RAMAN SPECTROSCOPY-BASED OBSERVATION OF ANTIOXIDANTS IN FRUITS AND VEGETABLES

APPLICATION NOTE RAMAN-007 (A4)

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Abstract

It is commonly recognized that eating a diet rich in fruits and vegetables is healthy. The basis of this statement is epidemiological research showing a decreased risk of chronic diseases including cancer and cardiovascular disease in people who consume high levels of plant foods.[1] Behind this observation is the fact that the presence of fruits and vegetables in an individual's diet is sometimes displacing more harmful things, such as refined sugars, fatty meats and processed foods. Another part of this complex story is that fruits and vegetables contain pigments, many of which are potent antioxidants in the human system. This is important because of the ability of these antioxidants to scavenge reactive oxygen species, linked to development of both cancer and cardiovascular disease, from the body. Carotenoids in general are potent antioxidants, and lycopene in particular has been shown to be the most effective singlet oxygen scavenger in vitro.[2] Most lycopene consumed by Americans is in the form of tomatoes and tomato products.

Tomato ripening involves the visible breakdown of chlorophyll and a concurrent increase of carotenoids, including lutein, β-carotene (Figure 1) and lycopene (Figure 2), so that monitoring their appearance is a reasonable way to establish maturity. Loss of lutein and β-carotene and the accumulation of lycopene has been monitored in a research environment with spatially-offset Raman by observing the carotenoid peak at 1525 cm^{-1} (lutein) fade away, to be replaced by the feature at 1513 cm^{-1} (lycopene).[3] Raman spectroscopy has also been used to develop a freshness discriminant, which according to one paper, can estimate the freshness of tomatoes correctly about 86% of the time.[4]

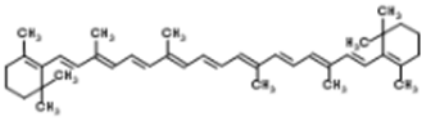

Figure 1.
Structure of beta-carotene (11 C=C double bonds).

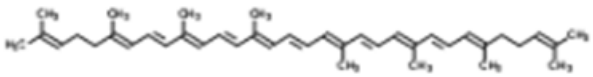

Figure 2.
Structure of lycopene (13 C=C double bonds).

The fruit is mainly composed of water, soluble and insoluble solids, organic acids (mostly citric acid) and micronutrients like carotenoids, and vitamins A and C. Many of these components have infrared and Raman active modes, enabling biochemical analysis on both macro- and microscopic scales. Water is present in high concentrations in fruit, so IR analysis is not an optimal method for making these analyses on the unprocessed fruits. Raman spectroscopy, which is relatively insensitive to the influence of water, is a much better technique for this analysis, as it can provide a detailed picture of the molecular environment within intact cells and tissues, nondestructively.

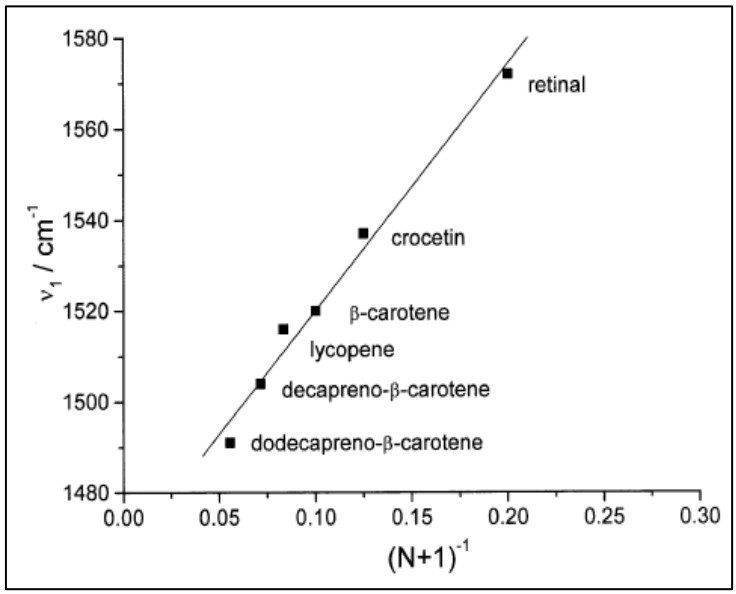

Figure 3. Relationship between the ν_1 band location and number of double bonds in the carotenoid's backbone.

The Raman spectrum of crystalline lycopene, for example, is composed of three main bands, which are located at 1,518, 1,155, and 1,005 cm^{-1} and called the ν_1, ν_2, and ν_3 bands, respectively. Normal coordinate analyses have assigned these three bands to C=C in-phase stretching, C-C stretching, and methyl in-plane rocking modes, respectively.[4] The weak band around 960 cm^{-1} in the solid state is called the ν_4 band and is assigned to the C-H out-of-plane wagging.[4] Other carotenoids have a ν_1 band location that is a function of the number of double bonds in the polyconjugated main backbone of the molecule. This effect, shown in Figure 3, has been used to estimate molecular structure of unfamiliar carotenoid compounds.

The frequency differences observed in Raman spectra of these compounds are subtle, especially in carotenoids that do not have very different number of double bonds in the backbone of the molecule. A TSI ChemLogix™ ProRaman-L was used with 785 nm excitation to endeavor to separate these features. Raman features of lycopene and β-carotene can be easily seen in Figure 3, along with features related to the structure of the skin of the tested tomato. These extra features originate in the waxy cuticle of the tomato skin (C-C at 1050 cm^{-1}, CH_2 twist at 1305 cm^{-1}, CH_3 asymmetrical at 1450 cm^{-1} and C=C phenolic at 1600 cm^{-1}), and are; therefore, not present in the carrot spectrum, shown here to demonstrate the location of the β-carotene features.

