

Rapid Raman Mapping for Chocolate Analysis

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Raman microspectroscopy mapping capabilities have advanced significantly and have been applied to cell and pharmaceutical tablet formulation analysis. Bulk Raman investigations of food and their constituents have been carried out but little work exists on the application of Raman mapping capabilities to food. Here, we assess the applicability of Raman microspectroscopy mapping to the analysis of chocolate and examine both white and milk chocolate samples. It was found that the sucrose, lactose and fat constituents of white chocolate could be extracted and spatially resolved, indicating that the sucrose and lactose formed particles within a matrix of 'fats'. Fluorescence from cocoa solids present in milk chocolate prevented chemical mapping with the instrumentation used. Raman mapping should provide a powerful analytical technique for the analysis and development of food products.

Introduction

Raman mapping can give a wealth of information about the spatial distribution and sizes of constituents within a complex sample. It has been used for the spatial analysis of active pharmaceutical in the excipient matrix within a final tablet¹⁻⁴ and has also been applied to *in vivo* studies where either a cell or a tissue section is analysed.^{5,6} The quality of data obtained has improved significantly as technological advances have been incorporated into the latest Raman microscope systems. Raman mapping can now be routinely carried out at a resolution set by the optical diffraction limit. However, one area where the possibilities of the information rich technique of Raman mapping has not yet been fully exploited, is within the food industry.^{7,8} Raman investigations of constituents and final food products have been carried out by obtaining spectra at individual points.⁹⁻¹³ However, this approach provides low resolution data and gives no indication of particulate sizes or distribution within final products.

Since Cadbury's introduced the first chocolate bar in 1842, the global chocolate market has grown to an estimated value of \$145bn/annum.¹⁴ Several analytical techniques have been applied to the analysis of chocolate or its constituents. Solid phase micro-extraction (SPME) followed by gas chromatograph toroidal ion trap mass spectrometry has been used to analyse the volatile and non-volatile compounds in cocoa beans, cocoa butter and final chocolate products.¹⁵ The free fatty acids in chocolate were probed with liquid chromatograph – mass spectrometry (LC-MS)¹⁶ while the majority of reported work has been conducted with infrared spectroscopy.¹⁷⁻²⁰

Diffuse reflectance near-infrared Fourier transform spectroscopy (DRIFTS) was used to monitor the nutritional parameters of chocolate.¹⁷ Sucrose, lactose, fat and moisture levels within bulk chocolate or cocoa powder have all been reported.¹⁸⁻²⁰ However, to the best of our knowledge, there have been no reports in the primary literature of chemical mapping of chocolate.

Raman spectroscopy has been used to investigate the individual ingredients present within chocolate,^{9,21,22} as well as their phase changes;¹⁰ however, the final product has not been the focus of analysis. We report the application of rapid Raman microspectroscopy mapping to final chocolate products. This technique provides a powerful development tool, not just for chocolate, but for the food industry as a whole.

Experimental

Materials

The individual raw constituents were obtained from Mars Chocolate Ltd. These included; sucrose, lactose, whey, skimmed milk powder, anhydrous milk fat, vegetable fat, lecithin, cocoa butter and cocoa mass. Whey consists of ~80% lactose, skimmed milk powder contains whey, lactose and anhydrous milk fat. Cocoa mass is approximately 50% cocoa solids and 50% cocoa butter. Final preparation white and milk chocolate samples were also provided. All materials were used without further purification.

Instrumentation

A WITec Alpha 300R confocal Raman microscope was used for all spectral acquisitions with independent spectrographs for the two laser wavelengths investigated; 532 and 785 nm. The gratings were 600 g/mm (BLZ = 500 nm) and 300 g/mm (BLZ = 750 nm) and were coupled to thermoelectrically cooled charge-coupled devices (CCDs) which gave spectral resolutions of 4 cm⁻¹ and 3.7 cm⁻¹ respectively across a spectral range of 3936 cm⁻¹ and 3324 cm⁻¹. Unfocussed laser powers at the sample were measured at ~37 mW and ~80 mW respectively.

Reference and map spectra were recorded using an Olympus LMPlanFI 50x/0.50 long working distance objective. The cocoa mass reference had to be recorded using a Nikon EPlan 10x/0.25 LWD objective and accumulated for 5 seconds to prevent sample degradation and detector

saturation. All reference spectra of the remaining pure constituents were accumulated for 60 seconds.

