WHITE PAPER

RAPID MILK ANALYSIS

Qualitative and Quantitative Analysis of Highly Fluorescent Animal Source Milk and Plant-Based Milk Products by Time-gated Raman Technology

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Introduction

Raman spectroscopy has been gaining ground in the last decade for food systems analyses. The vibrational fingerprint offers a wealth of information on the content and structure of the studied sample.

Quality control of both the raw ingredients and the end products in the food industry are of paramount importance. With the exponential increase of lifestyle diseases, more and more people are moving towards vegan food alternatives. A recent study says that for every three per cent increase in calories from plant protein risk of death is reduced by ten per cent. One of the main products in food industry is milk. Due to lactose intolerance and various allergies associated with animal source milk, plant-based milk products are flourishing. Raman spectroscopic technique has been employed for characterizing the milk products and its ingredients. Fluorescence is big challenge for Raman spectroscopic technique since plant-based products have high fluorescence compared to its animal-based counterparts.

In this work, we have compared continuous wave Raman spectroscopic technique to time-gated Raman technology on different varieties of milk and milk products. First, we analyzed and compared animal-source milk with soy and oat milks. Further we tested cheese, lactose, whey and vegan protein supplementary powders. To exemplify the quantitative efficacy of this technique, cow milk with different fat and lactose content were analyzed.

Instruments and methods

Raman spectroscopy provides non-destructive chemical analysis and throws light on the structure, phase, polymorphism and molecular interaction of the samples. Furthermore, another added advantage is that this technique does not require sample preparation. Despite these advantages the use of this technology is still restricted by the influence of fluorescence. It is a well-known fact that amino acids have a strong fluorescence signal and proves to be a great challenge. Time-gated Raman technology exploits the time delay in the emission of fluorescence and the Raman scattered photons are detected before the advent of most of the hampering fluorescence signals. Time-gating enables the study and separation of the Raman scattered photons of interest, as long as there is a time difference between the studied response and other (interfering) emission as illustrated in figure 1. This technique of fluorescence suppression has been studied for decades in research laboratories and yet there have not been any commercially available systems owing to the high cost and complexity of the other time-gated Raman technologies. The patented CMOS-SPAD electrically gated detector offers a commercially viable solution for time-gated Raman spectroscopy. Thus, this groundbreaking innovation is set to take where fluorescence has restricted the entry of Raman technology.

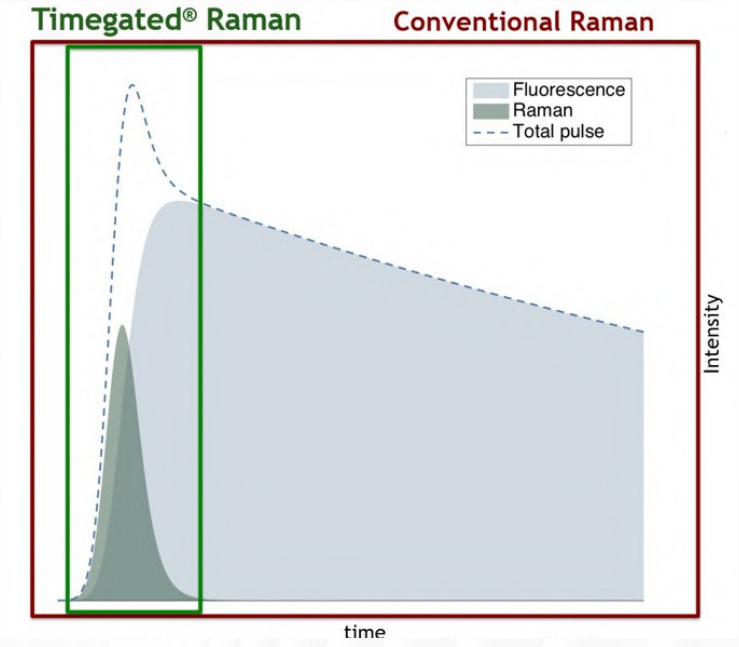

Figure 1. Schematic comparison of the process of the conventional Raman and Time-Gated Raman spectroscopy

Continuous wave (CW) Raman spectra were obtained using Thermo Scientific DXR2xi Raman imaging microscope employing both 532 nm and 785 nm lasers and compared to the measurements obtained from Timegate Instrument PicoRaman 532 nm, a time-gated Raman spectrometer. A 10x probe was used in all the measurements. The number of accumulations for the continuous Raman spectroscopy was set to 3. The time duration for every measurement was restricted by the saturation of the CCD. The total time duration to acquire every spectrum in PicoRaman was set to 3 minutes.

