



A REVIEW ON RAMAN SPECTROSCOPY AND ITS APPLICATION IN FOOD TECHNOLOGY

***Shital S. Bhabad¹, Het B. Bhandari², Pradnya U. Patil³, Rishikesh S. Bachhav⁴**

¹Department of Pharmaceutical Quality Assurance, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik

²Department of Pharmaceutical Quality Assurance, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik

³Department of Pharmaceutical Quality Assurance, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik

⁴Department of Pharmacology, R.G.Sapkal College of Pharmacy, Anjaneri, Nashik

ABSTRACT: Food safety in supply chains is greatly aided by food detection technologies. Traditional procedures for finding biological, chemical, and physical contaminants in food are labor-intensive, expensive, time-consuming, and frequently cause food samples to change. These drawbacks need the creation of more useful food detection instruments that can identify pollutants from all three classes, according to the food industry. Raman spectroscopy can provide widespread food safety screening in a quick, simple, sensitive, and easy way. Recent improvements in Raman spectroscopy techniques have increased the ability to identify food pollutants, greatly expanding the applicability of these techniques in food safety. In this review, we describe the fundamentals of surface-enhanced Raman spectroscopy (SERS), micro-Raman spectroscopy, and imaging while also outlining recent developments in the detection of biological, chemical, and physical hazards in foods. We also discuss the drawbacks and potential applications of Raman spectroscopic techniques for food safety surveillance. The goal of this review is to highlight possible applications for Raman spectroscopic methods as an innovative way for determining food safety.

KEYWORDS: Raman spectroscopy; SERS; Raman imaging; food hazards; rapid detection

INTRODUCTION:

Due to the vulnerability of our food chain, food safety has become a crucial concern. Food contamination has been demonstrated to have a negative impact on economic growth as well as cause serious health issues and even death. Unsafe food causes 600 million illnesses and 42,000 fatalities annually, according to the World Health Organization. Food quality assessment techniques are now more necessary than ever as consumers' concerns about food quality have grown. As a result, all stages of food production, such as the content or quality of the items, their origin, and how they have been handled, processed, and stored, have become more significant. Food analysis employs a variety of techniques, such as microbial methods, sensory analysis, biochemical methods, and physicochemical methods. Three types of contaminants can unintentionally or purposefully contaminate food throughout the food supply chain: microorganisms (bacteria, viruses, and fungi), chemicals (toxins, pesticides, adulterants, and allergies), and physical contaminants (e.g., metals, glass, plastic, human hairs, and rocks). To ensure that food is safe, it is essential to find pollutants early in the food supply chain. One of the few methods is Raman spectroscopy, which is quick, sensitive, non-destructive, and reasonably priced at detecting contaminants from all three classifications. The spectroscopic techniques used for food analysis include UV-Vis spectroscopy, fluorescence spectroscopy, Raman spectroscopy, infrared spectroscopy (IR), circular dichroism (CD), X-ray spectroscopy, nuclear magnetic resonance, electron spin resonance, dielectric spectroscopy, and photoacoustic spectroscopy. Raman spectroscopy uses bonding to

identify the many kinds of chemical and organic molecules as well as their physical structures. In order to evaluate food quality systems while they are being handled, processed, and stored, Raman spectroscopy is a viable technique. Raman spectroscopy has many benefits that make it a great tool for drug analysis, including ease of use, little sample handling, and considerable changes in scattering strengths between packaging materials, tablet excipients, and active medication ingredients.

Principles of Raman spectroscopy-

An inelastic scattered light phenomenon called the Raman influence was initially described by Sir C.V. Raman. A tiny number of photons with known polarisation and frequency are scattered from the sample when it is subjected to a laser beam. Elastic and inelastic scattering both occur in the scattered radiation. The majority of the scattering is known as Rayleigh scattering, which has the same frequency as the incident light, and it occurs when light is elastically scattered. In contrast, Raman scattering occurs when light is inelastically scattered, which makes up a much smaller portion of the total scattered light. The vibrational energy, which is lowered or increased as a result of the interaction between photons and molecules, is used in this process to change the energy or frequency of the Raman scattering from that of the incident light. The Raman shift is the phrase used to describe frequency differences between incoming and dispersed radiation. Anti-Stokes scattering is the term used to describe the increase in photon energy from incoming light. The opposite is known as Stokes scattering, which is the loss of photon energy from incident light. It's unusual for there to be scattering. Raman spectroscopy offers a number of benefits, including lower costs, non-destructiveness, immune to water interference, and the requirement of only a small sample for analysis. Raman spectroscopy has been used in food analysis primarily for two purposes: determining the composition of food and determining any conformational or structural changes that take place as a result of food processing.

