

Fast determination of minor components in rosemary leaves by near infrared spectroscopy

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Introduction

For some years near infrared (NIR) spectroscopy has gained great success in the determination of main components in food and agricultural applications while the analysis of minor components was still the domain of classical analytical methods. First results about the utilisation of NIR for the analysis of essential oil components in fresh and dried peppermint and sage leaves, as well as some umbelliferae fruits (fennel, caraway, dill, coriander), have recently been reported.^{1,2} Good results have been also achieved with NIR on the quantification of non-volatile compounds in tea plants as, for example, phenolic substances in green tea (*Camellia sinensis*) leaves.³ Generally, fast and reliable analysis of minor components in spice, tea and medicinal plants is very important for industrial quality and in-process control as well as for breeding purposes.

Rosemary (*Rosmarinus officinalis*) is of great interest because it exhibits strong antioxidant properties in comparison to other herbs. The most relevant compounds responsible for this effectiveness are phenolic diterpenes.⁴ According to the present knowledge carnosic acid appears to be the main substance which is oxidised to artefacts with γ - or δ -lactone structure, as for example carnosol, rosmanol and epirosmanol.⁵⁻⁷ Rosemary extracts are mostly used as antioxidative food additives. Furthermore the essential oil of rosemary is frequently used in the flavour industry as, for example, in tooth paste because of its strong odour in conjunction with health promoting properties.

Material and methods

The NIR measurements were performed with a dispersive grating spectrometer manufactured by Foss Instruments, Rodgau BRD, on 106 rosemary samples. The spectral range was from 1100 to 2500 nm with a resolution of 2 nm. The spectra were obtained from reflection mode measurements of the untreated dried rosemary leaves. Clean-up procedures as, for example, pulverisation does not lead to significant better analytical results. The whole spectral range, excluding only the water signals, was used as basis for the calibration procedure. The spectral data were processed using the NIR 2 programme from Infrasoft International, Port Mathilda USA. The accuracy of the NIR analyses is characterised by the standard error of calibration (*SEC*) values and controlled by internal cross-validation.

The analytical results were achieved with a standard HPLC method for carnosic acid.⁴ The essential oil content was determined by hydro-distillation in combination with subsequent gas chromatographic analysis according to a procedure described earlier.⁸

Results and discussion

Best results for the determination of minor components in rosemary leaves were achieved with the application of a modified partial least squares (MPLS) algorithm to weighted scatter-corrected and third-derivative processed spectra (Figure 1 shows a choice of the untreated spectra). In Table 1, the statistical results for the NIR determination of carnosic acid, the essential oil and individual terpenoids are presented. To elucidate the meaning of the achieved standard error of cross-validation (*SECV*) values the quotient of standard errors and mean values is also listed. The correlation schemes for carnosic acid and the essential oil content are plotted in Figures 2 and 3.

In most cases NIR analyses lead to results comparable to the usually applied HPLC/GC methods. In particular, the most interesting compound carnosic acid and also the essential oil content are successfully determined very well. Furthermore, it is suggested, that a *SECV*/mean quotient of ≤ 0.15 is quite acceptable for a reliable determination under these conditions, as the reference values are similarly distributed. Even values ≤ 0.25 lead to semi-quantitative results which are accurate enough to support breeding research studies. Only the amounts of β -pinene, bornyl acetate and camphor could not be determined very well. On the one hand, this could refer to the low concentration of these substances (mean value $< 3\%$ in the oil) or, on the other hand, it might be assigned to spectroscopic interferences with the plant material. To improve the overall determination results NIR studies were also performed with pulverised rosemary leaves but no significantly improved analytical values were observed. This agrees well with the assumption that the minor components, in particular the essential oil

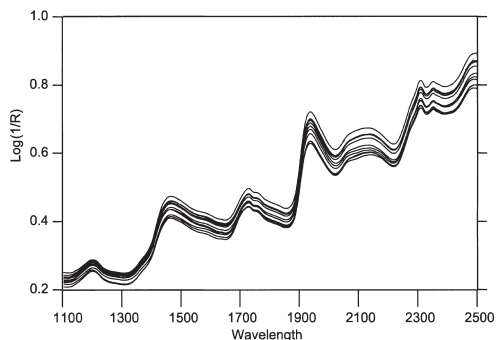


Figure 1: NIR reflectance spectra of untreated dried rosemary leaves

Table 1. Range and mean reference values, NIR calibration and cross-validation statistics for carnosic acid (%), essential oil content (mL 100 g⁻¹) and essential oil components (% in the essential oil).

	Range	Mean	<i>SEC</i>	<i>R</i> ²	<i>SECV</i>	<i>SECV</i> /mean
Carnosic acid	0.1–7.8	3.9	0.32	0.95	0.38	0.10
Oil content	0.2–4.1	1.7	0.17	0.95	0.21	0.12
α -pinene	1.6–37.7	22.6	1.73	0.97	2.45	0.11
1,8-cineole	3.1–58.8	18.7	2.52	0.93	6.70	0.21
Camphor	2.5–42.4	14.4	2.42	0.95	3.98	0.28
β -pinene	0.2–5.8	1.7	0.35	0.93	0.55	0.32
Camphene	3.2–8.0	6.1	0.43	0.90	0.59	0.10
Bornyl acetate	0.1–7.7	3.0	0.60	0.89	0.91	0.30
Borneol	1.6–9.9	4.0	0.72	0.84	0.91	0.23

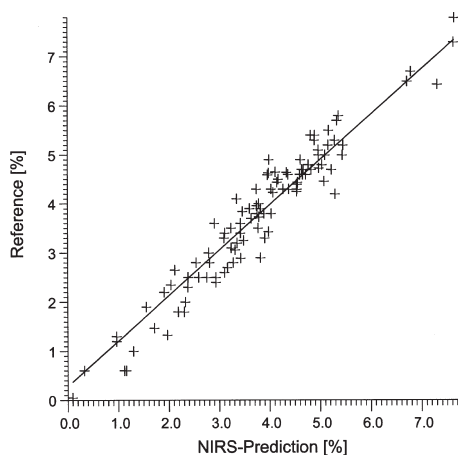


Figure 2. Reference values (HPLC) vs NIR prediction of carnosic acid (%) in dried rosemary leaves.

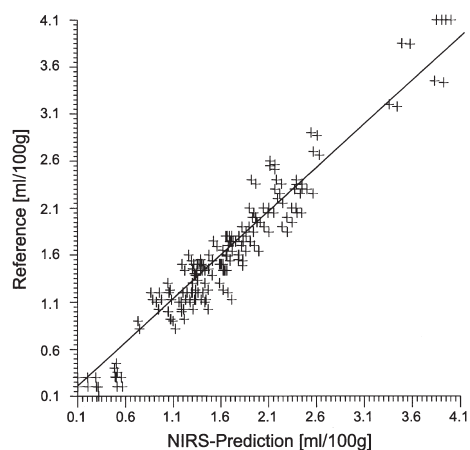


Figure 3. Reference values (solvent extraction/GC) vs NIR prediction of the essential oil content (%) in dried rosemary leaves.

substances, are predominantly located on the surface of the plant leaves, as already found in sage or mint species.

The results obtained for dried rosemary leaves lead to the assumption that even fresh plant material can be determined very well, as already demonstrated for peppermint and sage leaves, which is a very useful tool for breeding and cultivation purposes. Furthermore, another interesting topic will be the analysis of rosemary extracts. Herein, the control of carbon dioxide and solvent extraction processes as well as the determination of antioxidative substances in food additives by NIR could be an important feature in the near future.

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