

ESTIMATION OF MINOR COMPONENTS IN CARAWAY, FENNEL AND CARROTS
BY NIRS - COMPARISON OF RESULTS FROM DISPERSIVE
AND FOURIER-TRANSFORM INSTRUMENTS

H. Schulz, R. Quilitzsch, H.-H. Drews, H. Krüger

Federal Centre for Breeding Research on Cultivated Plants, Institute for Quality Analysis, Neuer Weg 22/23
D-06484 Quedlinburg, Germany

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A b s t r a c t. The purpose of the present study is to compare the prediction accuracy of some secondary metabolites occurring in caraway, fennel and carrot, recorded with two different NIR spectrometers. Based on the recorded spectra and the chemically determined reference values, chemometrical analyses were performed using a partial least squares (PLS) or a modified PLS algorithm. Very good calibration statistics (SECV, R^2) were obtained for the prediction of the essential oil content in caraway both with the dispersive spectrometer system (0.20, 0.93) and with the FT-IR instrument (0.19, 0.92). The results received for the essential oil content in two harvests of fennel (1996+1997) gave nearly the same accuracy: (0.47, 0.92) and (0.58, 0.91). Satisfactory calibration results were received for the NIRS determination of total carotenoids in two harvests of carrots (1997: 3.54, 0.82 / 1998: 3.45, 0.91) by using a FT-IR spectrometer. Applicable calibration results were received for total sugars in carrots (1997: 1.61, 0.67 / 1998: 1.14, 0.73).

K e y w o r d s: near-infrared spectroscopy, non-destructive measurements, caraway, fennel, carrot

INTRODUCTION

The near-infrared (NIR) region, which covers the spectral interval between approximately 14000 and 4000 cm^{-1} (0.7-2.5 μm), contains absorption bands corresponding to overtones and combinations of fundamental C-H, O-H and N-H vibrations. Near-infrared spectroscopy (NIRS) is widely used as a quantitative technique for predicting the chemical composition of various agricultural products. The fast and non-destructive operation is one of the main advantages of this technique. During

the last ten years, the growing interest in near-infrared spectroscopy has risen by improvements in instrumentation and in data analysis as well as by the introduction of optical fibers allowing delivery and transfer of NIR energy and information over long distances [6,10]. Today numerous different near-infrared systems are available on the market, ranging from simple filter systems to scanning systems that are able to scan a complete spectrum in the fraction of a second. After the first applications of the FT-principle to NIR in the late eighties of the 20th century [5] today FT-IR spectrometers are able to measure in the NIR range. Extraction of quantitative information from the rather featureless NIR-spectra has been a notable success of multivariate calibration techniques [4]. But today a significant limitation to the use of multivariate regression in the NIR spectrometry is the difficulty of transferring calibrations from one NIR instrument to another. Typical NIR spectra include not only the spectral variation caused by the chemical or physical properties to be measured, but also time-varying, unintended variation attributed to the particular instrumental system used for acquiring the spectra.

The main aim of this study was to compare the NIR predictions for the contents of minor components in the selected plant material measured with two different NIR instruments.

The NIR spectrometers used were different in the scanning principle as well as in the sampling schemes. The measured plant material was fruit of fennel and caraway. Moreover, spectral analyses are carried out in carrot roots applying the FT-NIR equipment.

fruit of caraway (*Carum carvi* L.) and fennel (*Foeniculum vulgare* MILL.) are widely used in the industry because of their positive properties for human health. In this context, the breeding of medicinal and aromatic plants is a considerable importance [3,7,8].

In carrots (*Daucus carota* L.) the content of carotenoids in the roots has been regarded with increasing interest. During the last years breeding research activities have focused on selecting carrot genotypes with a carotenoid content as high as possible [9].

MATERIALS AND METHODS

Genotypes of caraway and fennel as well as different carrot varieties were grown in the experimental garden of the Federal Centre of Breeding Research on Cultivated Plants in Quedlinburg, Germany. Samples selected for calibration and validation covered a wide range of individual secondary metabolites for analysis. The investigations were carried out on 225 caraway fruit plants (harvest in 1997), 200 fennel fruit (harvest in 1996) and 150 fennel plants (harvest in 1997). In the case of carrot, investigations were carried out on 100 different roots of 5 varieties (harvest in 1997) and on 200 different roots of 10 varieties (harvest in 1998).