Raman Spectroscopy Techniques-

Raman spectroscopy uses an inelastic scattered light phenomenon to detect analytes by measuring the vibrations of molecular bonds. In this case, incident light photon energy is either obtained by converting molecules' energy into photons (anti-Stokes Raman scattering) or lost by converting photons' energy into molecules (Stokes Raman scattering). An analyte can be identified molecularly by means of a unique vibrational fingerprint produced by each Raman peak in the spectrum, which is distinctive for a particular molecular bond. Raman imaging, surface-enhanced Raman spectroscopy, and micro-Raman spectroscopy are the Raman spectroscopic methods most frequently employed for determining food safety (SERS).

I. Raman Microscopy and Micro-Raman Spectroscopy-

Raman microscopy is the name for the combination of Raman spectroscopy and an optical microscope. Raman scattering is accomplished in a Raman microscopy setup by aiming the laser light via an objective lens towards the material. In contrast, confocal Raman microscope setup is more difficult and requires the use of pinhole apertures. In confocal micro-Raman spectroscopy, a point light source is focussed into a diffraction-limited area of the material by an objective lens after being directed by a source pinhole and splitter beam. The same objective lens collects and collimates the light that is scattered or emitted from this area before it is directed through a tiny detector pinhole and onto a spectrometer. Since the detector pinhole only gathers the signal from the focal point and rejects light from out-of-focus regions, it serves as a depth selector. Due to their improved depth resolution and increased picture contrast from the suppression of stray light, confocal microscopes have grown in value as analytical tools over the past few years.

II. Raman Imaging-

Raman imaging is used to show how components are distributed in a sample according to their chemical properties. Spatial data and Raman spectroscopy are combined in Raman imaging. As a result, each pixel in the image correlates to a Raman spectrum, which is compared to a well-established Raman database to identify a particular analyte or spectral background observations in this area. Typically, no sample preparation is necessary for Raman imaging.

Raman imaging can be carried out using either scanning or wide-field techniques. There are two ways to accomplish scanning imaging, both of which include a confocal microscope. (1) Point scanning sequentially gathers a Raman spectra at each spatial position. The lateral and axial coordinates of the sample are controlled

by a high-precision stage as it is moved from one specified spot to the next. High spectral resolution and complete spectral coverage are benefits of this approach. It takes a lot of time and damages samples because of the laser. (2) In contrast to point scanning, line scanning increases the spatial range of each scan by applying a laser line, acquiring a line of spatial and spectral data for each measurement. On a robotic stage, the sample is moved perpendicular to the laser line that is being incident. Line scanning provides a higher spectrum resolution and is quicker than point scanning, but producing a weaker Raman scattering signal as compared to point scanning due to a lower laser power per region. In wide-field Raman imaging, the complete sample region is illuminated by laser light, and its spatial information is collected in a single scan without any relative movement of the laser and the sample.

III. SERS-

The extremely weak Raman scattering signal is the main drawback of traditional Raman spectroscopy. Raman spectroscopy may be unable to detect molecules at low concentrations because only a very small portion of incident photons are scattered inelastically, which restricts its use in the food sector. A Raman spectroscopy approach known as SERS uses SERS substrates to overcome the intrinsically weak Raman scattering signal. The low-concentration single-molecule Raman signal can be amplified by many orders of magnitude, typically between 10^7 and 10^{14} , by these metallic nanostructures.

Label-free (direct) and label-based (indirect) SERS are the two concepts on which current SERS methodologies are built. The label-free SERS approach immediately recognises an analyte's intrinsic fingerprint by relying on their mutual interaction with the SERS substrate. This technology has various benefits over label-based SERS technologies, including simplicity, high speed, cheaper cost, and no interaction with other components because no SERS tags are needed. Label-based SERS techniques employ SERS tags, which include target recognition components for capturing analytes and particular Raman reporting molecules for attaching to the SERS substrate. Multiplex detection, greater sensitivity, and repeatability are benefits of label-based vs label-free SERS systems.

APPLICATION OF RAMAN SPECTROSCOPY IN FOOD ANALYSIS:

1. Raman spectroscopy for the detection of viruses and microorganisms-

The detection of bacteria and viruses can be done using a variety of analytical techniques. Although standard microbiological plate count techniques like PCR, immunological, and serological techniques have been widely employed for this purpose, Raman spectroscopy is gaining popularity because of the above-mentioned benefits, including high sensitivity, dependability, and non-destructiveness. Hepatitis A, Norwalk, Polio, Astro, Enteric adeno, parvo, and rotaviruses have also been identified in foods as contaminants, in addition to microbes that naturally occur in food.

Viruses-

The use of Raman spectroscopy for structural characterisation of viruses has been reported in numerous publications. For instance, numerous research has been carried out using structural data from Raman spectroscopy to create antiviral medications. The analysis of foodborne viruses using Raman spectroscopy has only been studied in a very small number of cases. Hepatitis A, the most common foodborne virus, has only been the subject of one study that used Raman spectroscopy. A cysteine protease known as hepatitis A 3C proteinase is recognised to be essential to the life cycle of this virus and in charge of converting the polyprotein precursor into mature viral proteins. In the aforementioned study, acyl groups in the enzyme's active region were examined using Raman spectra. A number of studies have used Raman spectroscopy to investigate Hepatitis viruses, but none of them have been identified as foodborne viruses.