Qualitative characterization of milk and milk products

Three different milk varieties were studied. The first milk was from animal origin with reduced lactose content, the second was oat milk and the third was soy milk. All these were commercially available in the local supermarket. The figure 2 compares the obtained raw spectra of the samples from both CW Raman and PicoRaman.

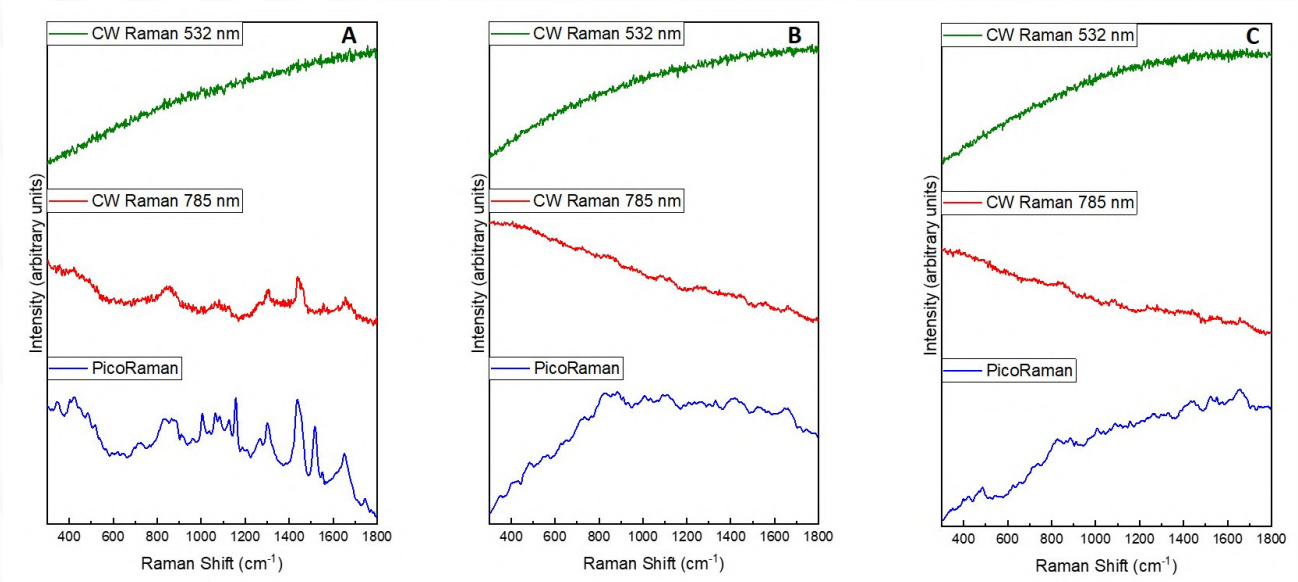

Figure 2. Raw Raman spectra of (A) cow milk (B) oat milk and (C) soy milk measured from continuous Raman spectroscope at 532 nm and 785 nm laser excitation, and 532 nm laser excitation of PicoRaman with Y offset for clarity.

It is clear from figure 3 that plant based milk products are highly fluorescent and it is nearly impossible to obtain meaningful data from the fluorescence plagued Raman spectra from CW Raman spectrometer. Whereas with effective removal of fluorescence, we can clearly characterize the Raman spectra and analyze both quantitatively and qualitatively. The protein content in milk comes from casein and whey. Hence to clearly understand the spectral features of milk, cheese (80% of casein), lactose, vegan protein and whey protein were measured (figure 4).

