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Fast determination of carrot quality by spectroscopy methods in the UV-VIS,NIR and IR range.*

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Summary

Some optical spectroscopic methods are able to determine the main quality parameters of fresh carrots in a fast and nondestructive manner. Reflection measurements in the visible range and the use of colorimetric calculations supply characteristic colour coordinates and evaluation of the colour homogeneity of a specific variety. Near infrared spectroscopy (NIRS) in combination with multivariate regression, e.g. the method of partial least squares (PLS), provide a fast and nondestructive method to predict the contents of α-carotene, β-carotene and dry matter content. Measurements of carrot juice samples in the mid-infrared range (MIR) applying a diamond-ATR-equipment and PLS-algorithm for chemometric interpretation supply reliable predictions of sugar contents. The application of Raman spectroscopy on carrot slices and the method of Raman mapping reveals the relative carotenoid distribution in cross sections of various carrot roots.

Introduction

Carrot cultivars represent an important vegetable for fresh consumption, production of juices and wet preserves as well as industrial isolation of natural carotenoids. In this context breeding activities are necessary to adapt the quality parameters (colour homogeneity, carotenoids, sugars, dry matter contents, aroma substances, bitter compounds) to the individual demands. In order to select the appropriate single plants, spectroscopic methods can be a very helpful tool for nondestructive analysis of the fresh carrot tissue. Generally, optical spectroscopy occupies with systematical research of interactions between electromagnetical radiation and atoms, molecules, liquids or solids. For this the absorption or the scattering of radiation by a sample is measured. In the range of ultraviolet and visible light (UV-VIS) absorptions will be caused by electronic transitions. In the mid infrared (MIR) range the absorption bands can be interpreted by various vibration modes of analysed molecules. The absorptions in the near infrared range (NIR) are caused by overtones and combination bands of fundamental vibrations registered in the mid infrared range. Raman spectroscopy is a complementary technique to mid infrared spectroscopy. Illumination of samples with light of a certain wavelength supplies inelastic scattering by the sample producing characteristic Raman signals.

Reflectance measurements at surfaces or tissue layers of fresh fruits are usually performed in the wavelenght range from 400 to 15 000 nm, as Fig. 1 shows for a fresh carrot root. This whole wavelenght range is divided in the visible light range from 400 nm to 800 nm, the NIR range from 0.8 µm to 2.5 µm (in wave numbers from 12000 cm^{-1} to 4000 cm^{-1}, respectively) and the MIR range from 2.5 µm to 15.0 µm (in wave numbers from 4000 cm^{-1} to 667 cm^{-1}, respectively). In most cases the goal of reflectance measurements in the visible range (VIS) is the determination of surface colour (VÖLZ, 1990; SHAW, 1991). The nondestructive analysis by near infrared spectroscopy (NIRS) has already history for more than thirty years. Numerous applications of NIRS exist in agricultural, horticultural and food sectors (SIESLER et al., 2002; ROBERTS et al., 2004; QUILITZSCH and HOBERG, 2003). For MIR spectroscopy, development of the attenuated total reflection (ATR) – technique, which was introduced in the 1980s, offers several advantages. It is mainly used for measurements of liquid samples but a significant breakthrough for general-purpose ATR analysis of solid samples occurred in the late 1990s with the introduction of a new diamond internal reflection element. Recent IR instrumentation developments lead to a combination of this diamond ATR accessory with a complete FT-IR spectrometer (COATES and SANDERS, 2000). Generally for the prediction of quantitative results it is necessary to supply reference data and multivariate statistics for calibration. The first step is to perform a calibration experiment which is crucial in quantitative NIR and MIR spectroscopy. This involves collecting a set of reference or calibration samples which should contain all chemical and (or) physical variations to be expected in the unknown samples. The purpose of a calibration experiment is to establish a mathematical relationship between the NIR or MIR spectrum and the physical

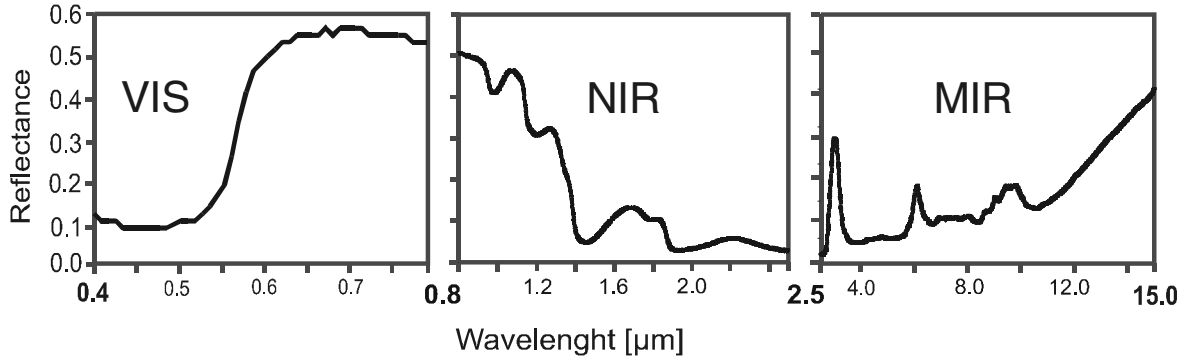

Fig. 1: Reflectance spectra of carrot surface, measured with different spectrometers