Microorganisms-

It is possible to detect the presence of microbes using Raman and its derivatives, including UV-RR, FT-Raman, microRaman, and confocal Raman. It is feasible to recognise and distinguish between various microorganisms by carefully choosing which Raman approach to use, leveraging neural networks, and using chemometric techniques to make qualitative distinctions between spectra.

The bacterial species *Bacillus* and *Brevibacillus* produce spores. Particularly virulent *Bacillus* species can also be employed as a biological weapon and result in severe cases of food poisoning.

Micro-Raman spectroscopy was used to track the transition of these bacteria from the spore to the vegetative form and to examine the impact of calcium and manganese dipicolinate on spore formation.

A class of harmful bacteria (Enterococci and Staphylococci) was identified in a different investigation using confocal Raman microscopy. Raman measurements were collected from various areas of a microcolony of each culture and analysed using chemometric techniques. Additionally, the identification of *Legionella*, *Klebsiella*, *Micrococcus*, *Bacillus*, *E. coli*, *Pseudomonas*, *Staphylococcus*, *Listeria*, *Yersinia*, and *Salmonella* species was carried out using Raman spectroscopy in conjunction with various chemometric techniques.

Chemometric techniques were also used to differentiate between various varieties of *Lactarius* mould using micro-Raman spectroscopy. Since lipid and amylopectin are typical substances for amyloid reactions of *Lactarius* spores, they were observed using Raman spectroscopy. Due to its edible nature, *lactarius* mould is valued highly both ecologically and economically and is well-known throughout much of the world.

Raman spectroscopy for toxin and chemical detection-

Unintentionally introduced ingredients to food are referred to as contaminants. These compounds may be found in food as a result of contamination during any phase of manufacture, packaging, transit, or storage. They may also be the result of environmental pollution. Numerous analytical techniques have been developed for the identification and quantification of these substances since contaminants in general have a detrimental impact on food quality and pose a concern to human health.

Toxins-

In order to find small levels of surface-remaining pesticides, different fruits and vegetables were analysed using micro-Raman and near-infrared FT-Raman spectrometry. Atrazine, prometryn, and simetryn herbicides were studied using their solid forms in both polar and apolar solvents to determine their Raman spectra by Bonora et al. The experimental and theoretical spectra acquired from Raman and surface-enhanced Raman spectroscopy (SERS) experiments were compared. Aflatoxin generated by *Aspergillus* in maize was analysed qualitatively and quantitatively using Raman spectroscopy in conjunction with LDA. Depending on the amount of aflatoxin in the samples, different Raman bands were seen.

Chemicals-

Most plants, such as grass, tonka beans, sweet clover, and woodruff, contain coumarin, a naturally occurring benzopyrone.

Prior to its direct use being outlawed over worries that it might have liver-damaging effects in animal tests, it was employed as a flavouring food ingredient. Polycarbonate plastics, food cans, and food storage containers all contain the estrogenic substance bisphenol A (BPA). Because of the usage of fertilisers and manufacturing processes, ground waters may get contaminated with perchlorate ions.

A useful real-time detection technique for perchlorate was discovered to be Raman spectroscopy. Due to their capacity to promote carcinogenesis, polycyclic aromatic hydrocarbons (PAH) pose a potential risk to human health.

Using UV-RR spectrometry, it was possible to identify PAH (such as naphthalene, anthracene, phenanthrene, and pyrene) in tiny amounts. Alajtal, Edwards, and Scowen employed FT-Raman spectroscopy to examine how spectral resolution affected the Raman spectra of several polyaromatic hydrocarbons.

Different spectrum resolutions of Raman measurements were performed on the molecules of naphthalene, anthracene, and pyrene as well as beta-carotene naphthalene, anthracene, and pyrene. In this study, the impact of spectral resolution on the resulting Raman spectra was assessed.

IV. Raman spectroscopy to detect food adulteration-

Raman spectroscopy, a vibrational technique, is one of the analytical instruments and is gaining popularity since it can quickly, non-destructively, and inexpensively analyse food goods while also providing fingerprint features. Furthermore, by combining Raman spectroscopy with multivariate data analytics, it is possible to collect both quantitative and qualitative data. To identify between authentic olive oil and oil tainted with inferior oils, Zou et al. used a portable Raman spectroscopy. The relevant investigation has successfully identified adulterated olive oil that contains as much as 5% (v/v) or less of other edible oils. Zhang et al. examined extra virgin olive oils contaminated with soy, corn, or sunflower seed oil by describing their Raman spectra in the 1000–1800 cm^{-1} region. The oil samples' CH₂ band served as the standard for normalising the Raman spectra. In a separate investigation by Zhang et al., the degree of adulteration in a collection of olive oil samples that contained 5% or more of various oils, including soybean, rapeseed, sunflower, and corn oil, was successfully identified. The authenticity of different extra virgin olive oils and their adulteration with hazelnut oil were also tested by Lopez-Diez et al. using Raman spectroscopy. The resulting Raman spectra were normalised using the band's frequency, which corresponds to the scissoring-bending mode of -CH₂ groups.

