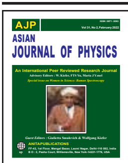

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The role of Raman spectroscopy in food and beverage analysis

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Demands on food and beverage (F&B) production and innovative food products have brought unprecedented challenges to the food and beverage industry. To address these demands, the industry has adopted increasingly scientific approaches to growing food, as well as in food processing and storage. Automation and a scientific risk-based approach to analysis called Process Analytical Technologies (PAT) are being adopted by the food industry to support an increasing number of complex foods being produced. Raman spectroscopy is a composition measurement tool that can be used in the laboratory or process environments. In this paper, we review the literature in Raman spectroscopy of food manufacturing, safety, and understanding. We also describe our feasibility studies in using of dispersive Raman spectroscopy at 1000nm to measure the quality of salmon to highlight the capabilities of Raman in solids measurements. Extension of PAT principles to the F&B industry can provide new avenues to ensure consistent product quality, support innovative manufacturing approaches, enable real-time product release, and support sustainability goals. Raman is an established PAT for laboratory and process monitoring uses within the chemical and life sciences industries and is being adopted more by the food industry. We provide a perspective on future directions with enthusiasm toward increased adoption of the PAT and Raman spectroscopy in F&B. © Anita Publications. All rights reserved.

Keywords: Raman spectroscopy, Food, Process Raman, Salmon, Protein, Fats.

1 Introduction

The changing landscape in food and beverage (F&B) production

From seed to table, the food and beverage industry affects every human. Its success is essential on a practical level because it must feed the increasing global population, and optional on an esoteric level because it also must meet the expectations of ever-changing consumer demands. Addressing these two aspects has necessitated the industry to adopt more scientific approaches to growing, processing, and distributing food. In one example, the seemingly simple process of growing a vegetable for large-scale production is the result of scientific optimization of the seed itself, soil chemistry, and growth conditions. In another example, the plastic packaging of food is specially designed to optimize air flow, protect against high moisture levels, and preserve the product's taste profile. These are just a couple of examples that underscore how the food industry has adopted scientific principles to improve the quality of its offerings.

Ongoing adoption of science-based approaches has significantly impacted the F&B industry, with intense efforts toward innovative manufacturing practices. In order to be globally competitive, food manufacturers are adopting new strategies in manufacturing that will improve efficiency, assure quality in real-time, and enable manufacture of high-value products. One strategy is adoption of "Internet of Things" and Industry 4.0 principles for improved connectivity, digitalization, and automation. This new approach encompasses automated, data-based control of the process and "smart sensors" that not only form the basis of automated feedback control of the process but also indicate when sensor recalibration or replacement is necessary.

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Another strategy to improve manufacturing is adoption of the Process Analytical Technology (PAT) and Quality by Design (QbD) framework [1,2]. While originally developed for pharmaceutical manufacturing, the principles and framework are gaining traction in food manufacturing. This framework is meant to shift manufacturing to a risk-based approach where "quality should be built into a product with a thorough understanding of the product and process by which it is developed and manufactured along with a knowledge of the risks involved in manufacturing the product and how best to mitigate those risks". Other benefits of QbD/PAT are real-time process understanding, an ability to make in-process corrections, and enabling novel processes. Despite these benefits, QbD/PAT has been slowly adopted in the F&B industry until recently. A more current interpretation of QbD/PAT in F&B is that it represents "a silent revolution in industrial quality control in food processing [3]". One industry perspective discusses important aspects of the PAT framework in F&B which focuses on technology and collaboration:

> "*Finally, the food industry needs to be made more aware of PAT as a framework for innovative process manufacturing and quality assurance. More collaboration between industry, academia, and regulators is required to unify the disperse efforts currently underway. Adoption of PAT as a strategy would bring together process engineers, food scientist, technologists, and microbiologists under one umbrella with the goal of providing the industry with a manufacturing framework for the twenty-first century. Here, we can learn from and cooperate with other industries such as the pharmaceutical and petrochemical to further develop the strategy* [4]".

Cullen, O'Donnell, and Fagan rightfully point out that risk-based analysis is already commonplace within the food industry under the context of Hazard Analysis Critical Control Point (HACCP) for food safety hazards and the use of PAT can be supported under the purview of HACCP, cGMP, and Critical Control Points (CCP's) [5,6]. A PAT approach to food analysis includes a comprehensive examination of the process variables, implementation site, type of analysis, data management, chemometric modeling, and government regulations. As an example of PAT, Johnsen *et al* provides a dairy industry perspective on the challenges and promise of adopting PAT [7].

