



In the food industry, various ingredients, additives, and biopolymers such as emulsifiers, stabilizers, carbohydrates, or thickeners are commonly used in order to optimize the texture or the flavor of food. The distribution and the microstructure of the ingredients strongly determine the properties of the final product. Therefore, research and development as well as quality control require powerful analytical tools for studying the distribution of the various compounds in the food. Because Confocal Raman Microscopy provides the ability to non-destructively image the chemical composition of a sample and requires only minimal sample preparation, it can be used for three-dimensional analysis of emulsions, suspensions, or solids. In this study, Confocal Raman Microscopy is used to investigate chocolate and an instant gravy thickener.

Confocal Raman Microscopy

Raman spectroscopy has become widely used for the characterization of materials in terms of chemical composition. Optical microscopy, on the other hand, is capable of providing spatial resolution down to 200 nm using visible light excitation. In a confocal microscope, only light from the focal plane is detected while out of focus light is rejected, thus providing depth resolution and a strongly reduced background signal. Images are recorded point by point and line by line while scanning the sample through the excitation focus. With this technique, the specimen can be analyzed in steps along the optical axis and even a depth profile or 3D image can be generated. Because the images consist of tens of thousands of spectra, very short acquisition times for a single Raman spectrum are essential. With the Confocal Raman Microscope alpha300 R used for this study, the acquisition time for a Raman spectrum is in the range of 10–100 ms.

Experiment and Results

In the first experiment, instant gravy thickener particles have been imaged with a scan range of $50 \times 50 \mu\text{m}$ and 150×150 pixels (22,500 spectra, 70 ms/spectrum) using a 532 nm Nd:Yag laser for excitation. Figure 1a shows the color-coded Raman image obtained by evaluating dedicated peak characteristics of the corresponding spectra shown in Figure 1b. The red region shows the distribution of starch surrounded by an excipient (blue), whereas lipids (yellow) are located between the starch grains together with other additives. In a second experiment, white chocolate was investigated. The scan range was $50 \times 50 \mu\text{m}$ at 150×150 pixels (= 22,500 spectra) and the measurement used an integration time of 40 ms for each spectrum. One can clearly see a distinct phase separation in the chocolate material. Saccharose (blue) and additives (red)

Food Analysis with Confocal Raman Microscopy

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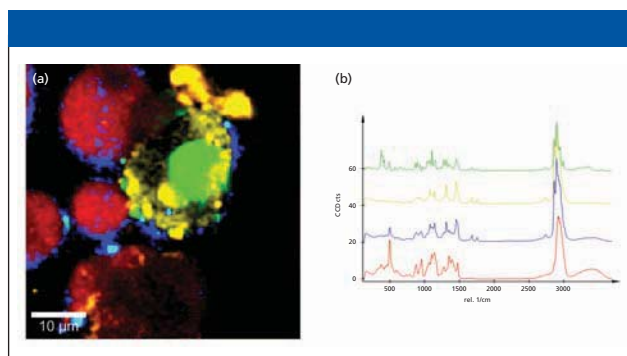


Figure 1: (a) Raman image of instant gravy thickener particles. (Scan range: $50 \times 50 \mu\text{m}$, 150×150 pixels, 22,500 spectra, 70 ms/spectrum, excitation: 532 nm Nd:Yag.) (b) Corresponding Raman spectra.

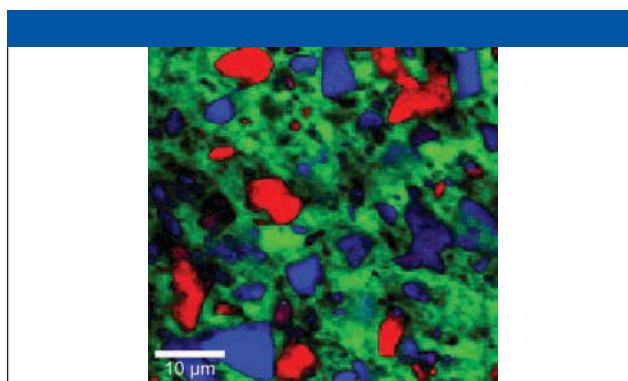


Figure 2: Raman image of white chocolate (scan range: $50 \times 50 \mu\text{m}$, 150×150 pixels, 22,500 spectra, 40 ms/spectrum, excitation: 532 nm Nd:Yag.). Saccharose (blue) and additives (red) particles are embedded in a fatty matrix (green).

particles are embedded in a fatty matrix (green). The size of the saccharose particles varies between 650 nm and $10 \mu\text{m}$.

Conclusion

This work shows the utility of Confocal Raman Microscopy when applied to food products. It was demonstrated that various food compounds or different structures can be easily imaged and distinguished.

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