Through the use of NIR, FT-IR, and FT-Raman spectroscopy, it was possible to evaluate whether extra virgin olive oil was adulterated with olive pomace oil. Utilizing portable Raman spectroscopy in conjunction with PLS regression, the quantitative adulteration of milk powder containing melamine was identified. Using the distinctive bands of melamine, which are found at one strong band at 673 cm^{-1} and a weak band at 982 cm^{-1} , melamine adulteration was observed. The melamine band, which is found at 676 cm^{-1} , was used in a similar investigation to test the adulteration of milk powder with melamine. The milk powder samples that had calcium carbonate injected into them were successfully used to identify adulteration. Raman chemical imaging combined with mixture analysis methods allowed the simultaneous detection of multiple adulterants, including ammonium sulphate, dicyandiamide, melamine, and urea, which were present in the milk powder samples. Researchers were able to identify and separate these four chemical adulterants by comparing the Raman spectra of these samples.

Raman spectroscopy in food additive analysis-

The detection of food additives has also been done using Raman spectroscopy, and many methods have been utilised for this. Raman spectroscopy was used to examine the two main carotenoids, astaxanthin and canthaxanthin, which are responsible for salmon's red-orange colour. Raman spectroscopy was used to examine carbon black, another colourant created by burning hydrocarbons. Amaranth is a frequently used food industry colouring pigment that had its molecular structure studied by Snehalatha et al. Tartrazine is a synthetic dye that has the potential to trigger allergic reactions. Peica et al. looked at the molecular structure of this substance.

Although curcumin is a substantial contributor to human health and is used as a natural colouring and stabilising factor in food, its low solubility and stability limit its potential uses. Cyclodextrin encapsulation was used to increase the solubility and stability of curcumin, and Raman spectroscopy was used to characterise this complex.

Aspartame as an artificial sweetener was examined using Raman spectroscopy in a different investigation by Peica et al. The ability to identify the excess azodicarbonamide additive in our samples using IR, Raman, and SERS was assessed. The degree of chitosan's deacetylation determines its chemical and physical characteristics, including solubility, biodegradability, and biocompatibility. In this regard, Zajac et al. showed that it was possible to determine the degree of deacetylation of chitosan by calculating a number of bands from the spectra of relevant Raman and IR wavelengths. Mannitol is a different food additive that is employed in the creation of low-calorie foods as well as in the pharmaceutical sector and other lyophilized goods. A study looked at the changes that occur in the ice, water, and mannitol bands as mannitol is lyophilized. At the spectral ranges 150–250 cm^{-1} and 1000–1170 cm^{-1} , respectively, ice and mannitol's Raman bands were seen. During the lyophilization process, mannitol showed a variety of polymorphic forms.

Raman spectroscopy in raw material analysis-

One of the most significant applications for quality control in the food sector is the quick and on-the-spot analysis of raw materials. Before beginning the food production process, businesses can save time and money by evaluating the quality of the raw ingredients. The identification of raw materials is particularly crucial because it has a significant impact on the end product's quality. Raman spectroscopy has been extensively employed in the examination of raw materials, particularly in the differentiation of food samples, the observation of chemical and biological processes, the compositional characterization of food samples, and the authenticity of meals. Principal component analysis (PCA) is an example of an unsupervised chemometric method. Supervised chemometric methods include partial least square (PLS), partial least square discriminant analysis (PLS-DA), principal component regression (PCR), and artificial neural networks (ANN).

Honey-

Raman spectroscopy was combined with PCA and ANN in a study by Goodacre, Radovic, and Anklam to distinguish distinct honey samples from different European nations with diverse oral and geographical origins. The results show that 13 out of 14 honey samples were correctly categorised, but the nation of origin could not be precisely predicted because there were not enough honey samples. Ozbaldi et al. have recently conducted additional research on honey. By using chemometric techniques on the Raman spectra of honey samples, the sugar contents of the honey samples were measured in this study. Carvucci et al. used Raman spectroscopy to separate the honey samples obtained from various places, much like the first study discussed in this review. By applying PCA to the Raman spectra they had collected, scientists were able to identify the plant and geographic origins of the honey and match them to the pollen composition of real honey.

Coffee-

There are three studies that have been done that use Raman spectroscopy to distinguish between Arabica and Robusta green coffee. FT-Raman spectroscopy was employed to conduct the initial Raman investigation on this important subject. The lipid samples taken from the coffee samples were analysed using Raman spectra. In the Arabica coffee extract, which is unique to this variety of coffee, there were two peaks of kahweol (1567 vs. 1478 cm^{-1}) that are typical of it. Additionally, using the chemometric technique PCA, they successfully distinguished between these two varieties of coffee with a success rate of 93%. However, it is different from the earlier study in that the samples' Raman spectra were obtained without the use of any chemical or physical processes on the coffee beans. The "spectral kahweol index," which was calculated using the spectra of samples with various geographic origins, was used to distinguish between the different coffees. The samples' Raman spectra were acquired using visible micro-Raman spectroscopy, and the discriminating of the coffees was successful 93% of the time using two separate PCA models.

