

Understanding in-situ generated resonance Raman spectra of carotenoids in pollen with the help of high performance thin layer chromatography

^{1,2}F. Schulte, ³J. Mäder, ³L.-W. Kroh, ^{1,2}U. Panne, ^{1,2}J. Kneipp

¹Humboldt Universität zu Berlin, Chemistry Dept., Brook-Taylor-Str. 2,
D-12489 Berlin, Germany

²Federal Institute for Materials Research and Testing, I.42, Richard-Willstätter-Str. 11,
D 12489 Berlin, Germany

³Berlin University of Technology, Institute of Food Technology and Food Chemistry,
Department of Food Analysis, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany

Hyphenated methods combine the benefits of two or more analytical methods. Here, the examination of carotenoids contained in tree pollen was accomplished by combining HPTLC and resonance Raman spectroscopy. Extracts containing carotenoids derived from pollen were separated with High Performance Thin Layer Chromatography (HPTLC) prior to analysis by resonance Raman spectroscopy. The resulting spectra were compared to *in-situ* generated Raman spectra. The information obtained from the separated carotenoids can be found as well in the spectra of the overall carotenoid composition derived from whole pollen grains. As Resonance Raman spectroscopy with an excitation wavelength of 488 nm was used, carotenoids were detected more sensitive with Raman spectroscopy than with HPTLC. Also combination tones and overtones could be observed in the range from 2000 cm⁻¹ to 4000 cm⁻¹. For the separation by HPTLC, a new highly effective multiple development protocol for HPTLC was established using a gradient of methylenchloride, tetrahydrofurane and n-hexane as mobile phase. Raman spectra were measured directly on the HPTLC plates. The carotenoid composition of six tree pollen was analysed (horse chestnut, large-leaved linden, european ash, sallow, mahaleb cherry, tree of heaven), and the concentrations of four prominent carotenoids (beta-carotene, cryptoxanthin, lutein, zeaxanthin) in these pollen species were determined.

The Raman data obtained from the HPTLC-separated carotenoid species provide evidence that the *in situ* Raman spectra really represent an average of the overall carotenoid constitution in pollen. In principle, a full interpretation of average pollen carotenoid signals should be achieved by an RR exploration of the plethora of pollen carotenoids. Comparing the results of the different analytical approaches and taking into account the great instability of the carotenoid molecules, the Raman spectral information obtained *in situ* is clearly of advantage. In particular, the *in situ* spectra already include spectral features that may arise due to association with the biological matrix.

References

F. Schulte et al. "Characterization of pollen carotenoids with in situ and HPTLC-supported resonant Raman spectroscopy", *Analytical Chemistry* **in press**