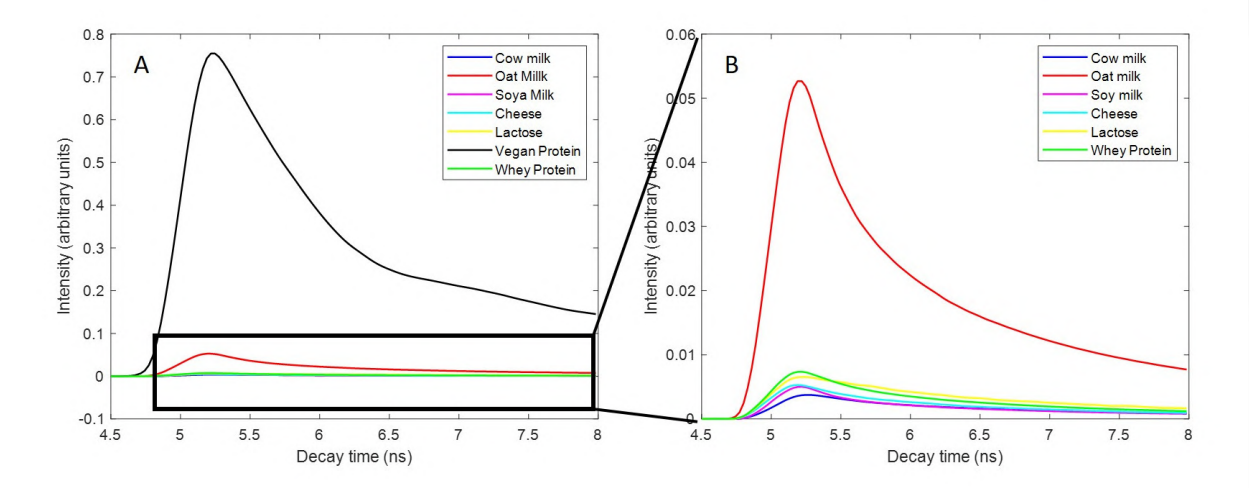

Figure 3. Fluorescence decay characteristics of (A) cow milk, oat milk, soy milk, cheese, lactose, vegan protein and whey protein. (B) Zoomed in plot of fluorescence decay characteristics.

A peek into the scientific data that PicoRaman offers

Time-gated Raman spectra of oat milk reveals the β sheet structure of aromatic amino acids like tryptophan and phenylalanine at 1672 cm-1. Further the amide I α helical band at 1654 cm-1, amide II at 1567 cm-1, amide III β sheet band between 1238 to 1245 cm-1, amide III random coil structure at 1250 cm-1 and amide III α helical band at 1260 to 1300 cm-1 can be exploited to quantitatively analyze the protein content of the sample. The content of saturated fatty acids can be accessed by the peaks at 1303 and 1443 cm-1. The triglycerides exhibit a peak at 1748 cm-1 and the peak at 1654 cm-1 are assigned to unsaturated fatty acids. Whereas the peak at 1087 cm-1 is indicative of lactose. Thus protein, fat and lactose content can be quantified based on the Raman spectra with the help of statistical tools. Furthermore, carotenoid content can also be calculated based on the peak at 1520 cm-1.

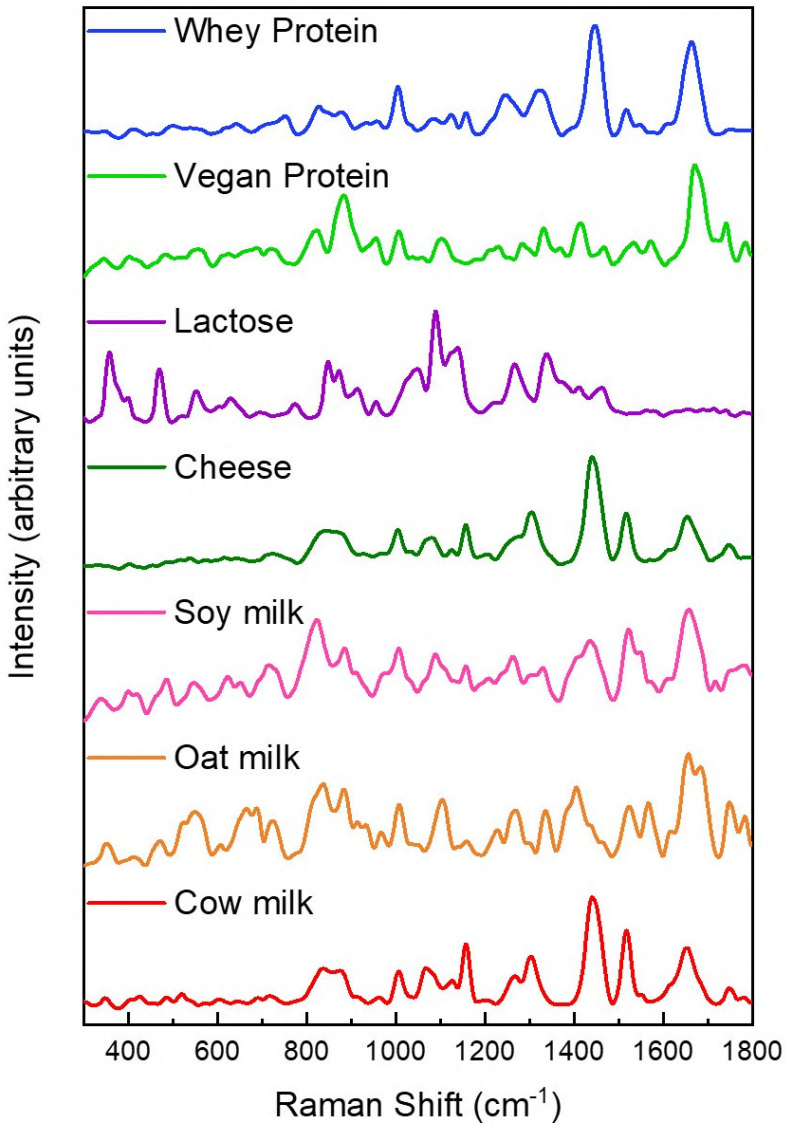

Figure 4. Raman spectra of cow milk, oat milk, soy milk, cheese, lactose, vegan protein and whey protein measured using PicoRaman (Y offset for clarity).