Lipid-

Yang et al. conducted a study on the differentiation between various edible oils and fats. Spectra from FT-Raman spectroscopy were compressed in this work using PLS and PCA, and the processed data were then used for linear discriminant analysis (LDA) and canonical variate analysis (CVA). One of the most crucial quality characteristics in the production of olive oil is the quality of the olive fruit, which has also been assessed using Raman spectroscopy. It was suggested in a different study on olive oil that oxidation states of oils may be determined using a low-resolution portable Raman equipment. In this work, it was shown that field-based, portable Raman spectroscopy can be used to assess the quality of olives used to make olive oil. Gouvinhas et al. generated extra virgin olive oil, in contrast to prior studies on olive oil, by extracting samples from three different types of olives at various stages of ripening. Through the use of qualitative Raman spectral analysis techniques, they were categorised according to their types and ripening times. Different forms of edible fats were subjected to the same analysis. By using PCA, it was possible to clearly identify between fats from fish, poultry, pigs, and cows. According to how many cycles of freezing and thawing the fish samples had experienced, Velio glu et al. utilised Raman spectroscopy to determine how fresh they were.

Fermentation products-

Raman spectroscopy can also be used to identify substances produced by procedures like fermentation, including ethanol, lactic acid, and acetic acid. Using Fourier transform-middle infrared (FT-MIR) and Fourier transform-Raman spectroscopy, Sivakesava et al. monitored the ethanol fermentation of *Saccharomyces cerevisiae* (*S. cerevisiae*). Chemometric techniques were used in this investigation to examine the amounts of glucose, ethanol, and optical cell density produced by *S. cerevisiae* throughout fermentation. Fourier transform-near infrared (FT-NIR), Fourier transform-Raman (FT-Raman), and Fourier transform-mIR (FT-MIR) spectroscopy were used in a different study to measure the same parameters as those used in the *Lactobacillus casei* study previously (*L. casei*). The consumption and production of glucose, glycerol, and ethanol during wine fermentation were observed by Wang et al. using Raman spectroscopy. The validation analysis was conducted using HPLC. During the making of yoghurt, the fermentation process was monitored using micro-Raman spectroscopy. Based on the gathered Raman spectra as a function of the incubation time, the chemical conversion of lactose and inorganic phosphorus into lactic acid and organic phosphorus as well as the synthesis of exopolysaccharides were observed.

Other food-

To assess the protein and oil levels of soybeans, a dispersive Raman spectroscopy technique was created. Another subject investigated using Raman spectroscopy is food characterization. The characterisation of Marama beans from Southern Africa was done for this reason using FT-Raman spectroscopy. Data on the carbohydrate, protein, amino acid, and aromatic component composition of marama bean oil, both quantitative and qualitative, were gathered. To gather spectral information on the primary organic components of ripe and unripe tomato fruit samples, a portable and confocal Raman microscope was used for the analysis. To maximise the amount of spectral data obtained, two separate laser excitation wavelengths were applied during confocal microscopy measurements. Additionally, ethanol, lactic acid, and acetic acid produced during food fermentation and/or spoiling as well as chemical and metabolic alterations can be observed using Raman spectroscopy.

Raman spectroscopy for the detection of food components-

Because it has a significant impact on quality, nutritional value, and economic worth, as well as contributing to the characteristics of the finished product, the composition of food samples is very important. Both favourable and unfavourable effects on food components are possible as a result of environmental and processing-related factors. Therefore, it is crucial to closely monitor these changes at each stage of the food manufacturing process. There are numerous methods for identifying these changes in food ingredients, and during the past few decades, Raman spectroscopy has gained popularity for this purpose. This section provides an overview of the quantitative and qualitative study of food components using Raman spectroscopy.

Proteins-

Researchers are able to gather comprehensive data on the structural characteristics of proteins using Raman spectroscopy. Since proteins are one of the key food ingredients and have a significant impact on the qualities of food, studies on food proteins have been undertaken for a number of decades and continue to be essential. Since proteins are huge polypeptides with hundreds of amino acids each, their Raman spectra are made up of a complicated arrangement of overlapping bands. Strong Raman scattering from polypeptide chains and aromatic amino acids also plays a role in the development of the distinctive bands seen in the Raman spectra. Chi et al. made use of UV-resonance Raman spectroscopy (UV-RR spectroscopy), which has the benefit of allowing for the selective analysis of the secondary structures of diluted protein and peptide solutions. Raman spectroscopy makes it simple to monitor the deamidation of proteins, which is another process. Wong et al. examined the degree of deamidation in dietary proteins using soy and whey protein isolates as well as spray-dried egg white powder. Raman spectroscopy was used to look at the protein structures' conformational changes. In the study by Ferrer et al., the gluten protein was chemically altered using the emulsifier sodium stearoyl lactylate (SSL), and the impact of the alteration on the secondary and tertiary structure of this protein was examined using FT-Raman spectroscopy. Perisic et al. investigated the impact of various salts on the hydration characteristics of structural proteins by combining vibrational spectroscopic methods, such as NIR spectroscopy and FT-IR, NIR, and Raman microspectroscopy. Investigations on interactions between salt cations and aromatic amino acid residues and their significance to the final protein structure were made.