Individual reference analyses were performed as follows:

Fully ripe umbelliferae fruit were extracted with acetone and the resulting essential oils were analysed by gas chromatography (GC) with FID as described earlier [2]. Analyses were performed on a Hewlett Packard GC (HP 5890) applying the following conditions:

25 m x 0.25 mm i.d., 0.25 μ m Optima 225 (Machery & Nagel); temperature programme: 120 °C hold for 2 min; rise to 200 °C by 25 °C min⁻¹, hold time of 1 min; carrier gas He; split 1:40.

Reference data for carrot samples were determined from freshly prepared carrot juice. Individual content of saccharose, fructose and glucose was analysed by HPTLC. After solid phase extraction (SFE), the contents of α - and β -carotene were analysed by RP-HPLC according to Chen *et al.* [1] applying the following conditions: 3 ml of the SFE eluate were concentrated and then a volume of 20 μ l was injected on the HPLC column. The detection was carried out at 444 nm and 450 nm.

Dispersive NIRS measurements of umbelliferae fruit (caraway, fennel) were carried out on a near-infrared scanning monochromator NIRSystems 5000 (FOSS-Deutschland GmbH, Rodgau, Germany) in the reflectance mode. A few cm³ of intact fruit were transferred into rectangular cups (51 x 64 mm, 11 mm depth), placed in a transport unit, moving the sample up and down at right-angles to the incident radiation. Each sample was measured twice with 32 scans each time. After transforming to log (1/R), the data were processed with a commercial spectral analysis programme (NIRS2 4.0, Infrasoft Int. Inc., Port Matilda, PA, U.S.A.).

The wavelength region for all the calibrations was 1100-2490 nm to produce a total of 700 points per spectrum. At first, spectral data were treated with a weighted multiple scatter correction (WMSC) to eliminate interferences of scatter, and then transformed with the second- or third-derivative processing. A modified partial least square (MPLS) algorithm was used onto spectra and reference values for predicting the content and composition of the individual essential oil. The optimum number of MPLS factors used for the respective prediction was determined by cross validation. The overall error between modeled and reference values, the standard error of cross validation (SECV), and the multiple coefficient of determination (R^2) were used to describe the accuracy of the calibration models.

Analysis of the same umbelliferae fruit were performed on a FT-IR spectrometer system IFS 55 EQUINOX (Bruker GmbH, Karlsruhe, Germany), equipped with a light source,

beam splitter, detector and Y-shaped fused silica fiber suitable for NIR region. A few cm³ of intact fruit were transferred into a beaker. In all the experiments, a fiber optics probe was placed with a constant distance above the sample material, so that the measuring spot had a diameter of approx. 8 mm. In all the cases the spectrum was averaged from 12 interferometer scans. The wavenumber region used for the analysis was 4000-12000 cm⁻¹. The spectral measurements were carried out at 5 different parts on the flat surface of several fruit poured into the beaker. Based on the received average spectra, the chemometrical analyses were performed using the commercial software programme OPUS 2.2/QUANT 2 (Bruker GmbH).

The calibration programme was set up with the full wavenumber range applying the PLS (partial least square) algorithm. The optimum number of PLS ranks used for the respective prediction was determined by cross validation. The accuracy of the calibration statistics was described by the overall error between modeled and reference values, the root mean square error of cross validation (RMSECV), and the mul-

tiple coefficient of determination (R^2). The RMSECV is comparable with the above mentioned SECV.

NIRS measurements of carrot roots were performed only on the FT-IR spectrometer system. The spectral measurements were carried out on the untreated surface of a carrot at 5 different points of equal distances. Based on the recorded average spectra for each carrot sample, chemometric analyses were performed as described above.

