dairy sector. [7] These functional dairy food products have become more widely used in daily life, and their global popularity can be related to mounting evidence of numerous favorable health impacts. Yogurt, a result of lactic milk fermentation, is one of most popular functional dairy products. Lactic acid fermentation is the conversion of milk sugar lactose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) into lactic acid (C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>) by lactobacillus, leading to drop pH level. Caseins are class of phosphoproteins present in milk. Depending on the pH, three phases could be seen throughout this procedure. When the pH is first decreased from 6.7 to 6.0, just a little quantity of colloidal calcium phosphate is dissolved. When the pH is reduced to 5.0, the colloidal calcium phosphate is entirely dissolved in the second step. Finally, when the pH falls below the casein's isoelectric point (pH 4.6), the casein microcapsules consolidate and form the yoghurt gel matrix. Automatic monitoring and management of the yoghurt fermentation process could have significant effect on the dairy sector in terms of product quality and economic benefits. [8, 9]

**Methods of fermentation:-**In a microwave oven, 500 mL UHT sterilized milk was prewarmed to a temperature of 38 C. The milk was inoculated with 10 mL yogurt and stirred for 2min at 1500 rpm using a magnetic stirrer. The inoculated milk was then placed in a quartz cuvette cell with a 1 mm path length for spectrum measurement and evenly distributed to 17 test tubes containing 10 mL samples for pH measurements. The spectrometer had a TE-InGaAs detector and a temperature controller that was attached to the cuvette cell holder to keep the stable temperature. The sample in the cuvette cell was fermented in the spectrometer at 38<sup>0</sup> C, and absorbance spectra of the same sample were continuously recorded every 15 minutes for 8 hours. In the incubator, the samples in the test tubes were fermented at same temperature. During the fermentation, pH measurements were taken every 30 minutes, and at each time point, a new tube was opened and the pH was measured. [10, 11, 12]

- 2) Arishta fermentation process by **UV Spectroscopy:-**

Medicinal plants are known to possess a variety of bioactive phytoconstituents that are utilized all over the world to prevent and treat a variety of diseases. Arishtas are popular ayurvedic products that have a better level of stability and palatability. These are alcoholic medicines made from herbal decoctions fermented with sugars added. As a result, this approach employs solvent extraction of ethanol from arishta, accompanied by spectrophotometer measurement of alcohol using acid dichromate solution.



Chromium ions oxidize ethanol in an aqueous solution, and these ions are reduced from the +6 oxidation state to +3, changing the color from orange to green. [13, 14]

## Methods of fermentation:-

### *Solvent extraction*

The tri-n-butyl phosphate (TBP) was chosen as the solvent for ethanol extraction from arishta. It has a density of 0.9727 and 0.576 is a distribution coefficient for ethanol in water. During dichromate oxidation, it is possible to see dramatic color development for alcohol. 2.5 ml arishta sample was diluted with an equal quantity of distilled water and vigorously vortex-mixed with 5 ml TBP for 15 minutes. The upper layer of these tubes was utilized for dichromate oxidation, while the lower layer was put aside for phase separation. Standard ethanol solutions were made by diluting a set amount of absolute alcohol with distilled water and processing them in the same way. [15]

### *Preparation of dichromate reagent*

The dichromate reagent needed for the experiment was made by dissolving 40 g potassium dichromate in 200 mL distilled water. Then, with caution, 270 mL concentrated H<sub>2</sub>SO<sub>4</sub> is added, the solution is cooled, and the volume is adjusted to 500 mL by adding sufficient distilled water.

### *Dichromate oxidation and spectrophotometric analysis*

3 ml TBP layer was transferred to the new tube and mixed with 3 ml dichromate reagent for 10 minutes while shaking at 150 rpm. After that, the bottom layer was separated and the absorbance at 595 nm was measured using a spectrophotometer. [16]

## 4.1 CONCLUSION

Although India offers a wide variety of fermented foods, most of these activities are regional and mainly exclusive to a single culture.

For quantitative evaluation of ethanol in fermented ayurvedic formulation arishta, dichromate oxidation is a consistent and accurate approach. Alcohol measurement may be done with a smaller sample volume, which makes it excellent for small study batches.

Raman spectroscopy at 785 nm was used to continuously monitor the fermentation of a synthetic fermentation broth and a lignocellulosic hydrolysate. These findings suggest that Raman spectroscopy could be a useful technique for improving the efficiency of current bioconversion systems.

NIR spectroscopy and aquaphotomics have been proposed for non-invasive automated yoghurt production control and a better knowledge of the lactic acid bacteria fermentation process. NIR spectra efficiently recorded both chemical and physical fluctuations that occur during the fermentation process, according to the findings.

## 5.1 ACKNOWLEDGEMENT

We would like to thanks principle Dr. S V Patil and Assistant professor Pranali P Patil Ashokrao Mane College of Pharmacy, Peth-Vadgaon for their guidance and support during this work.

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