Carbohydrates-

Since they make up the largest class of organic substances, carbohydrates require careful structural characterisation. In the structural investigation of carbohydrates employing Raman spectroscopy, the vast number of atoms in the repeat unit and the lack of a clearly defined entity raised the significance of correct vibrational mode assignment. The in-depth analysis of the structural elements of the food samples also made use of Raman spectroscopy. The Roman et al. study assessed the components of wild carrot root in situ, without sample preparation, including starch, pectin, cellulose, lignin, and even bioactive polyacetylenes. Using a Raman mapping technique, they also demonstrated component accumulations that were particular to certain tissues. The distribution of these elements in the structure of the wheat grain was also examined using Raman imaging. Raman spectroscopy can be used to distinguish minute changes between extremely similar structural types and identify the sources of carbohydrates. Raman spectroscopy was utilised by Scudiero and Morris to distinguish between soft and hard wheat flour samples. Using a Raman imaging approach, Wellner et al. investigated the content and physical structure of starch granules discovered in wild-type and mutant maize kernels. Raman spectroscopy has also been used to examine the impact of food processing on carbohydrates, including the alteration of starch. The gathered Raman spectra were used to track how high temperatures affected the structural makeup of polysaccharide molecules. In contrast to the previous study, Raman spectroscopy was used in this one to determine how freezing treatment alters the structure of wheat bread dough.

Lipids-

The most complicated molecular structures to be analysed are lipids, one of the three main dietary components. The various characteristics of lipids have been extensively studied using Raman spectroscopy. For instance, Sade-ghijorabchi et al. proposed a method that use FT-Raman spectroscopy to assess the overall degree of unsaturation in oils and fats. This non-destructive measurement technique was used to get the Raman spectra of culinary oils, margarine, mayonnaise, hydrogenated fat, and butter using a near-infrared Raman spectrometer. In order to measure the components of milk powder, specifically the protein and fat in samples of skim and whole milk, McGoverin et al. used Raman spectroscopy. Lactose, milk proteins, and milk lipids are thought to be the culprits for the overlapping bands found in Raman spectra. Raman spectroscopy has been used to study lipid oxidation, one of the most significant food quality indicators. Muik et al. investigated the chemical alterations that took place during lipid oxidation in edible oils. Using Raman spectroscopy, Kathirvel et al. tracked the development of lipid oxidation in mechanically separated turkey by observing the oxidative bleaching of β -carotene. FT-Raman spectroscopy was utilised by Sanchez-Alonso et al. to track the oxidation of hake fillets' lipids during frozen storage. A study was conducted utilising linoleic acid, a crucial fatty acid in the diet of humans. This study used high pressure to treat linoleic acid. Using Raman spectroscopy, the phase transition and structural changes of linoleic acid under high pressure may be seen in real-time. Another essential oil made from Lamiaceae plants exhibits different chemical profiles depending on their genomic properties and has a significant biological activity. Using dispersive Raman spectroscopy and FT-IR, the chemical structures of the essential oils were established in light of the aforementioned information.

Vitamins-

To analyse vitamins in food samples, a range of analytical techniques have been employed. These approaches were said to have two key drawbacks: lack of specificity and matrix effect. Raman spectroscopy has grown in significance since it is highly accurate and has a strong signal-to-noise ratio for analysing vitamins. In the 1970s, Raman spectroscopy was used to study vitamins. Characterizing the isomeric forms and obtaining the distinctive Raman spectra of vitamins were the main objectives of these early studies. According to Rimai et al., who measured the retinal, retinol, and transretinoic acid Raman spectra in octanol solution, it may be possible to identify the terminal group on vitamin A type molecules and isomers by their distinctive bands at roughly 1580–1590 cm^{-1} and 1100–1400 cm^{-1} . Additionally, the determination and localization of vitamins in biological samples were made possible by the use of Raman microscopy. When vitamins were present at micro molar amounts, Kim and Carey employed riboflavin to distinguish between free vitamins and vitamins linked to protein-vitamin complexes. In a different investigation, Beattie et al. employed Raman spectroscopy to pinpoint α -tocopherol, which is known to be the main form of vitamin E in biological materials. Vitamin concentrations in powdered mixes and solutions were also measured using chemometric methods.