An attractive benefit is integration of PAT into an advanced process control approach for real-time control of a particular processing step or to predict endproduct quality earlier in processing. PAT principles can support analyses in a variety of implementation sites, shown in Fig 1, ranging from off-line laboratory measurements to direct inline measurements. We see the most benefits from an inline implementation, because that allows for 24/7 monitoring without needing to collect samples. With inline PAT implementation, there are additional benefits including the ability to quickly gain product or process knowledge, implement advanced control strategies, realize real-time product release, in-process corrections, and ensure process and product quality in real time.

Other important considerations are the business value, assessing sources of risk in the instrumentation, and the type of analysis. It is important to remember that the PAT framework is not prescriptive, but rather encourages innovation through the use of risk-based manufacturing and integration of analytical technologies. There is no single technology that can suit the needs of a process in all steps and identifying the most appropriate technology (or technologies) is part of applying PAT. Laboratory-based food analysis typically focuses on biological and chemical aspects of the sample, while inline PAT encompasses physical and chemical process measurements. A non- exhaustive list of chemical analysis tools used in the food industry includes titration, extraction, ultrasound, mass spectrometry, chromatography or electrophoresis, nuclear magnetic resonance, and vibrational spectroscopy. The reader is referred to are several excellent textbooks on food analysis [8], new biosensors and nanotechnology [9], and innovative analysis approaches [10]. Specifically for inline purposes, analyses include physical process parameters and chemical process

parameters [4]. Physical parameters such as turbidity, viscosity, flow, level, color, conductivity, pressure, and temperature provide necessary information on the process operation. Color, pH, conductivity, turbidity, and viscosity provide indirect information on the sample chemistry. For more specific chemical parameters, such as the presence of organic molecules, proteins, lipids, sugars, amino acids, or diatomic species, then optical or electrochemical PAT tools can be used. Direct identification of chemical species is most often achieved inline through electrochemical probes or opticalprobes.

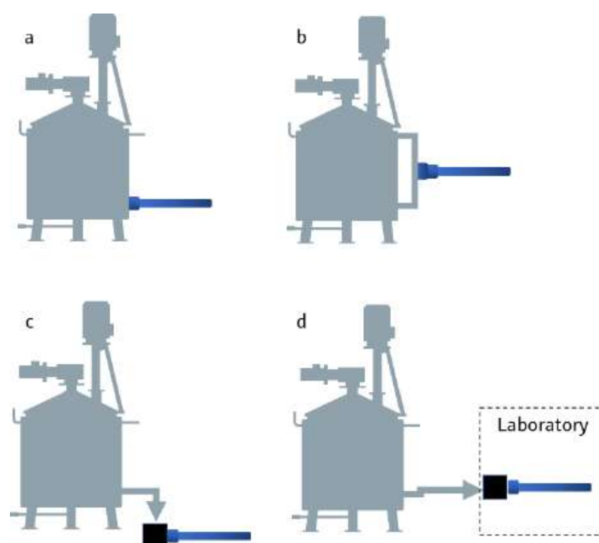

Fig 1. Process Analytical Technology (PAT) tools can be implemented in a variety of installation sites. The most robust, most resilient PAT approach looks at lab and process using fit for purpose technology. An inline installation (panel a) is where a sensor or probe is placed directly into a reactor or stream, or through a site glass, and measurements are continuously performed. At-line measurement (panel b) performs continuous measurement similar to in-line but measures the sample as it is diverted using a slip stream or diverting line. For an at-line (panel c) or off-line (panel d) implementation, a sample is taken from the reactor or stream and measured either next to the line or in a laboratory.

Within the in-line PAT “toolbox”, vibrational spectroscopy (near-infrared, infrared, and Raman) provides valuable chemical information without needing to prepare, extract, or destroy a sample. These techniques are useful for three reasons: qualitative (identification), quantification (how much), and monitoring and controlling change. For qualitative uses, molecular spectroscopy can be used to identify the chemical composition of a material and molecular structure. The identification usage of molecular spectroscopy is broadly applied to measure composition of solids, liquids, or gaseous materials for:

- Understanding multiple aspects of a biological tissue such as meat or fish.
- Understanding protein or sugar molecular structure.
- Understanding moisture content in agricultural products or processed foods.
- Identifying contaminants in a mixture.

For quantification uses, molecular spectroscopy can provide the concentration of single or multiple components or the ratio of those components. The quantification capabilities can be used to monitor change of a process, and that aspect is especially useful for knowing when to end a processing step or knowing when to add more ingredients to a long-running process. Quantification by molecular spectroscopy can be

applied to solids, liquids, and gases. Some examples of quantification or change monitoring by molecular spectroscopy include:

- Measuring feed and byproducts in fermentation or cell culture media.
- Monitoring the relative amount of starting material and end material in a chemical reaction.
- Measuring unsaturated and saturated bonds in fats.

