Application of Novel Microscopic and Spectroscopic Techniques in Assessing Food Quality and Safety

Indrajith C Senevirathne¹ and Reshani N Senevirathne²*

¹Department of Geology and Physics, Lock Haven University of Pennsylvania, United States of America
²Research and Development, Godshall’s Quality Meats Inc., United States of America

*Corresponding Author: Reshani N Senevirathne, Research and Development, Godshall’s Quality Meats Inc., Telford, Pennsylvania, United States of America.

Received: November 10, 2019; Published: December 06, 2019

Abstract

Globalized, interconnected food industry requires both careful assessment and management of food quality and safety. This begins at the point of origin i.e. the farm to the point of exit i.e. at hand of the consumer. Atypical investigative techniques that are extensively used in other disciplines such as physics, chemistry, engineering and medicine can provide highly localized, detailed, diverse, and time dependent data about food products, both efficiently and effectively. Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-Ray Spectroscopy (EDS), Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI) can be used in Food Science and Engineering because both are inherently interdisciplinary in nature. These techniques would be a valuable addition to the existing set of tools and techniques available to industry practitioners and the academia alike. This is a review of the science, technology and application aspects of these techniques.

Keywords: Food Safety; Food Quality; Food Science; Food Engineering; Food Nutrition; Scanning Electron Microscopy (SEM); Transmission Electron Microscopy (TEM); Energy Dispersive X-Ray Spectroscopy (EDS); Nuclear Magnetic Resonance (NMR); Magnetic Resonance Imaging (MRI); Critical Point Drying (CPD)

Abbreviations


Introduction

We live in an age where food trade is globalized unlike in any other time in the history. Today people in northern hemisphere can consume off-season peaches and tropical mangoes while those who live in the tropics can enjoy fresh apples and grapes which come from more temperate climates. These trade partnerships span continents and different time zones. As an example of the economic impact, in 2016 the United States exported US$99 billion food and beverage products while importing US$119 billion (this does not consider re-exports of food and beverages from the US). United States is a major player in global food trade hence an indicator of the trade volume in food and beverages [1] products. These complex transactions adds layers of intricacy in building and maintaining production volumes, convoluted supply chains, changing trading routes, and most importantly, increased difficulty in assessing food quality & safety [2].

A foodborne outbreak is an ultimate nightmare for a business: E. coli outbreak in 1993 at Jack in the Box, the E. coli outbreak in 2006 at Taco Bell, and the E. coli, STEC 026, norovirus outbreaks in 2015 at Chipotle Inc. can be considered [3]. These episodes also
had a significantly detrimental effect on the sales (lost sales), customer health (lost customers, legal woes, customer anger and apathy, etc.) and trust (sometimes lasting damage to the brand and to the reputation of the company) [4]. In 2017 there was an incident of egg contamination by fipronil, an insecticide used for killing lice and ticks on farm animals. This affected consumers in 16 European countries and China (Hong Kong) and had a significant effect on the poultry industry [5,6]. Therefore, not only food quality and safety issues have immediate, significant, material effect on businesses, but also often they are long lasting and could be felt across entire sectors.

All these episodes invariably point to the urgent need of efficient and reliable, techniques to both assess the quality, consistency, and contamination episodes in food. These techniques and technologies need to be implemented along the critical points in the food product supply chain to mitigate such episodes. There are many such techniques and technologies both current and under development. Some of these technologies can be multi-platform sensing technologies which include real-time recognition of the presence of biological, chemical and physical contaminants including degradation of nutrient and deterioration of sensory aspects over time. Sensing Technologies are new, and they are evolving at a rapid clip.

Another category is the spectroscopy and microscopy techniques, which are our focus. They would help in implementing structural and material changes to mitigate such quality and safety issues, control problems originating from food source supply chains and to improve processes to manufacture quality products to gain competitive advantage.