High resolution mapping was carried out on an area measuring 80 x 80 μm . 160 points/line and 160 lines/image were recorded to give a resolution of 0.5 μm . Spectra were recorded for 50 ms per point. Laser powers were reduced for milk chocolate samples by means of attenuators on the fibre optic coupling on the laser heads. Data analysis was carried out using WITec Project software, version 2.02.

Results and Discussion

Using 532 nm laser excitation it was found that skimmed milk powder, cocoa mass, whey and lecithin (an emulsifier) all fluoresced, figure S1 and contained no vibrational spectral features suitable for component identification. The other raw ingredients produced spectra with good signal-to-noise levels. These constituents contained vibrational bands that could be used for component extraction, figure 1.

The band at 636 cm^{-1} was used for identification of sucrose, while the band at 468 cm^{-1} identified lactose. Vegetable fat, anhydrous milk fat and cocoa butter all showed very similar spectral features and were therefore classified generally as 'fats' with the band at 1295 cm^{-1} , assigned to $-\text{CH}_2$ deformation,⁹ used for identification.

Initial mapping experiments were performed on white chocolate samples due to the reduced risk of sample degradation. It was found that spectra could be recorded at the maximum laser power without observable sample damage occurring. Figure 2 shows the individual Raman maps extracted for each of the three main components; sucrose, lactose and 'fats', using the bands identified in figure 1. The combined map is also shown, which clearly shows that the sucrose and lactose particles were found within a matrix of 'fats'. These maps contain 25,600 individual full range spectra and were recorded in approximately 40 minutes. [The time discrepancy is largely due to stage movements.](#)

Raman mapping did not only give spatial distribution information about the constituents, it also provided sizing information. In the example shown the lactose and sucrose particles were measured to be $4.9 \pm 2.1 \mu\text{m}$ and $4.4 \pm 1.5 \mu\text{m}$ respectively. This was in agreement with the particle sizes of the raw materials measured via light scattering and the final crystal sizes observed under electron microscopy (data not shown), although significantly it gave non-destructive, unambiguous chemical information of what the crystals were made from within the final product.

Milk chocolate samples proved significantly more difficult to map due to sample damage from the laser excitation. The unfocussed laser power at the sample had to be reduced to < 0.1 mW to prevent degradation of the chocolate surface. Once sample degradation was minimised sample fluorescence became the overriding problem. It has already been mentioned that skimmed milk powder, cocoa mass, whey and lecithin all fluoresce at 532 nm. However, cocoa mass was responsible for the observed fluorescence as this was the only fluorescent constituent missing from white chocolate, in which no problems with sample fluorescence were observed. Cocoa mass contains cocoa butter and cocoa solids. Since, the

spectra from cocoa butter has already been recorded and showed no fluorescence, this means that the detrimental fluorescence was obtained from the cocoa solids component. It should be noted that although skimmed milk powder, whey and lecithin are used within white chocolate their fluorescent signals did not overwhelm the signals from the other constituents.

In an attempt to extend Raman mapping to milk chocolate samples the wavelength was increased to 785 nm. Figure 3, shows the spectra recorded from the non-fluorescent constituents. Lactose, sucrose and the 'fats' could be discriminated with the same bands as those used for the 532 nm results, this resulted in similar maps being produced for white chocolate, indicating no wavelength dependence of the results, figure S2. Skimmed milk powder and whey did not give fluorescent signals, although they did not contain individual vibrational bands which could be used for their discrimination without further data analysis such as modified alternating least squares regression.²³ Unfortunately, cocoa mass and lecithin continued to be fluorescent at this wavelength.

Milk chocolate mapping at 785 nm suffered from laser induced sample damage and the power had to be significantly reduced from 80 mW to ~2 mW to ensure no observable sample degradation occurred. Figure 4 shows the map extracted using the lactose peak, it shows some structural features however, these turned out to be fluctuations within the fluorescence signal rather than any 'real' features. Cocoa solids are again responsible for the poor Raman mapping of milk chocolate. We have observed that cocoa mass, containing cocoa solids, continues to show a fluorescent response, even when 830 nm laser excitation is used (data not shown). Therefore, either Raman mapping using longer wavelengths, such as 1064 nm, is required or more sophisticated fluorescence rejection techniques employed. Although it has not been possible to map milk chocolate samples, we have successfully demonstrated Raman mapping of white chocolate samples.