Finally, quantification and change monitoring can lead to process control applications. Some examples of process control applications include:

- Automated timing for adding new media into a cell culture or fermentation.
- Stopping a processing step based on the amount of product formed.
- Sorting meats or fish for fresh or processed products based on their fat content.

The identification, quantification, and change monitoring and control capabilities of molecular spectroscopy make these techniques useful for many applications. The reader is referred to several textbooks and review articles on near-infrared [11-16] and infrared [17-22] spectroscopy in food applications including product quality, adulteration, and process monitoring applications. The remainder of this paper focuses on food applications of Raman spectroscopy.

History of Raman spectroscopy in food analysis

Raman spectroscopy is a molecular optical spectroscopy technique that uses visible or near-infrared light to measure composition and molecular structure. Raman spectroscopy is based on light interacting with molecular vibrations resulting in the light becoming inelastically scattered. The change in wavelength of the light that results from inelastic scattering is invisible to the eye and specialized scientific equipment is needed to measure these small changes in color. Inelastic scattering is a rare occurrence and considered a “one-in-a-million” phenomenon. The paucity of the inelastic scattering phenomenon begs the question: why is inelastic scattering even used? The answer is because the change in wavelength from inelastic scattering is very specific to the part of the molecule that scattered the light. That type of specificity is akin to a fingerprint, which is a very powerful tool for identification, quantification, and change monitoring uses. In short, a Raman spectrum provides a “molecular fingerprint” of the sample, enabling highly specific information about the chemical composition and molecular structure without sample preparation. Raman provides measurements in aqueous system because the Raman water signal is weak. The chemical specificity, minimal to no sample preparation, ability to measure in aqueous systems, and sampling flexibility of Raman spectroscopy enabled fundamental and applied studies in a variety of molecular classes. The value of the technique was quickly understood, leading to its use despite its difficulty to carry out. The 1939 book “The Raman Effect and its Chemical Applications” by James Gibben reviewed 1987 journal articles published between the report of the phenomenon in 1928 by Raman and Krishnan [23] and the book’s publication [24]. Today, Raman spectroscopy is a valuable and practical tool for chemical analysis in laboratory, field studies, and manufacturing environments.

Raman spectroscopy is growing within the food industry and there is a long history of relevant literature to support new applications and techniques. We were pleasantly surprised to realize that many of the first feasibility studies were much earlier than widely recognized! In the 1930’s to the 1970’s, there were reports of food-relevant natural molecules such as amino acids, polypeptides, lipids, carbohydrates, carotenoids, and polysaccharides. These early studies adapted techniques from the biophysics community and infrared spectroscopy to establish feasibility and make band assignments [25-30]. The early 1970’s brought about the first lasers and the intense monochromatic light source provided by a laser made Raman spectroscopy more practical for more complex molecules. In this era, there were emerging applications in biopolymers and biomacromolecules in the 1970’s. Three papers by Koenig [31], Rippon *et al* [32] and Freeman [33] in the

early 1970's reviewed the Raman literature for molecular structure of functional groups such as alkene bonds, organosulfur groups, aromatic groups, acyclic groups. The papers also reviewed the current understanding in peptides, polymers, pigments, carbohydrates, and nucleic acids. Two series of papers that were started in the mid-1970's provided in depth examination of a topic. At Case Western Reserve University, the Koenig and Blackwell groups published a series of papers on infrared and Raman spectroscopy of carbohydrates [34-39]. A series of papers on "Laser-Excited Raman Spectroscopy of Biomolecules" by the Lord laboratory at Massachusetts Institute of Technology establishes Raman spectroscopy as an important tool for measuring molecular structure of proteins and enzymes under native and denaturing conditions [40-56]. These papers extended the use of Raman toward understanding molecular structure and form a basis for applying Raman in food macromolecules.

Papers in the 1970's to 1990's are of more complex molecules in more complex systems. Starting in the mid 1990's, technologies such as robust solid-state red lasers, holography-based optical components, integrated spectrograph designs, commercial microscopy instrumentation, charge coupled device detectors, and fiber-optic probes enabled Raman spectroscopy outside an academic laboratory [57-59]. These technologies enable dispersive Raman spectroscopy and we see a transition in the literature from the early-mid 1990's that largely uses Fourier-transform Raman to late 1990's onward that mostly uses dispersive Raman. In 1996, E C Y. Li-Chan published the first review of Raman spectroscopy specifically discussing food science [60]. The paper highlighted applications in fundamental understanding of composition or molecular structure, quality assurance, and identification of adulteration. Since then, Raman has been used in a variety of food analysis applications.