RESULTS AND DISCUSSIONS

The results presented in Tables 1 and 2 as well as Fig. 1(a, b) demonstrate that the NIRS technique is suitable well for the non-destructive and reliable determination of the essential oil content and the composition of essential oil substances in the intact fruit of caraway and fennel. Regarding the most valuable statistical parameters, i.e., R^2 and SECV (or RMSECV), only small differences between the results of Fourier-transform and dispersive measurements were found. More than the optical configuration itself, the sampling schemes seem to

Table 1. Range and NIRS correlation statistics for the essential oil content (ml 100 g⁻¹) and components (ml 100 g⁻¹) in the fruit of caraway received by FT-NIRS and dispersive NIRS (C = number of calibration samples)

Component	Caraway (C = 225 / 1997)					
	FT -NIR spectrometer			Dispersive NIR spectrometer		
	range	RMSECV	R^2	range	SECV	R^2
Oil content	3.07 - 6.87	0.19	0.92	2.63 - 6.89	0.20	0.93
Carvone content	1.29 - 3.78	0.16	0.82	1.29 - 4.03	0.13	0.92
Limonene content	1.34 - 3.31	0.16	0.82	1.34 - 3.31	0.11	0.92

Table 2. Range and NIRS correlation statistics for the essential oil content (ml 100 g⁻¹) in the fruit of fennel (harvests in 1996 and 1997) received by FT-NIRS and dispersive NIRS (C = number of calibration samples)

Harvest/Year	Fennel (C = 200 / 1996; C = 150 / 1997)					
	FT - NIR spectrometer			Dispersive NIR spectrometer		
	range	RMSECV	R^2	range	SECV	R^2
Harvest 1996	2.48 - 8.34	0.58	0.76	3.19 - 8.22	0.47	0.83
Harvest 1997	3.49 - 11.22	0.63	0.84	1.26 - 11.99	0.50	0.92
Harvests 1996 +1997	2.48 - 11.22	0.58	0.91	1.26 - 11.22	0.47	0.92

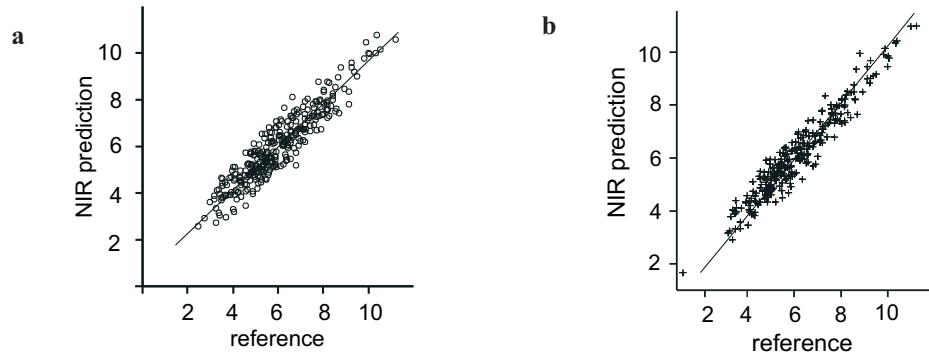


Fig. 1. Reference values (solvent extraction) versus FT-NIRS (a) and dispersive NIRS (b) predictions of the essential oil content ($\text{ml } 100 \text{ g}^{-1}$) in fennel fruit (harvests in 1996 + 1997; $C = 350$).

be of major concern for the reliability of the calibration results. Especially for non-destructive measurements of whole umbelliferae fruit, the moving reflectance cup achieved a better homogeneity of the sample material than using fused silica fiber optics. A comparable homogeneity would be achievable with a great number of fiber optics measurements on the same sample. Nevertheless the results point out that two NIR spectrometers working with different scanning principles and sample handling obtain multivariate calibrations with comparable accuracy for the same samples.

On the other hand, measurements on the surfaces of intact carrots are only possible applying a Y-shaped optical fiber. In connection with the FT-NIR technique acceptable analytical results were obtained for the prediction of α - and β -carotene and consequently of the total carotene content in carrot roots (Table 3, Fig. 2). Table 3 also shows the well known fact that a greater number of samples supplies better calibration results.

Contrary to the above, less analytical reliability was reached for the NIRS estimation of individual sugars. Nevertheless, the prediction of the total sugar content in single carrots (Table 3) can be successfully introduced as a useful screening method in breeding experiments. Finally, it should be mentioned that high water content of fresh carrots does not permit to

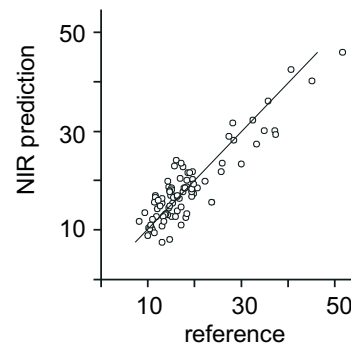


Fig. 2. Reference values (HPLC determination) versus NIRS prediction (FT-NIRS) of the total carotene content ($\text{mg } 100 \text{ g}^{-1}$) in carrots (harvest 1997, $C = 100$).