REFERENCE-

1. Fung, F.; Wang, H.-S.; Menon, S. Food Safety in the 21st Century. *Biomed. J.* 2018, 41, 88–95. [CrossRef]
2. World Health Organization. Food Safety. Available online: <https://www.who.int/westernpacific/health-topics/food-safety> (accessed on 5 May 2021).
3. Yang, T.; Wang, P.; Guo, H.; He, L. Surface-Enhanced Raman Spectroscopy: A Tool for All Classes of Food Contaminants. In *Reference Module in Food Science*; Elsevier: Amsterdam, The Netherlands, 2017; ISBN 978-0-08-100596-5.
4. A. M. Herrero, *Food Chem.*, 2008, 107, 1642–1651.
5. Ellis G, Hendra PJ, Hodges CM, et al. Routine analytical Fourier-transform Raman spectroscopy. *Analyst* 1989;114:1061-6.
6. Raman, C., & Krishnan, K. (1929). The production of new radiations by light scattering. Part I. *Proceedings of the Royal Society of London. Series A Containing Papers of a Mathematical and Physical Character*, 122(789), 23-35.
7. Qin, J., Chao, K., & Kim, M. (2010). Raman chemical imaging system for food safety and quality inspection. *Transactions of the ASABE*, 53(6), 1873-1882.
8. Herrero, A. M. (2008). Raman spectroscopy a promising technique for quality assessment of meat and fish: A review. *Food Chemistry*, 107(4), 1642-1651.
9. Li, Y.-S., & Church, J. S. (2014). Raman spectroscopy in the analysis of food and pharmaceutical nanomaterials. *Journal of Food and Drug Analysis*, 22(1), 29-48.
10. Yaseen, T.; Sun, D.-W.; Cheng, J.-H. Raman Imaging for Food Quality and Safety Evaluation: Fundamentals and Applications. *Trends Food Sci. Technol.* 2017, 62, 177–189. [CrossRef]
11. Lorenz, B.; Wichmann, C.; Stöckel, S.; Rösch, P.; Popp, J. Cultivation-Free Raman Spectroscopic Investigations of Bacteria. *Trends Microbiol.* 2017, 25, 413–424. [CrossRef] [PubMed]
12. Jones, R.R.; Hooper, D.C.; Zhang, L.; Wolverson, D.; Valev, V.K. Raman Techniques: Fundamentals and Frontiers. *Nanoscale Res. Lett.* 2019, 14. [CrossRef] [PubMed]
13. Bodelón, G.; Montes-García, V.; Pérez-Juste, J.; Pastoriza-Santos, I. Surface-Enhanced Raman Scattering Spectroscopy for Label-Free Analysis of *P. Aeruginosa* Quorum Sensing. *Front. Cell. Infect. Microbiol.* 2018, 8. [CrossRef] [PubMed]
14. Yaseen, T.; Sun, D.-W.; Cheng, J.-H. Raman Imaging for Food Quality and Safety Evaluation: Fundamentals and Applications. *Trends Food Sci. Technol.* 2017, 62, 177–189. [CrossRef]
15. Giridhar, G.; Manepalli, R.R.K.N.; Apparao, G. Chapter 7—Confocal Raman Spectroscopy. In *Spectroscopic Methods for Nanomaterials Characterization; Micro and Nano Technologies*; Thomas, S., Thomas, R., Zachariah, A.K., Mishra, R.K., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 141–161. ISBN 978-0-323-46140-5.
16. Wang, J.; Yang, M.; Yang, L.; Zhang, Y.; Yuan, J.; Liu, Q.; Hou, X.; Fu, L. A Confocal Endoscope for Cellular Imaging. *Engineering* 2015, 1, 351–360. [CrossRef]
17. Stewart, S.; Priore, R.J.; Nelson, M.P.; Treado, P.J. Raman Imaging. *Annu. Rev. Anal. Chem.* 2012, 5, 337–360. [CrossRef]
18. Bodelón, G.; Montes-García, V.; Pérez-Juste, J.; Pastoriza-Santos, I. Surface-Enhanced Raman Scattering Spectroscopy for Label-Free Analysis of *P. Aeruginosa* Quorum Sensing. *Front. Cell. Infect. Microbiol.* 2018, 8. [CrossRef] [PubMed]
19. Lorenz, B.; Wichmann, C.; Stöckel, S.; Rösch, P.; Popp, J. Cultivation-Free Raman Spectroscopic Investigations of Bacteria. *Trends Microbiol.* 2017, 25, 413–424. [CrossRef] [PubMed]
20. Zhao, X.; Li, M.; Xu, Z. Detection of Foodborne Pathogens by Surface Enhanced Raman Spectroscopy. *Front. Microbiol.* 2018, 9. [CrossRef] [PubMed]
21. Liu, Y.; Zhou, H.; Hu, Z.; Yu, G.; Yang, D.; Zhao, J. Label and Label-Free Based Surface-Enhanced Raman Scattering for Pathogen Bacteria Detection: A Review. *Biosens. Bioelectron.* 2017, 94, 131–140. [CrossRef]
22. V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa and C. Adley, *Biotechnol. Adv.*, 2010, 28, 232–254.
23. M. Koopmans, C. H. von Bonsdorff, J. Vinje, D. de Medici and S. Monroe, *FEMS Microbiol. Rev.*, 2002, 26, 187–205.
24. P. Rösch, M. Schmitt, W. Kiefer and J. Popp, *J. Mol. Struct.*, 2003, 661, 363–369.
25. H. Yang and J. Irudayaraj, *J. Mol. Struct.*, 2003, 646, 35–43.