2 Highlights in current uses of Raman spectroscopy in food analysis

Better product understanding

Food is a spatially heterogeneous product with composition heterogeneity that can be observed at the micro-level to the bulk level. Heterogeneity can be observed across samples as well. The ability to manufacture food products in light of this compositional variety is a challenge in the food industry, and assessment of bulk and spatially-resolved composition is a topic of intense research. The sampling flexibility of Raman enables microspectroscopy and bulk composition measurements of materials [61,62]. We see the use of Raman for measuring beef [63-66], pork [67-72], apple sugars [73], salmon [74] and dairy [75] using a variety of Raman instrumentation including Raman microscopy, fiber-optic Raman, and portable Raman.

Food safety

Food safety is an umbrella term that encompasses the extent of contamination by three major sources: microbes, physical, and chemical. Chemical aspects of food safety focus on source authenticity and the presence of additives or adulterants. Adulterants are low-quality, but nearly indistinguishable, ingredients that are intentionally added or substituted into a food to mask poor quality or increase the volume of high-value foods. In addition to the safety hazards of using potentially dangerous ingredients, adulteration has important implications for labelling and product pricing. Because adulteration is typically performed using ingredients that are indistinguishable by sight, taste, touch, or smell, the use of analytical tools is necessary to identify adulteration. A recent book chapter by Esteki *et al* reviews analytical methods of food authentication, which encompasses chromatographic, mass spectrometry, and spectroscopy techniques for commonly-adulterated foods [75]. Petersen *et al* has recently published a review paper on Raman spectroscopy in food safety [76] and the reader is referred to this paper for its excellent overview of Raman variants used in food safety applications and its focus on bacterial and chemical contaminants. There is additional work in chemical contaminants on food authenticity and adulteration that were not highlighted by Petersen *et al*, and those will now be reviewed.

There is much research into using Raman spectroscopy for identifying chemical adulterants because of Raman's specificity and its ability to measure in solids or liquids without sample preparation. Particular attention has been paid to Raman identification of chemical adulteration in commonly adulterated foods such as honey [77-79], milk [80-85], paprika [86], and olive oils [87]. Honey adulteration can be achieved using other sugar sources such as high fructose corn syrup, hydrolyzed inulin syrup, glucose syrup, maltose, and inverted sugar. Raman spectroscopy was used in laboratory studies to identify adulterations and quantify them in honey. We highlight the paper by Oroian *et al* as an example of using Raman spectroscopy to identify adulterants and verify authenticity. The paper discussed the use of Raman spectroscopy for honey authenticity and adulteration purposes [78]. The study examined five honey sources (acacia, sunflower, tilia, polyfloral, and honeydew) and 5-40% adulteration using fructose, glucose, inverted sugar, malt must, or hydrolyzed inulin syrup. Samples were corrected to 65 ˚Brix and measured at 55°C with a 785 nm Raman analyzer equipped with a probe to measure samples in a 1cm quartz cell. Spectra were preprocessed by an optimal combination of air PLS and auto-scaling. Raman data were modeled by the type of analysis. Three analyses were performed on the data: authentic vs. adulterated classification using Partial Least Squares-Linear Discriminant Analysis (PLS-LDA), type of adulterant using PLS-LDA, and the amount of adulterant using Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR). Classification of authentic vs adulterated groups were correctly classified with a cross-validation model accuracy of 96.54%. Identification of adulterant, independent of its concentration, was achieved with a cross-validation model accuracy of 90.00%. Quantification of adulterants in each of the honeys using PCR yielded an R^2 in the prediction set varying from 0.903-0.995, and an R^2 in the cross-validation set varying from 0.903-0.996 using PLSR. The Raman spectrum of the adulterant, and potential overlap with honey bands, appeared to be the major factor in the PLS-LDA model parameters.

A form of adulteration in processed foods containing natural products like meat, fish, or milk is substitution from other species [88]. For example, trout may be used to mimic salmon or adulterate salmon-based products and horse or offal meats may be used to adulterate hamburger meat. In a report of salmon adulteration or substitution by trout, differences in the fats were used to discriminate trout from salmon and quantify the amount of adulteration. Notably, the trout spectra did not contain carotenoid bands while the salmon spectra did contain carotenoid bands, and that is another potential basis for discrimination [89]. Two approaches have been presented for beef adulteration, where the beef muscle is directly measured [90,91] or fat is extracted from beef and then measured by Raman [92,93].

3 Raman in the food processing environment

There are a variety of laboratory applications of Raman spectroscopy for food authenticity, product quality, and product understanding. Translating the technology to a plant environment can be facilitated through industry- government partnerships or consortia. The Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima) is a food research institute and have reported on spectroscopic analyses in food. The papers reviewed describes extension of a "toolbox" approach by Nofima researchers, using different spectroscopy techniques to control industrial food processes, identify adulterants, and measure product quality.