This review focuses on recent trends of microscopic and spectroscopic techniques commonly used in other disciplines but can be utilized successfully in the food research and industry.

**Electron beam techniques and instruments**

Scanning Electron Microscope (SEM) is a microscopic technique which have been used extensively in nanotechnology, physics, chemistry material science, various domains of engineering and biology. One important aspect in SEM is that it is a surface analysis technique, meaning it will not be revealing details about what’s under the surface.

A beam of electron with low to medium energy (5-40 keV) [7] is used to image the sample specimen [8]. SEM images give very high resolution and unprecedented detail. The superior resolution is a direct consequence of using electrons which have lower (de Broglie) wavelength [9] in contrast to visible light which is used in a typical optical microscope. Using electrons however makes this a destructive and invasive technique. In addition, one must take care not to alter the sample by employing higher energy electrons which could lead to radiation induced degradation.

Electrons also require the instrument to be held under vacuum. Typical food samples contain a significant amount of water. Samples therefore must be dried/dehydrated prior to placing in the SEM. This is because removal of water under vacuum (and any other volatile organics that could get released) could contaminate the instrument, impair the image quality and reduce the lifetime of electron gun filament.

Uncontrolled dehydration tends to collapse small structures leading to loss and/or distortion of critical morphological features. Hence, care must be taken in the dehydration process. There are many techniques, to control the dehydration process and to get dry samples without altering morphology. One that is prevalently used in biology and that can be transferred readily to food samples is Critical Point Drying (CPD). In CPD liquid Carbon Dioxide (CO₂) (typically) is used at its surface tension zero point at a temperature of 31.1°C and a pressure of 1072psi [10]. There are many commercially available relatively inexpensive CPD systems used in various laboratories around the world.

Dried sample needs to be applied a conductive coating (typically a gold or platinum film about 5 - 10 nm in thickness which is sputter deposited on the dehydrated sample) because certain metals have high electron outputs under an applied electron beam.
Having a conductive surface also facilitates dissipating heat generated due to sample-electron beam interactions, thus reducing thermal degradation. Most importantly electrons, being negatively charged, would otherwise charge the sample and make imaging impossible [11].

The samples are then attached/fixed via double sided conductive tape, conductive silver or copper paint onto a small Aluminum sample holder. Typical SEM sample holder has about a 1 cm diameter. This sample holder is then loaded into the chamber housing the SEM. Once oriented under the electron beam and imaged under the electron beam. The beam is systematically swiped (process is also called rastering) on the surface left to right and top to bottom, line wise covering the entire surface (this region can be as small as a couple of hundred nanometers in length) region of the sample under investigation. In the process of electron beam interacting with the surface, a significant portion of the electrons emerge/scattered back bringing localized surface information about the sample. These electrons are collected by electron detectors within the SEM. Nearly all SEMs have a Secondary Electron (SE) Detector which give much desired highly resolved images. A few SEMs also have a Back Scattered Electron (BSE) Detector which provides marked difference in contrast between regions heavy atoms (high atomic numbers) vs. light atoms (low atomic numbers) (consider calcified-bone, mineral deposited regions vs. soft tissue regions) [8].

When imaging soft materials, such as many food products or biological specimen the energy of the electron beam is set to lower energy values (5 - 10 keV) as it preserves the sample integrity.

SEM was used in studying grapevine berry ripening and dehydration after postharvest. Tornielli has studied differences in outermost cell layers of the skin of grapevine varieties using SEM [12]. Torriani and Felis have used SEM to study cultures of single bacteria, yeast and molds with their intricate arrangements. They also have imaged *Zygosaccharomyces gambellarensis*, a novel yeast isolated from an Italian “passito” style wine and gave a detailed morphological description using SEM [13].

SEM was also used study the agglomerations of molds, yeasts, and bacteria which are critical in the first months of ripening of San Daniele ham leading to its proper drying. Samples of *Botrytis cinereal*, necrotrophic fungus was continuously tracked and taken from the surfaces of grapevine berries during process of withering. These were studied under an SEM and found to be integral to the unique sensory attributes of the resulted wine [13].