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parameters or chemical components under investigation previously determined by an independent technique. The method assumes that systematic variations observed in the spectra are related to different concentrations of the individual analyte. However, the correlation between the analyte concentration and change in the infrared signal must not be linear. Once the mathematical model is established, the analyses of the unknown samples can be performed on the basis of their NIR or MIR spectra with reference to the chemical or physical properties of interest. More details of chemometric calibration algorithms can be found e.g. in SIESLER at al. (2002) or in QUILITZSCH and HOBERG (2003).

Raman spectroscopy principally allows the non-destructive identification of various components in fresh plant material if characteristic key bands of the individual analyte molecules can be found in the spectrum. Especially NIR-FT-Raman spectroscopy has been described as a valuable method for *in vivo* investigations because fluorescence and thermal decomposition of the plant tissue can be reduced to a minimum (BARANSKA et al., 2004). In combination with Raman spectroscopy, mapping techniques are a powerful tool to study the chemical composition of plant samples not only in a single point but also within a larger area. The two-dimensional Raman maps provide detailed information regarding the distribution of specific compounds occurring in the surface layer of the plant tissue of the size ranging from about 0.01 mm^2 to 35 cm^2.

The aim of this paper is to show the special advantages of mentioned spectroscopic methods for nondestructive determination of various quality parameters on carrots.

Materials and methods

Samples

The carrots were grown in the experimental garden of the BAZ. From 1997 to 2004 the experiments were carried out on the varieties "Beta III", "Cyrano", "Lange Rote Stumpfe", "Sempra", "Gonsenheimer Treib", "Vitaminaja", "Icon", "Karotan", "Marktgärtner", "HCM" and three backcross populations. Spectral measurements were carried out in the visible light range on 120 carrot samples (6 varieties), in the NIR range on 260 carrot samples (13 varieties), in the MIR range on 360 carrot samples (4 varieties) and the Raman measurements were caried out on carrot slices of the variety "HCM".

Determination of homogeneity of carrot varieties by colorimetry

The homogeneity in all individual root colours of a carrot variety is an external quality parameter. The obvious quantification of surface colour of any object is possible by measurement of diffuse reflection spectrum (400 nm to 700 nm) of it and the afterwards calculation of three values by a algorithm. The tristimulus values X, Y, Z of object surface will be calculated from spectral diffuse reflection $\phi(\lambda)$ of object surface by use of C.I.E. distribution functions x (λ), y (λ), z (λ). The chromaticity co-ordinates in the C.I.E. colorimetric system $x = X/(X+Y+Z)$ and $y = Y/(X+Y+Z)$ will be represented by the C.I.E. standard table. The C.I.E. standard system allows not the direct determination of colour differences of identical sensations. This imperfection is removed by the CIELAB system by means of transformations between the tristimulus values X, Y, Z and the colour coordinates L*, a* and b*, which are derived from the Munsell system (VÖLZ, 1990). Nowadays the CIELAB system is the most used system of colour determination.

The spectral measurements were carried out on three points of every root of 20 carrots per variety by a spectrometer SpectraPro-500 (Acton Res. Corp., Acton, Massachusetts, USA), equipped with a fiber optics probe. The calculation of colour coordinates followed with a special software part. For further calculations were used the mean colour coordinates of every carrot. The distinction between e.g. carrot 1 (L_1*, a_1*, b_1*) and carrot 2 (L_2*, a_2*, b_2*) is determined by the colour difference $\Delta E_{1,2} = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$.

Near infrared spectroscopic determination of carotene and dry matter contents of carrots

In the quantitative NIR spectroscopy a wide range of the reference value, to be calibrated, is a very important condition for a working calibration and after that the working validation. For the NIR measurements, carried out on carrots, the fibre optics probe was found to be the best suitable equipment. The used spectrometer was a FT-IR spectrometer EQUINOX 55 (Bruker Optics GmbH, Ettlingen, Germany). The components, e.g. source, detector, beam splitter were switched on for the NIR range. In all experiments the fibre optics probe was placed in a constant distance above the sample, so that the measuring spot had a diameter of approx. 8 mm. In all cases the spectrum was averaged from 12 interferometer scans. The wavenumber region used for analysis was 4000 to 12000 cm^{-1}. On one root three spectral measurements were carried out. The average spectral data and the subsequently analysed reference values of a root, such as α-carotene, β-carotene and dry matter content were put into the calibration algorithm. For calibrations concerning carotenoid and dry matter content of carrot root the reference methods are high performance liquid chromatography (HPLC) analysis and gravimetric analysis.