Two studies apply various spectroscopy techniques for in-line fat, protein, and ash weight identification. A 2018 paper by Wubshet *et al* describes the use of NIR imaging, micro NIR, fluorescence, and Raman spectroscopy to measure fat, proteins, and ash weight of meat byproducts as received and after enzymatic hydrolysis under simulated process conditions [94]. The end goal of these studies was integration of spectroscopic PAT as a feed-forward control basis for enzymatic hydrolysis of byproducts. There was no single technique that could predict molecular weight and total protein, and each of the techniques could

predict a set of parameters well. An understanding of the underlying chemistry and how each technique can measure that chemistry gives context to the paper's major findings. Raman spectroscopy was found to predict ash weight better than NIR or fluorescence because of the strong symmetric stretch of bone's carbonated apatite phosphate group at ~ 960 cm^{-1}. Raman was sensitive to differences in molecular weight because the amide III and amide I envelopes reflect differences in molecular structure. A combined NIR and fluorescence approach was the best approach for predicting protein yield because of collagen specific fluorescence emission at 440nm and a CH overtone at 1200 nm in the NIR. The importance of this paper as the first known paper describing in-line spectroscopy to control an industrially relevant process cannot be overstated.

A follow on study in 2019 by Wubshet *et al* used the Raman experimental approach developed for meat byproducts to predict calcium and ash weight in mechanical deboned chicken meat [95]. During the deboning process, small pieces of bone may be produced and is an undesirable component of deboned meat. Calcium and ash are used as surrogates for the presence of bone, and they are typically measured using acid extraction and atomic absorption, titration, or incineration. Raman spectroscopy is an excellent tool for measuring bone and is an established technique for understanding bone composition in health and disease [96,97]. The carbonated apatite mineral has two main Raman bands: ν_1 PO_4^{3-} at ~960 cm^{-1} and ν_1 CO_3^{2-}. The phosphate band at 960 cm^{-1} is very strong, unique to bone in musculoskeletal tissues, and provides good contrast between bone and unmineralized soft tissue. Thus, the authors hypothesized that Raman spectroscopy could be used to non-destructively identify residual bone in mechanically deboned chicken meat. Mixtures of mechanically deboned chicken meat and mechanical deboning residue were prepared to mimic various amounts of bone content. Large volumetric Raman spectra were collected in all 79 samples and compared to reference measurements of calcium and ash weight. Raman-based partial least squares regression of ash and calcium yielded a model with an R^2 of 0.894 and prediction error of 0.634 g/100g for % ash and R^2 of 0.775 and prediction error of 0.333g/100g for % calcium. These promising initial results support further application development.

4 Dispersive Raman at near-to-shortwave infrared wavelengths

There are many Raman variants used to support the growing number of applications in basic understanding, product safety, and process control in food manufacturing. These variants include Fourier-transform Raman, surface- enhanced Raman, Raman microscopy, resonance Raman, spatially-offset Raman, transmission Raman, handheld Raman, and fiber-optic Raman. An addition to the growing number of Raman variants is dispersive Raman at 1000 nm. Dispersive Raman spectroscopy at higher wavelengths is a commonly-used approach to reduce fluorescence, but the benefit of fluorescence reduction is offset by a weaker signal since the scattering efficiency scales inversely with the excitation wavelength to the 4^{th}power [98]. As a result, 785 nm excitation has traditionally been a good balance between fluorescence reduction and speed of measurement. Moving to higher wavelengths without resorting to FT instrumentation or shifted-excitation approaches was traditionally a challenge because of suboptimal detector efficiencies [99]. At 830 nm excitation, the quantum efficiency of silicon-based detectors decreases disproportionately to the modest improvements in fluorescence reduction [100]. Advances in InGaAs detector efficiency in the 800 nm – 1.2μm region have enabled dispersive Raman at wavelengths in the ~1000 nm region. The use of 1000 nm excitation in dispersive Raman has been described for fermentation and cell culture monitoring applications [101-103]. We performed experiments to demonstrate feasibility of dispersive Raman spectroscopy at 1000nm for a range of solid foods. We describe a representative example in salmon meat. Salmon meat represents an interesting challenge to the F&B communities and its quality has been traditionally measured in offline or laboratory settings.

