CHARACTERISATION OF PEPPER AND PEPPER OLEORESINS BY FOURIER-TRANSFORM RAMAN SPECTROSCOPY

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Abstract: NIR-FT-Raman spectroscopy in combination with efficient chemometric algorithms was applied for rapid and non-destructive determination of piperine in ground pepper, green whole pepper berries as well as pepper oleoresins. Most of the well resolved Raman signals detected in the spectra of pepper and the relating oleoresins can be assigned to piperine which is known to be the main pungent principal in these products. According to these specific signals also selective Raman mappings were performed to determine *in situ* the distribution of piperine in the whole green berry and the dried peppercorn. It was found that the pepper alkaloid is predominantly located in the outer perisperm of the green fruit.

The main pungent principle in the green berries of pepper (*Piper nigrum* L.) is piperine. At least five other alkaloids, structurally related to piperine, also occur in smaller amounts. “Black pepper” is obtained from the unripe but mature green berries on sun drying while fully ripe dried fruits devoid of pericarp form the commercial “white pepper”. Generally the piperine content of black or white peppercorns lies within the range of 3 to 8 g/100g, whereas the content of the minor alkaloids piperyline and piperettine have been estimated as 0.2-0.3 and 0.2-1.6 g/100g, respectively [1]. The International Organization for Standardization (ISO) [2], the American Spice Trade Association (ASTA) [3] and the Association of Official Analytical Chemists (AOAC) [4] have published spectroscopic methods for piperine determination, involving UV-Vis absorbance measurements at the piperine absorption maximum near 343 nm. In order to get more specific determination of piperine (excluding the other pepper alkaloids) a number of HPLC methods have been described [5-7]. However all methods presently available for the estimation of piperine in pepper and its products are more or less time-consuming and need the use of organic solvents. Therefore the aim of this study was to develop a rapid and non-destructive Raman spectroscopy method which allows also to obtain 2- and 3-dimensional images presenting the piperine distribution in the plant tissue.

Raman spectra were recorded applying an NIR-FT-Raman spectrometer of Bruker (model RFS 100) equipped with a diode pumped Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The Raman mapping was performed by using an xy stage directly connected with the Raman spectrometer.

As demonstrated in figure 1 the Raman spectra obtained from intact green pepper berries, ground black pepper and black pepper oleoresin show predominantly the significant key bands of piperine. Apart from the intense –C–H aromatic bands between 2800 and 3100 cm^{-1} main Raman signals occur in the fingerprint range between 1100 and 1630 cm^{-1}. The spectra of black pepper and the relating oleoresin present very good resolution of the aromatic and aliphatic –C=C– as well as –O=C–N– stretching vibrations detected between 1580 and 1635 cm^{-1}. The signal to be observed at 1448 cm^{-1} is assigned as CH_2– bending vibration whereas the other bands in the range of 1100 and 1400 cm^{-1} are mainly due to –C–C– stretching and –C–H bending vibrations of the piperine molecule.

Feasibility studies performed to predict the individual piperine content in various ground pepper samples, spice mixtures as well as in relating pepper oleoresins by using Raman spectroscopy demonstrate high correlations between the Raman data and HPLC reference values. Based on such calibration equations it is possible to perform routine quality analysis of pepper and pepper extracts non destructively in less than 1 minute. Furthermore, Raman mappings obtained from fresh green pepper berries as well as peppercorns allow to determine the distribution of piperine *in situ*. In this context it has been shown that the pungent principle is predominantly located in the outer perisperm of the green fruit.

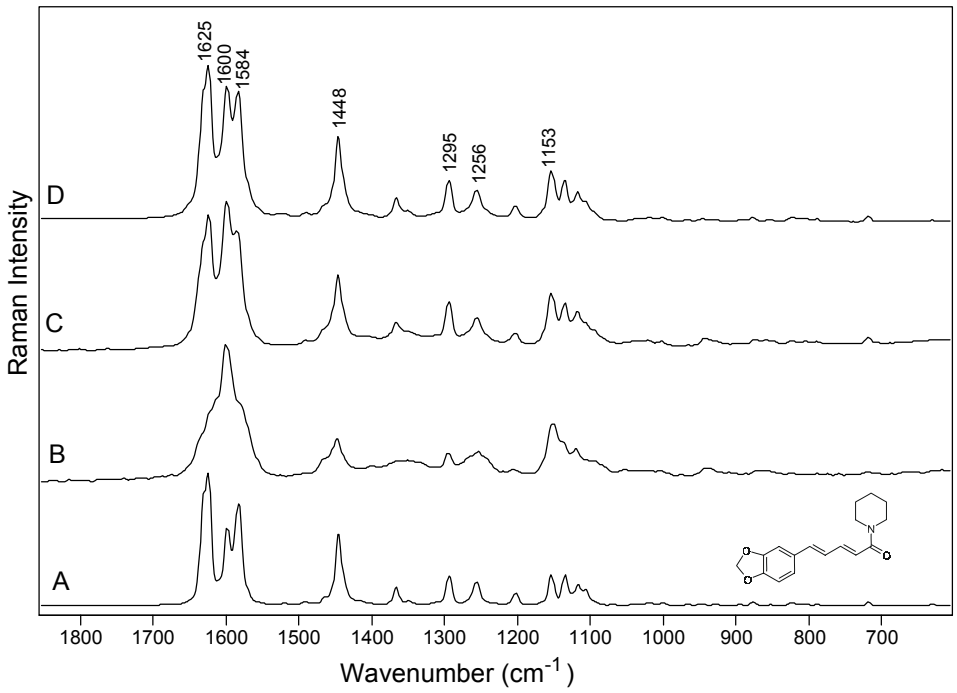

Fig. 1. FT-Raman spectra of pure piperine (A), intact green pepper berry (B), ground black pepper (C) and black pepper oleoresin (D).

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