Determination of sugar content in carrots by FT-IR diamond ATR spectroscopy

Over a long time IR transmission measurements of liquid samples were dominant. Nowadays the method of attenuated total reflection (ATR) is a wide spread reflection method (internal reflection). The light propagates in a medium with relative high refractive index (the ATR crystal) and falls on an interface to a medium with smaller refractive index (the sample). Total radiation is observed on this interface, if the angle of incidence is greater than the boundary angle of total reflection. At total reflection the radiation a little (some µm) penetrates into the adjacent medium. The intensity of the reflected beam is affected by the absorption intensity of the sample. The resulting reflection spectrum is considerable similar to the transmission spectrum. The wave numbers of absorptions characteristic for substances are the same by measurements in transmission. With this technique it is possible to measure spectra of substances, which are not translucent for IR radiation or are difficult to prepare. Generally, the spectra in the mid infrared provide more information of the analyte than near infrared spectra and usually a higher coefficient of determination in chemometric calculations is obtained. Measurements on carrot material in the mid-IR range were carried out with the "Travel-IR", a portable FT-IR spectrometer with a fixed mounted diamond-ATR accessory (SensIR Technologies, Danbury, Connecticut, USA). By means the diamond ATR equipment solid samples can be pressed with high pressure on the diamond crystal, so that infrared radiation can interact with the sample in the interface of the crystal. The diameter of the diamond crystal is 1.6 mm. So, only a few microliters are necessary to perform a reflection measurement. It is possible to measure liquid and solid plant samples with minimal preparation expense by this equipment. Juice samples of carrots were put on the diamond only with a pipette, tissue stripes of carrots were pressed on. The spectra were scanned in the wavenumber range between 750 cm^{-1} and 4000 cm^{-1}.The reference values of sugar components, necessary for a chemometrical calibration, were determined with high performance thin layer chromatography (HPTLC) analysis.

Determination of carotenoid distribution in carrot tissue by Raman spectroscopy

Raman spectra were recorded using a Bruker NIR-FT-Raman Spectrometer (model RFS 100) equipped with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The instrument was equipped with an xy stage, a mirror objective and a prism slide for redirection of the laser beam. Compared with the standard vertical sampling arrangement, the samples were mounted horizontally. Carrot root discs were placed between two glass slides to avoid their movement and deformation during the measurement.

Single measurements from carrot root were obtained with 128 scans and an unfocused laser beam of 200 mW. The spectra have been performed with a spectral resolution of 4 cm^{-1} in the range from 100 to 4000 cm^{-1}. 2-dimensional Raman maps of flat sample of the transversely cut carrot root were obtained point by point; x and y directions of the accessory were controlled by the spectrometer software. A quarter of carrot root was mapped at an area of 10.5 mm x 12.0 mm with a spatial resolution of 250 µm. The sample was irradiated with a focused laser beam of 200 mW of a diameter about 0.1 mm; eight scans were collected at each measured point. Spectra or 2-dimensional surface areas of the mapped samples were processed by the Bruker Opus/map software package.

Results and discussion

Quantitative comparison of plant populations concerning colour homogeneity is possible by determining the variance in colour differences between individual carrots of a variety. After the use of the colorimetric algorithm 20 number triples L*, a*, b* per variety are obtained. From these one gets 190 colour differences per variety. For a good colour homogeneity of a set of carrots the variance of colour differences must be low.

The reciprocal value of variance of colour differences is a measure for the colour homogeniety of a variety (Fig. 2). For the varieties "Sempra", "Vitaminaja" and "Cyrano" the colour homogeneity is approximative at the same level. This method of measurement of colour coordinates and the calculation of the reciprocal values of the variances of colour differences can be used as a objective and sensible method for homogeneity determination of a sample set.