Salmon quality

Salmon is a popular fish and there is high consumer demand for salmon worldwide. This demand has created a need for efficiency from the raising of salmon to its processing in the plant. Rapid measurements of fat, color, and firmness, the three main attributes of fish quality, can help achieve the goal of efficient processing. Fat content is associated with taste and mouth feel [104]. Salmon meat color influences consumer perception of fish quality, and consumers prefer a deep pink color [105]. Firmness affects consumer acceptance of both raw and smoked salmon products [106]. These three quality attributes have been traditionally measured in the laboratory using titration and chromatographic techniques or, in the case of color, by visual inspection against a color card. The increased demand for salmon is driving the use of novel analysis technologies that can keep pace with automated processing plants to provide a rapid and precise assessment of fish quality inline and in real-time. Fish meat measurements by Raman spectroscopy have been reported since the 2000's [64,107,108] with more recent work to measure fish quality transcutaneously [109] and to understand the effect of pH changes and cryopreservation on fish protein gelation properites [110]. Salmon has been extensively studied by Raman spectroscopy in the laboratory and government researchers since 2005, with new applications in salmon processing and storage, breeding, and adulteration identification [89,111-116]. Machine learning and automated model optimization are software based enablers, and many of the newer reports since 2017 use these data science approaches.

Feasibility of 1000 nm dispersive Raman was carried out through two experiments on store-bought samples of farmed Atlantic salmon, wild sockeye salmon, and smoked salmon. In the first experiment, laboratory tests were performed using a dispersive Raman Rxn2 analyzer operating at 1000 nm equipped with a Raman Rxn probehead and small-area contact probe (Kaiser Optical Systems, Inc.). The probe was manually placed at various locations on the surface of the fish and signal was collected for 10-60s. In the second experiment, mock processing tests were performed with a Raman analyzer operating at 785 nm equipped with a large volumetric non-contact probe (Kaiser Optical Systems Inc., Ann Arbor, MI USA) with a 3mm or 6mm spot size. Depending on the spot size, the probe was placed 12 or 25 cm away from the surface of the salmon and signal was collected for 1-5s on a single accumulation. Raman spectra were preprocessed in GRAMS/AI (ThermoFisher Scientific, Waltham, MA USA). Unless noted spectra were baseline corrected using a rolling window algorithm developed in-house and truncated to 400-1850 cm^{-1}. No smoothing algorithm was applied to spectra. Raman band areas and heights were calculated using an algorithm developed in-house.

Laboratory tests performed with the small area contact probe indicate feasibility of 1000 nm excitation for zonal heterogeneity measurements. Table 1 shows Raman band assignments for salmon fat, protein, and carotenoid components. While many fat and protein bands overlap in the 1000-1600 cm^{-1} region, there are bands unique to the component of interest that could be used to indicate that component's presence in the sampled volume. The v_s C=O band at ~1750 cm^{-1} was used to indicate fat content, the ring breathing band at ~1004 cm^{-1} was used to indicate protein content, and bands at ~1160 cm^{-1} and ~1520 cm^{-1} were used to indicate carotenoids.

Figure 2a shows representative Raman spectra from a cross section of Atlantic salmon, corresponding to measurements from fat-rich and muscle-rich zones. Spectra are shown without baseline correction to demonstrate the fluorescence reduction provided by 1000 nm excitation. Two qualitative observations can be made from the spectra of Atlantic salmon. The first observation is that the spectra from each zone contains features from the dominant macromolecule in that zone. Spectra collected in the fatty region do not have protein feature and spectra collected in the muscle regions do not have fat features. The contact probe's optical fibers are in a backscattered geometry, where the excitation fiber and collection fiber are placed with zero offset from each other, and thus collects signal from a small sampling volume. In the case of the salmon, the contact probe collects signal in a ~ 100µm spot size with an estimated sampling volume of 0.5-1mm^3. The

small sampling volume enables signal collection from the fat-rich and protein-rich zones without significant spectral interference from adjacent zones. Bands from fats or protein are apparent in the spectra. The top spectrum contains features from mostly lipids in fat, and the bottom spectrum contains protein features. The second observation is that there is a weak but observable signal from carotenoids at ~ 1159 and 1520 cm^{-1}. The second study was performed under simulated process conditions using a 785 nm Raman analyzer equipped with a large volumetric probe with an estimated sampling volume of 2-4mm^3. The mock process measurements were performed on the Atlantic salmon measured with the backscattered probe at 1000 nm. Figure 2b shows the large volumetric probe collecting measurements from the surface of the Atlantic salmon with Raman spectra collected at 1s, 3s, and 5s. A large volumetric probe was used because it suited the application needs of focus-free, rapid, and non-contact measurements. The probe uses a defocused laser excitation with a 3mm or 6mm spot size and 50 collection fibers. Thus, it has a larger sampling volume in both the axial and lateral directions. This aspect of the probe design informs on spectral interpretation and the resulting spectra had contributions from the fatty and muscle portions of the tissue. Measurement times were optimized to be compatible with conveyor belt speeds. As shown in Fig 2b, Raman spectra collected at 1s, 3s, and 5s are suitable for input into a univariate or multivariate model with good signal-to-noise and spectral resolution. Weak carotenoid bands were also observed, which was consistent with contact measurements collected with the backscattered probe at 1000 nm.