These spectra were obtained with about 200 mW of 785 nm excitation energy, and collected using the acquisition parameters present on legend on the graph. Raw produce material was obtained from a local grocery store and used without processing. The resolution in this instrument is about 8 cm^{-1} (nominal).

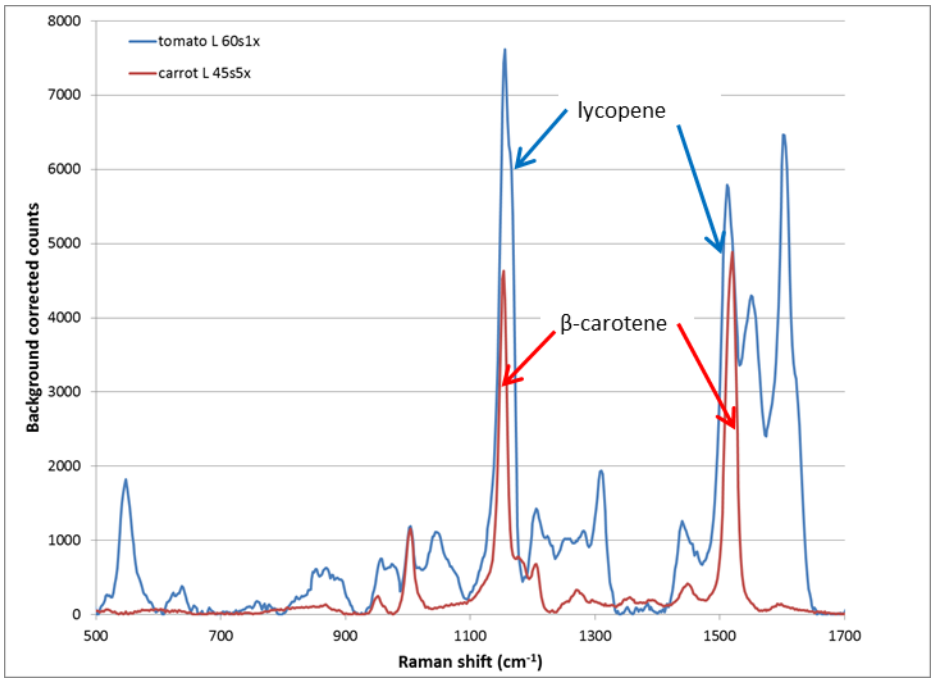

Figure 4. Spectra of tomato and carrot obtained with the ChemLogix ProRaman-L.

These spectra were also acquired with our least expensive portable instrument, the –NP. While the sensitivity of this device is not as high as the –L unit, it has slightly better resolution (due to a shorter spectral range on this particular instrument, 6 cm^{-1}). Figure 5 shows a spectrum acquired on a cherry tomato with a contact lens. The improved resolution enables clearer differentiation between lycopene and β-carotene.

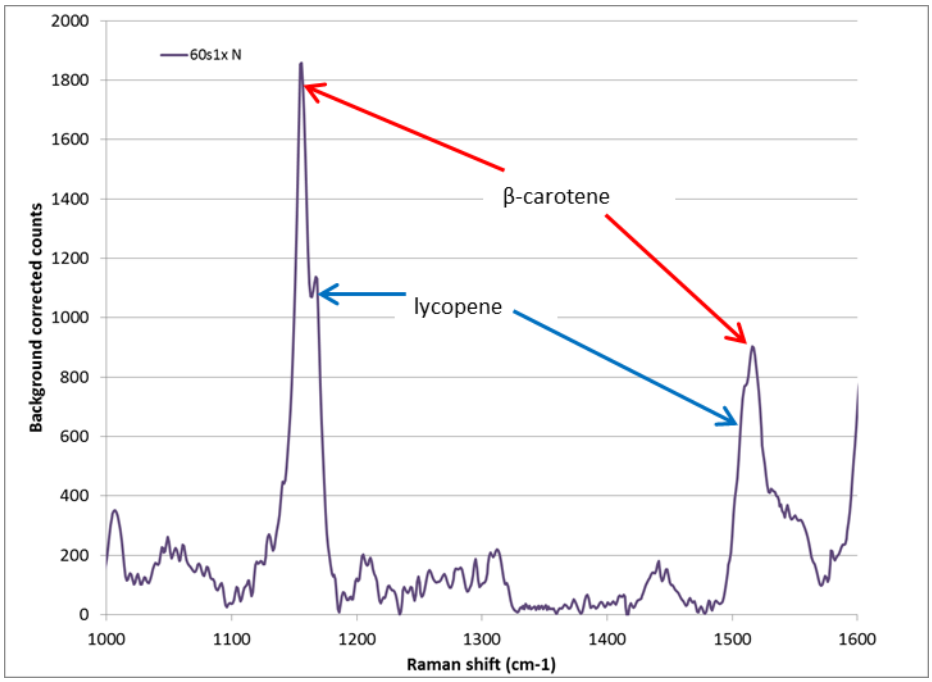

Figure 5. Spectrum of cherry tomato, acquired with a ChemLogix EZRaman-NP.

In conclusion, Raman spectra of raw tomatoes and carrots were collected with two types of TSI ChemLogix™ Raman spectrometer systems. In addition to demonstrating an interesting and important application, it also compares and contrasts two TSI systems, one with higher sensitivity and one with high resolution.

References

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