Table 3. Range and NIRS correlation statistics for the α -, β - and total carotene content ($\text{mg } 100 \text{ g}^{-1}$) as well as the total sugar content (%) in intact carrots (harvests in 1997 and 1998) obtained by FT-NIRS ($C =$ number of calibration samples)

Carotene and sugar contents	Carrots ($C = 100 / 1997$)			Carrots ($C = 200 / 1998$)		
	range	RMSECV	R^2	range	RMSECV	R^2
Total carotene	8.10 - 51.70	3.54	0.82	9.3 - 70.5	3.45	0.91
α - carotene	2.78 - 20.94	1.77	0.78	2.5 - 26.9	1.49	0.91
β - carotene	5.32 - 32.01	2.73	0.70	7.9 - 43.6	2.45	0.87
Total sugar	2.10 - 13.74	1.61	0.67	3.0 - 12.9	1.14	0.73

obtain better NIR based calibration results, related to broad water absorption bands in the near infrared range.

CONCLUSIONS

In breeding caraway and fennel for a high content of essential oil, application of NIRS is the only possibility to obtain information about its constituents, necessary for the selections in a non-destructive and time-saving manner. The compiled calibration equations with determination coefficients of 0.92 gave sound basis for precondition. When choosing the appropriate spectrometer, neither the FT-IR type nor the dispersive type showed prediction accuracy. That is why the time for sample handling shall be decisive. So the use of fiber optics can be of benefit, when compared to cuvette which must be filled, emptied and cleaned again and again.

In carrot breeding for high total carotene content, for example for industrial applications, NIRS proved to be a fast and non-destructive method of supplying information about differences in carotene contents of the cultivars. The compiled calibration equations with determination coefficients of 0.91 seem to be sufficient for this purpose.

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REFERENCES

1. **Chen B.H., Peng H.Y., Chen H.E.:** Changes of carotenoids, color, and vitamin A content during processing of carrot juice. *J. Agric. Food Chem.*, 43, 1912-1918, 1995.
2. DGF German Standard Methods for the Analysis of Fats and Other Lipids (in German). German Society for Fat Science (DGF) Münster, Wissenschaftliche Verlagsgesellschaft MbH, Stuttgart, 1991.
3. **Franz Ch.:** Züchtung und Anbau von Arzneipflanzen. In: *Pharmazeutische Biologie II, Biogene Arzneistoffe* (Ed. H. Rimpler), Georg Thieme Verlag, Stuttgart, New York, 16-33, 1990.
4. **Martens H., Naes T.:** Multivariate Calibration. John Wiley & Sons, Chichester and New York, 1989.
5. **McClure W.F., Davis A.M.C.:** Fast Fourier transforms in the analysis of near-infrared spectra. In: *Analytical Applications of Spectroscopy*. (Eds C.S. Creaser, A.M.C. Davies). Royal Society of Chemistry, London, 414-436, 1988.
6. **Murray I., Cowe I.A. (Eds):** Making Light Work: Advances in Near Infrared Spectroscopy. VCH Verlag, Weinheim, New York, Basel, Cambridge, 1992.
7. **Pank F., Krüger H.:** Development, growth type, and quality of crossbreeding progenies of sweet and bitter fennel (in German). Annual Report 1998, Federal Centre for Breeding Research on Cultivated Plants, Quedlinburg, Germany, 197-199, 1998.
8. **Pank F., Krüger H.:** Ability of biennial caraway cultivars as female parent in crossing experiments for the development of populations of annual caraway with improved variability of quality traits (in German). Annual Report 1998, Federal Centre for Breeding Research on Cultivated Plants, Quedlinburg, Germany, 199-200, 1998.
9. **Simon P.W., Wolff X.Y., Peterson C.E., Kammerlohr D.S., Rubatzky V.E., Strandberg J.O., Bassett M.J., White J.M.:** High carotene mass population. *HortScience*, 24(1),174-175, 1989.
10. The Proceedings of NIR-97. *J. Near Infrared Spectrosc.* NIR Publications, 6 Charlton Mill, Charlton, Chichester, West Sussex, UK, 6, 1-4, 1998.