SEM systems could be fitted with Energy Dispersive X-Ray Spectroscopy (EDS/EDX) systems to give a localized elemental information. An important aspect of this is the sensitivity of elemental recognition is, that it is limited to a depth of about 20 micrometers from the surface. Sectioning a sample could make it possible to investigate internal chemical signatures. Electrons when interacting with matter, emit X-Ray radiation. In the process of interaction, they carry elemental information of the interacted matter. EDS detector identifies the corresponding energy signatures and by measuring the intensities one can assess the relative concentrations of the elements. These could well be used to investigate the presence of trace amounts of certain minerals (to establish the locality of a product) or heavy metals (toxicity and contamination).

In a study SEM EDS was used to identify struvite crystals in salmon and cream of tartar crystals in grape juice, which were identified in the form of Calcium Tartrate [14].

Another electron beam microscopy technique that is prevalent in nanotechnology, material science, chemistry, physics, and various domains of engineering, but somewhat less so in biology is the Transmission Electron Microscopy (TEM).

In TEM, the same dehydration and CPD techniques applies to the sample preparation. In applying the conductive coating generally, a carbon coating is ideal. In TEM the electron beam is relatively much more energetic (100 - 400 keV) and is forced through a thin section of the specimen sample.
First step in preparing thin sections is fixing dehydrated sample in a resin (LR White, Lowicryl, etc.). Once hardened this is sliced in multiples steps to obtain a section with a diameter around 3 mm and a thickness less than 0.1 micrometer. This is achieved by the technique of Ultramicrotomy, where an especially designed thin sectioning tool with either glass or diamond blade or both is used in sectioning process. Once the thin section is prepared it is placed on a metal mesh stained with an agent that includes an electron rich, heavy atoms (Osmium, Lead, Uranium or Gold in immuno Gold labeling [15]) to get better image contrast by scattering the electrons in the electron beam away from the imaging system [16]. The electrons passing through the thin section carry information about the sample structure/density variations. These electrons are collected by the detector at the bottom. Early TEM electron detectors were electron micrograph films but subsequently changed to Charge-Coupled Devices (CCD) which is present in digital cameras among other various imaging devices. Recent technological advances have shifted these detectors to Direct Detection Device (DDD) which have much improved signal to noise ratios [17,18]. Generally, TEM images give an order of magnitude better resolution than a typical SEM. Like the SEM, TEM is also a destructive and invasive analysis technique.

According to Sbarbati, TEM has been used in investigating the state of preservation of various types of food, while giving insights into processing induced changes and geographic dependence of food sourcing [19]. Using TEM with immuno gold-labeled cryo-sections Schmidt and Buchheimi studied casein micelles in milk. In their study they showed that kappa-casein is localized on the surface while other caseins are distributed with the micelle [20]. Mendonca, Amoroso, and Knabel showed by using TEM that gram negative food-borne pathogens: E. coli O157:H7 and S. enteritidis ATCC 13706 underwent lysis at elevated pH of 12, while L. monocytogenes which is gram positive bacteria persisting. Cell lysis occurred via the disruption of the cytoplasmic membrane as was shown in the TEM and SEM studies [21].

**Magnetic resonance techniques and instrumentation**

Magnetic Resonance Imaging (MRI) and Nuclear Magnetic Resonance (NMR) are fundamentally the same and depends on the magnetic resonance of collective nuclear spin (of constituent protons) of the sample atoms. Magnetic resonance technique as applied in MRI and NMR follow four steps:

1. Sample is placed under a strong magnetic field. Under the magnetic field the collective spin (a quantum mechanical property) of the nuclei of the constituent atoms gets aligned parallel to the applied magnetic field.