26. S. Stockel, S. Meisel, R. Bohme, M. Elschner, P. Rosch and J. Popp, *J. Raman Spectrosc.*, 2009, 40, 1469–1477.
27. K. Maquelin, L. P. Choo-Smith, T. van Vreeswijk, H. P. Endtz, B. Smith, R. Bennett, H. A. Bruining and G. J. Puppels, *Anal. Chem.*, 2000, 72, 12–19.
28. D. Kusic, B. Kampe, P. Rosch and J. Popp, *Water Res.*, 2014, 48, 179–189.
29. S. Meisel, S. Stockel, P. Rosch and J. Popp, *Food Microbiol.*, 2014, 38, 36–43.
30. U. Munchberg, P. Rosch, M. Bauer and J. Popp, *Anal. Bioanal. Chem.*, 2014, 406, 3041–3050.
31. A. Silge, W. Schumacher, P. Rosch, P. A. Da Costa, C. Gerard and J. Popp, *Syst. Appl. Microbiol.*, 2014, 37, 360–367.
32. H. H. Wang, S. J. Ding, G. Y. Wang, X. L. Xu and G. H. Zhou, *Int. J. Food Microbiol.*, 2013, 167, 293–302.
33. K. De Gussem, P. Vandenabeele, A. Verbeken and L. Moens, *Spectrochim. Acta, Part A*, 2005, 61, 2896–2908.
34. E. W. Blanch, L. Hecht and L. D. Barron, *Methods*, 2003, 29, 196–209.
35. E. W. Blanch, I. H. McColl, L. Hecht, K. Nielsen and L. D. Barron, *Vib. Spectrosc.*, 2004, 35, 87–92.
36. R. Tuma and G. J. Thomas, *Biophys. Chem.*, 1997, 68, 17–31.
37. Z. Q. Wen, S. A. Overman and G. J. Thomas, *Biochemistry*, 1997, 36, 7810–7820.
38. D. Dinakarpandian, B. Shenoy, M. PusztaiCarey, B. A. Malcolm and P. R. Carey, *Biochemistry*, 1997, 36, 4943–4948.
39. H. Peters, Y. Y. Kusov, S. Meyer, A. J. Benie, E. Bauml, M. Wolff, C. Rademacher, T. Peters and V. Gauss-Muller, *Biochem. J.*, 2005, 385, 363–370.
40. P. Carmona, M. Molina and A. Rodriguez-Casado, *J. Raman Spectrosc.*, 2009, 40, 893–897.
41. A. Rodriguez-Casado, M. Molina and P. Carmona, *Appl. Spectrosc.*, 2007, 61, 1219–1224.
42. A. Rodriguez-Casado, M. Molina and P. Carmona, *Proteins*, 2007, 66, 110–117.
43. G. Font, M. J. Ruiz, M. Fern´andez and Y. Pic´o, *Electrophoresis*, 2008, 29, 2059–2078.
44. N. N. Brandt, A. Y. Chikishev, A. I. Sotnikov, Y. A. Savochkina, I. I. Agapov and A. G. Tonevitsky, *J. Mol. Struct.*, 2005, 735–736, 293–298.
45. P. X. Zhang, Z. Xiaofang, Y. S. C. Andrew and F. Yan, *J. Phys.: Conf. Ser.*, 2006, 28, 7–11.
46. S. Bonora, E. Benassi, A. Maris, V. Tugnoli, S. Ottani and M. Di Foggia, *J. Mol. Struct.*, 2013, 1040, 139–148.
47. K. M. Lee, T. J. Herrman and U. Yun, *J. Cereal Sci.*, 2014, 59, 70–78.
48. C. Sproll, W. Ruge, C. Andlauer, R. Godelmann and D. W. Lachenmeier, *Food Chem.*, 2008, 109, 462–469.
49. Y. Q. Huang, C. K. C. Wong, J. S. Zheng, H. Bouwman, R. Barra, B. Wahlstr´om, L. Neretin and M. H. Wong, *Environ. Int.*, 2012, 42, 91–99.
50. T. G. Levitskaia, S. I. Sinkov and S. A. Bryan, *Vib. Spectrosc.*, 2007, 44, 316–323.
51. S. A. Asher, *Anal. Chem.*, 1984, 56, 720–724.
52. A. I. Alajtal, H. G. M. Edwards and I. J. Scowen, *J. Raman Spectrosc.*, 2011, 42, 179–185.