Quantitative characterization of milk

Commercially available milk with different fat contents varying from 0 to 3,5 g per 100 ml of milk (figure 5) and lactose contents varying from 0 to 4.8 g per 100 ml of milk were measured (figure 6) using PicoRaman. All the spectra were preprocessed using the SHSGUI, a graphical user interface app which is provided by Timegate Instruments, and then vector normalized. The intensity at 1443 cm-1 was used to quantitatively compare the saturated fat levels contained in the ten milk samples. The quantitative analysis was based on the fat levels that were indicated on the carton of the commercially bought milk. A linear correlation was obtained with a high correlation coefficient of 0.95 (figure 7).

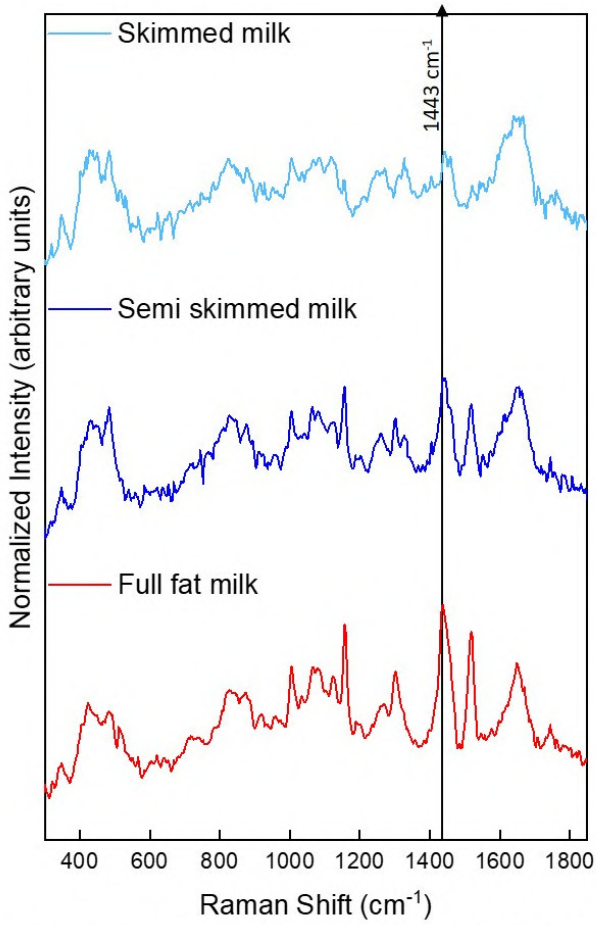

Figure 5. Mean Raman spectra of full fat, semi-skimmed and skimmed milk with Y offset for clarity

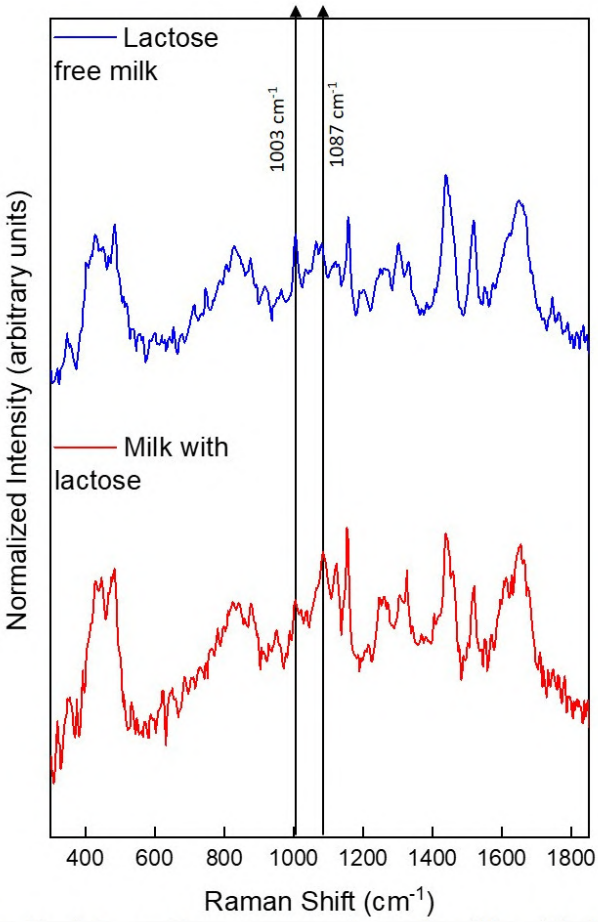

Figure 6. Mean Raman spectra of milk with lactose and lactose free milk with Y offset for clarity.