2. A radio frequency signal is applied to the sample where it disrupts the alignment of nuclear spins.

3. Once the radio frequency is removed the nuclei realign with the magnetic field by emitting electromagnetic waves (photons which are wave packets or light particles) in the radio wave frequencies. This realignment is also time dependent and therefore has relaxation time. These spin excitations are quantized and release energy via photon emission in the realignment process.

4. Frequency (which is correlated to the energy) of emitted photons (which can be shifted due to chemistry of the surrounding matter) is a marker for the types of molecules/bonds present in the sample. This frequency shift is used predominantly in NMR.

The intensity (i.e. the number of photons emitted) depends on the density and surrounding material properties. This is used principally in the MRI. In MRI, the system is attuned to protons (in Hydrogen) which make the water molecules. Since MRI is primarily focused on living biological specimen and water is abundant in such specimen. Photons released in the process of realigning of protons in Hydrogen provides spatially well localized, image with clear intensity.

A typical MRI system occupy large footprint and are designed for investigating large bodies (human diagnostics) thus many applications in food science uses small scale MRI systems designed for diagnosing small animals. These are shown to reach spatial resolution limits of 100 microns [22].

---

MRI is a non-destructive and non-invasive technique, it allows dynamic studies of food products while undergoing processing, and live fruits and vegetables to be studied as they undertake physical and chemical changes [23].

MRI has been successfully used to investigate physical state of water in fruit and vegetables, dehydration processes, brining cheese, measuring fat and muscle volume in Iberian ham processing, salting fish and the thermal processing of cereals [24].

MRI was also used in geotagging/origin of fresh cherry tomatoes. In the study cherry tomatoes from PGI (Protected Geographical Indication) was identified in contrast to non-PGI, cv. Naomi, and cv. Shiren cherry tomatoes varieties [25].

Thawing process of frozen meats, vegetables and margarine was also examined via MRI, where a transient thaw area could be identified correlated to the heat transfer process [26].

Advance MRI techniques which have been developed to investigating human metabolites in qualitative and quantitative manner. These have been extended and adopted to investigate lipids, proteins, fibers and others in food products. A variant of MRI, Diffusion Tensor Imaging (DTI) was used to investigate fiber make up and organization of celery and could be extended to other fibrous vegetables such as fennel [27]. Fluid transportation in cherry tomatoes was studied dynamically under nano particle Gadolinium contrasting agent using a Dynamic Contrast Enhanced (DCE) MRI [28].

Conclusion

Multitude of novel investigative techniques prevalent on other domains such as material science, physics, chemistry, engineering and medicine can be effectively and efficiently used to investigate both quality and safety of food products. The examples of using these techniques in food science and engineering are many and diverse.

Some of these techniques could be cost prohibitive, particularly to small scale manufacturers. There are many private technology outfits that specialize in providing such advance investigative services for a fee. However best strategy for small to medium scale manufacturers under budgetary constraints would be seeking out local higher educational institutions. These include research one institutions such as state universities or other big research institutions. They could also be other four-year universities that may have specialized programs with the right kind of instruments and the people with the technological know-how. Often the faculty and the administration in any such university would be more than willing to come to a partnership. In all these instances it is important to define your goal/s and problem/s clearly. It helps in communicating your needs effectively and understanding the capabilities of your partner institutions better. Our hope in pointing these opportunities out is, manufacturer would benefit from and improve her products in the competitive local and global food industry.

Bibliography

4. Whitten S. "Here’s what it costs restaurants when a foodborne illness outbreak occurs" (2018).
5. Gallagher J. "Eggs containing fipronil found in 15 EU countries and Hong Kong" (2017).
Application of Novel Microscopic and Spectroscopic Techniques in Assessing Food Quality and Safety


Application of Novel Microscopic and Spectroscopic Techniques in Assessing Food Quality and Safety


Volume 15 Issue 1 January 2020
©All rights reserved by Indrajith C Senevirathne and Reshani N Senevirathne.