Table 1. Raman band assignments for spectra of salmon collected using dispersive instrumentation and 1000 nm excitation. As expected, bands from fat, protein, and carotenoids were observed. In spectra collected using a large volumetric probe, overlapping bands from fat and proteins were observed, which affected the band position, width, and area of bands in the 1300-1700 cm^{-1}spectral region. Non-overlapping bands were used to identify the presence of spectral contributions including the ring breathing mode at ~ 1004 cm^{-1} for proteins, 1159 cm^{-1} and 1518 cm^{-1} for carotenoids, and 1745 cm^{-1} for fats

Raman shift (cm^{-1})	Assignment	Tissue component	Tissue (s)
620-640	Tyrosine	Protein	Muscle
850, 875	Hydroxyproline	Collagen	Muscle, skin
920	Proline	Collagen	Muscle, skin
1001	Phenylalanine ring breathing	Proteins	Muscle, skin
1065	νC-C skeleton, trans	Lipid	Fat
1080	νC-C skeleton, random	Lipid	Fat
1159		Carotenoid pigments	Meat
1230-1280	Amide III	Protein	
1300	CH_2 deformation	Lipid	Fat
1439	CH_2/CH_3 deformation	Lipid	Fat
1448	CH_2/CH_3 deformation	Collagen, protein	Muscle
1518		Carotenoid pigments	
1610-1690	Amide I	Proteins, lipids	Muscle, fat
1745	νC=O	Lipid	Fat

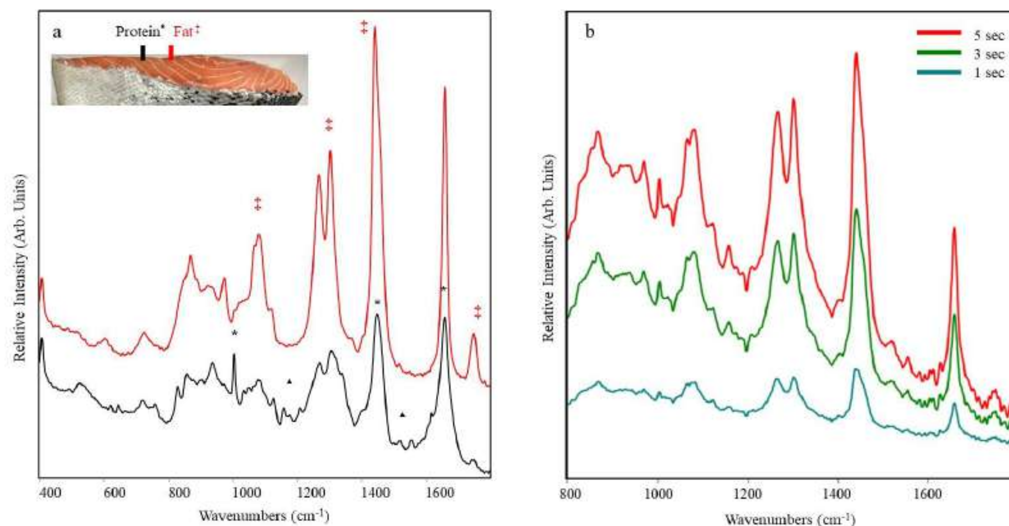

Fig 2. Spectra of Atlantic salmon collected with a contact backscattered probe (panel a) and a large volumetric probe (panel b) contain bands from protein and fats with weak signal from carotenoids. When the backscattered probe was placed at fat-rich (panel a, top spectrum) or protein-rich (panel a, bottom spectrum), the resulting spectra contained minimal interference from other zones. Spectra collected with the large volumetric probe was from a larger area and the spectra contained bands from both fat and protein-richzones.