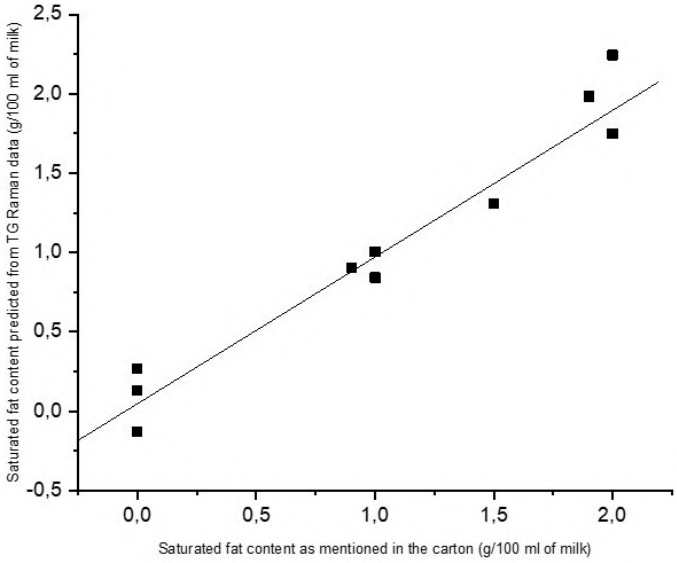

Figure 7. Calibration curve for saturated fat based on Raman spectra.

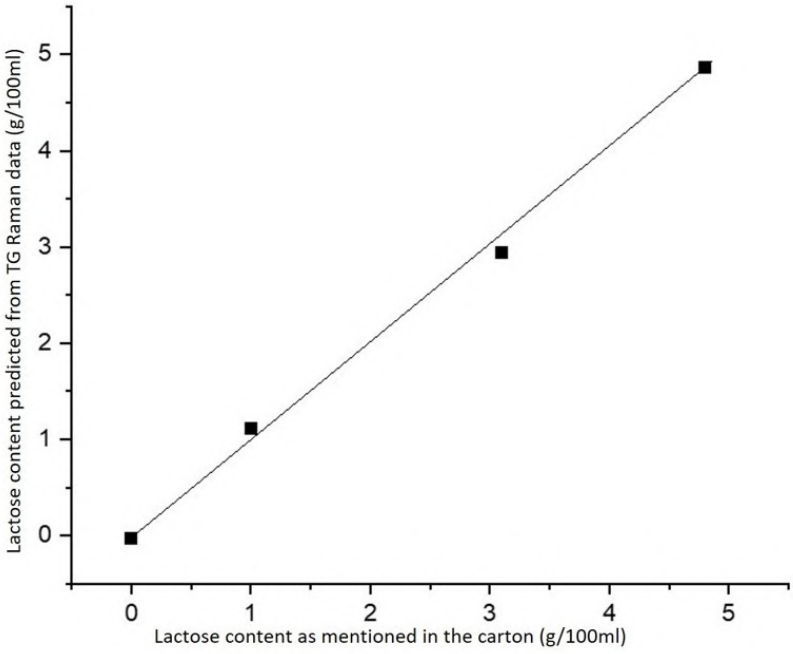

Figure 8. Calibration curve for lactose based on Raman spectra.

The lactose content was quantified using the C-O-H bending mode at 1087 cm-1. Phenylalanine, one of the essential amino acids has a strong peak at 1003 cm-1. Since the protein content of all the milk samples were comparatively similar, we took the ratio of Raman intensities at 1087 cm-1 to 1003 cm-1. A correlation coefficient of 0.99 was obtained as seen in figure 8.

Conclusion

Based on previous studies of animal-based milk products, it has been clearly established that Raman spectroscopy can be and has been successfully employed for quality assurance of online process in milk products manufacturing. But it has been curtailed to only samples where fluorescence does not mask the Raman signals. Hence there is an unmet need for qualitative analysis of the highly fluorescent vegan products. Further this technique can be effectively employed for online quantification of fatty acids, lactose and even proteins. Time-gated Raman technology can be effectively utilized for this purpose as demonstrated in this application note.