Conclusions

We have successfully applied rapid, high-resolution, large-area, Raman mapping to the analysis of chocolate samples. White chocolate was mapped with no special sample or instrumental alterations and showed that lactose and sucrose particles were embedded in a matrix of 'fats'. Laser powers had to be significantly reduced to prevent sample damage with respect to milk chocolate samples. Milk chocolate mapping also suffered due to inherent fluorescence from the cocoa solid constituent. Importantly, Raman mapping did not only provide spatial distribution of constituents, it also allowed the particles to be sized and their chemical nature to be assigned unambiguously. Raman microspectroscopy mapping should become a significant addition to the food analysts' toolkit.

Notes and references

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† Electronic Supplementary Information (ESI) available: Fluorescent constituents at 532 nm and 785 nm maps. See DOI: 10.1039/b000000x/

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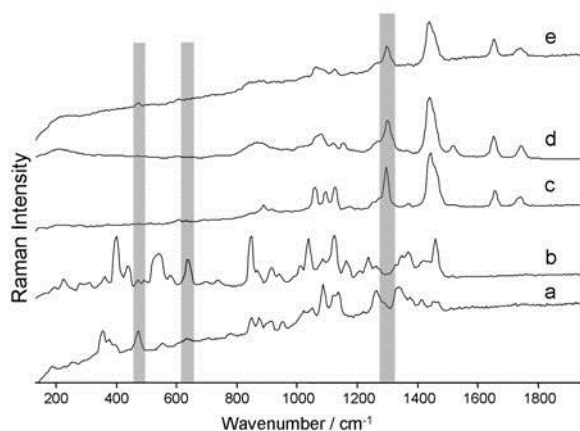


Fig. 1 Reference spectra of the non-fluorescent constituents of chocolate at 532 nm, accumulation time of 60 seconds. a – lactose, b – sucrose, c – cocoa butter, d – anhydrous milk fat, e – vegetable fat. The bands used for identification of the lactose, sucrose and 'fats' are identified.

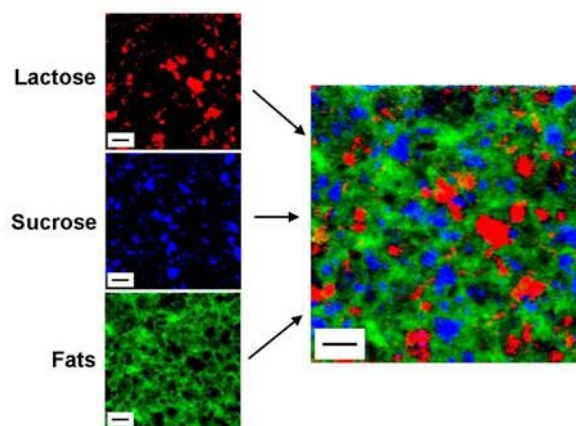


Fig. 2 Raman mapping of a white chocolate sample measured at 532 nm, constituent maps and the combined Raman map which shows the lactose and sucrose particles within a matrix of 'fats'. Scale bar in each image is 10 μm .

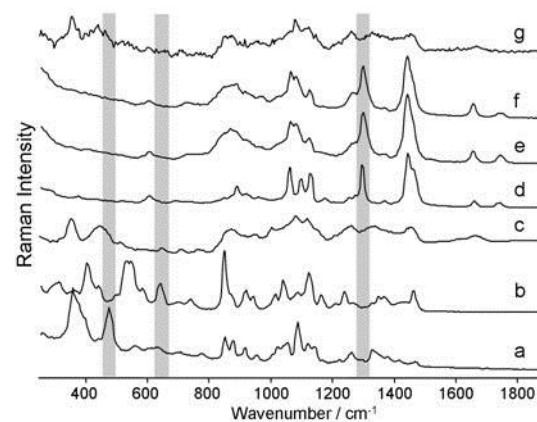


Fig. 3 Reference spectra of the non-fluorescent chocolate constituents measured at 785 nm. a – lactose, b – sucrose, c – skimmed milk powder, d – cocoa butter, e – anhydrous milk fat, f – vegetable fat, g – whey. All spectra were accumulated for 60 seconds. The bands used for identification of the lactose, sucrose and 'fats' are identified.