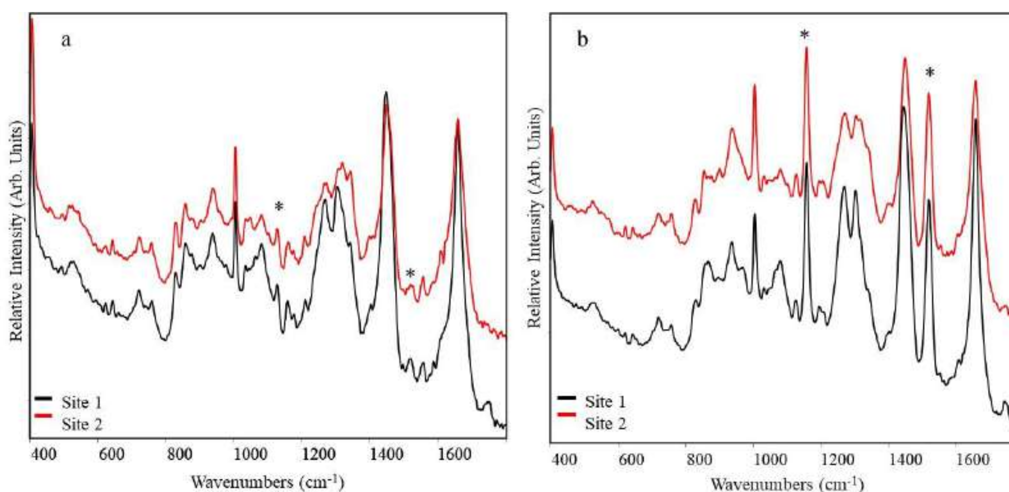

Fig 3. Raman spectra of smoked salmon (panel a) and wild sockeye salmon (panel b) using a backscattered Raman probe contained spectral contributions from fats and proteins, with varying intensities of carotenoids. Carotenoid bands at ~ 1159(*) and 1518(*) cm^{-1} were weak in spectra from smoked salmon and strong in wild sockeye salmon.

Raman spectra in Fig 3 are presented from several measurement sites of smoked and sockeye salmon samples. Spectra of wild sockeye salmon were collected from the surface of the loin, rather than the cross section, and contained signal from carotenoid pigments in addition to fats and protein bands. The spectra showed subtle zonal composition variations. The laboratory study using a contact probe at 1000 nm excitation yielded Raman spectra of fish fat, proteins, and carotenoids. Raman spectra of salmon are

rich with composition and molecule structure information on fats, proteins, and carotenoids. The ability to measure these parameters simultaneously, quickly, and without sample preparation is an appealing feature of Raman spectroscopy. Using a backscattered contact probe was useful when precise zonal measurements, which may be applicable for quality control or pricing purposes. A large volumetric probe was useful to collect a representative sample over a larger volume, which may be applicable for rapid scanning purposes. Salmon Raman band positions at 1000 nm excitation are consistent with 785 nm and 830 nm excitation with a reduction in fluorescence. However, a comparison of spectra collected with 1000 nm with those collected with 785 nm excitation (data not shown) showed only a modest decrease in fluorescence background. Thus, the use of 1000 nm excitation may not be necessary for salmon measurements to reduce fluorescence from farmed or wild salmon samples.

5 Conclusions and Perspectives

The Process Analytical Technology (PAT) framework is growing within the food industry to support rapid development of products, enable real-time product quality control, and ensure consistent product quality for natural, processed, and innovative cell-based or plant-based products. Raman spectroscopy is an important PAT in the chemical, pharmaceutical, and biopharmaceutical industries with growing adopting within the food & beverage industry. The plethora of food & beverages applications reported include understanding product quality and identifying adulterated products. We are particularly enthusiastic about a growing number of papers in spectroscopic PAT for industrial process monitoring and control.

We found that Raman studies on major macromolecules of food: proteins, fats, carbohydrates, and sugars, were performed much earlier than acknowledged in the literature. Papers dating to the 1930's directly support modern food studies and there is a rich history of papers and knowledge of band assignments and changes of the spectra under normal and variable conditions. These papers support newer studies on product quality, process control, and adulterant identification. Yet, we cannot help but observe a gap currently between the literature and industrial practice. Salmon analysis is an example of this gap. Despite a nearly 15-year record of excellent literature showing application feasibility, the availability of industrial Raman instrumentation, improved data science approaches in model development and maintenance, and improvements in the user interface to enable the non-specialist use, there are limited-to-no industrial reports of Raman for salmon measurements. This gap between the laboratory and processing environments highlights an opportunity to understand possible limiting factors of industrial adoption of the technology including implementation costs, data management, and personnel expertise. Technologies in spectroscopy hardware, data analysis, and digitalization continue to evolve. These aspects are important to reduce hardware costs, integrate into automation platforms, become more portable, and support use by non-specialists. We believe that these aspects will facilitate increased acceptance of Raman spectroscopy within the food and beverage industry.

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Statement on conflict of interest

The authors have no conflict of interest

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in elementary and secondary schools. As a volunteer tutor, she has tutored teachers, nurses, and struggling students of all ages. In her spare time, she enjoys adventures with her husband, to whom she has been married for 27 years, and their 9 children. Kayaking backward badly through rapids while screaming is her specialty.