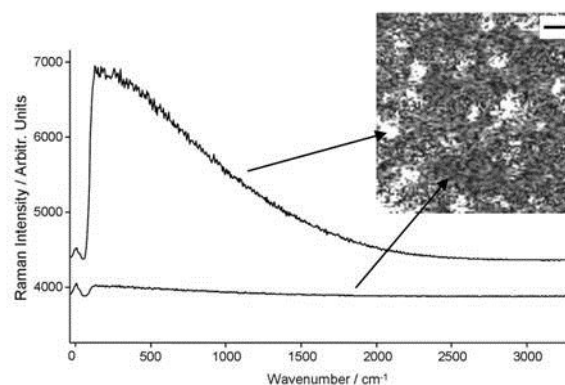


Fig. 4 An extracted 'lactose' map from a milk chocolate sample showing features. These turned out to be variations within the fluorescence signal intensity as shown. Scale bar in the map is 10 μm .

Supplementary Information

Rapid Raman Mapping for Chocolate Analysis

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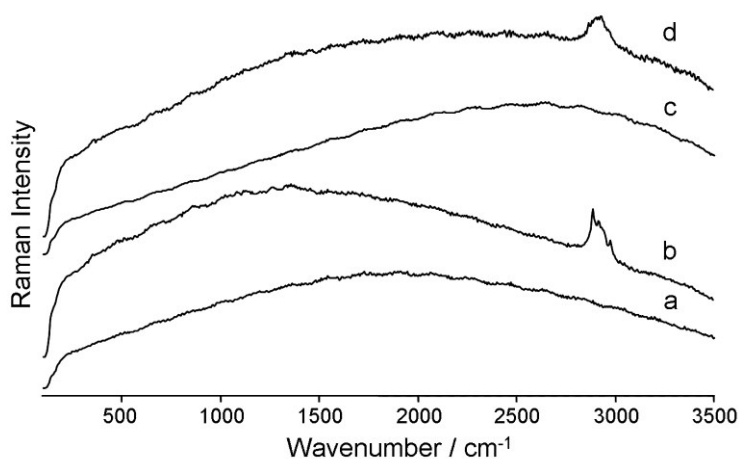


Figure S1. Reference spectra of the constituents which showed fluorescence at 532 nm laser excitation. a – lecithin, b – whey, c – cocoa mass, d – skimmed milk powder. Spectra were accumulated for 60 seconds apart from c which was accumulated for 5 seconds with a 10x objective, see main text.

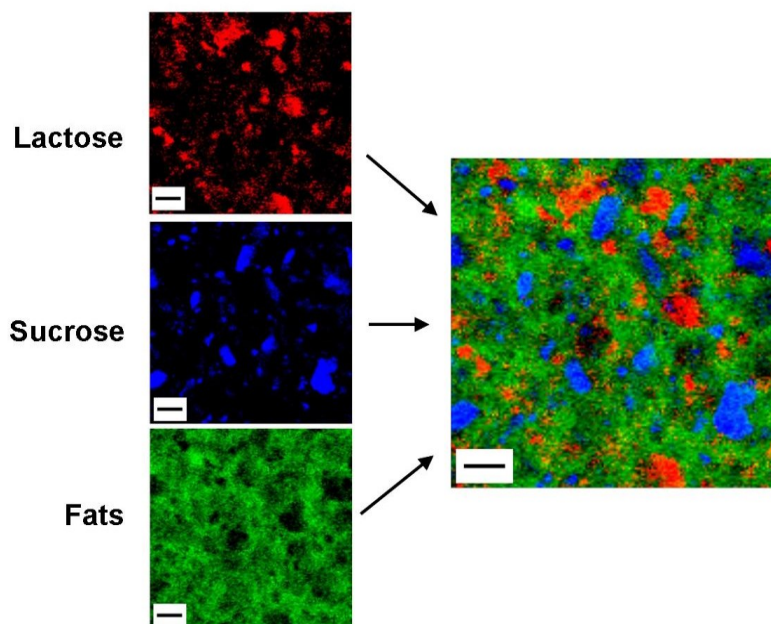


Figure S2. Raman mapping of a white chocolate sample measured at 785 nm, constituent maps and the combined Raman map which shows the lactose and sucrose particles within a matrix of 'fats'. Scale bar in each image is 10 μm .