The main objective of the NIR measurements is the formation of calibration models predicting the α-carotene, β-carotene and dry matter contents in fresh carrot roots. The OPUS/QUANT 2.0 software (Bruker GmbH, Ettlingen, Germany) was applied to the NIR spectra and reference data. A partial least squares (PLS) algorithm calculates the calibration model and a cross validation algorithm tests the model. The accuracy of the calibration statistics describes by the overall error between modeled and reference values, the **r**oot **m**ean **s**quare **e**rror of **c**ross **v**alidation (RMSECV) and the multiple coefficient of determination (R^2). The results of validations can be represented as

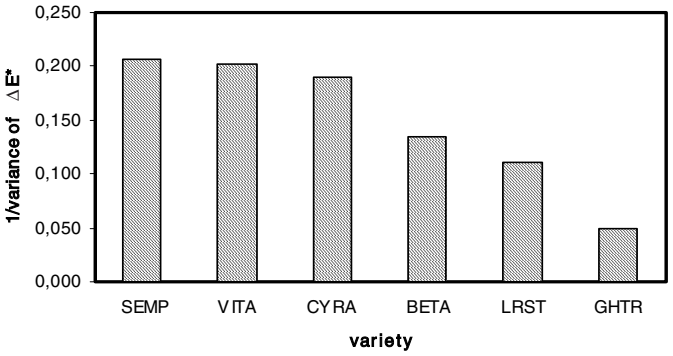

Fig. 2: Carrot varieties, arranged to here colour homogeneity (SEMP-Sempra, VITA-Vitaminaja, CYRA-Cyrano, BETA-Beta III, LRST-Lange Rote Stumpfe, GHTR-Gonsenheimer Treib)

validation plots or as table with the values of R^2, RMSECV and the range of predicted parameters. Tab. 1 shows the results of an experiment and two repetitions with 260 carrot roots in each case. In validation plots the predicted parameter values are shown versus the reference values. For example, Fig. 3 shows the cross validation plot for the spectroscopic prediction of dry matter content of carrot roots. For a hypothetical prediction with a coefficient of determination of value $R^2 = 1$, all values would be found on an ideal straight line with a slope of 1. The Tab. 1 and the Fig. 3 indicate, that near infrared spectroscopic determination of the main carotenoid content and the

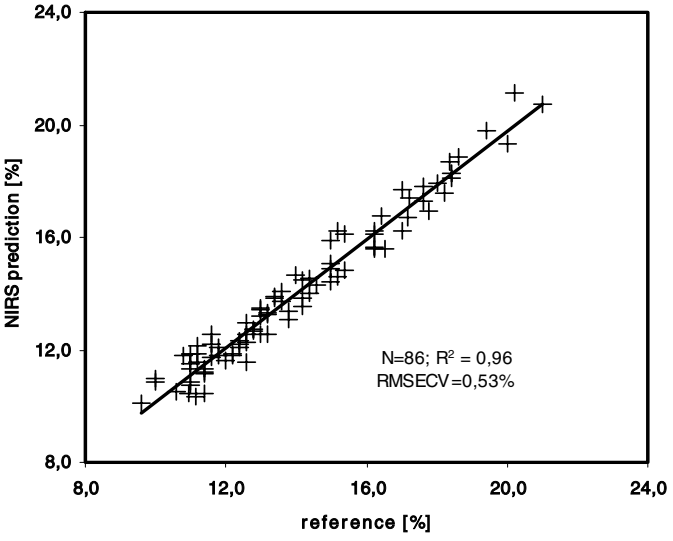

Fig. 3: Cross validation plot for dry matter content in carrot roots (N - number of spectra after outlier correction)

Tab. 1: Cross validation results for the spectroscopic prediction of carotenoid contents in carrot roots

	α-carotene			β-carotene			total carotene		
trial	*range [mg/l]*	*R^2*	*RMSECV [mg/l]*	*range [mg/l]*	*R^2*	*RMSECV [mg/l]*	*range [mg/l]*	*R^2*	*RMSECV [mg/l]*
t1	3.23 - 26.86	0.92	1.17	7.88 - 43.60	0.85	2.16	11.777 - 70.46	0.86	3.57
t2	2.51 - 24.98	0.92	1.11	6.74 - 47.74	0.88	2.34	9.25 - 72.72	0.92	3.35
t3	2.56 - 22.26	0.88	1.29	7.16 - 31.80	0.70	2.15	10.77 - 65.83	0.90	3.24

dry matter content of fresh carrot roots is possible reaching high determination coefficients. On the other hand the NIRS prediction of sugar contents in carrot roots can be performed only with lower prediction quality.

The main objective of the MIR investigations is the formation of calibration models predicting fructose, glucose, sucrose as well as total sugar contents of fresh carrot roots. The OPUS/QUANT 2.0 software (Bruker Optik GmbH, Ettlingen, Germany) was applied to the MIR spectra and reference data. Like in the case of NIR spectra a partial least squares (PLS) algorithm calculates the calibration model and a cross validation algorithm tests the model. For models which used the spectral measurements on carrot tissue the prediction quality is not so high as for those, which used spectral measurements on carrot juice. The results obtained for juice are summarized in Tab. 2. Cross validation data were calculated for a spectral range between 850 cm^{-1} and 2000 cm^{-1}. Apart from sucrose content the individual and total sugar contents are successfully predicted resulting in high determination coefficients.

Tab. 2: Cross validation results for the prediction of sugar contents in carrot roots by use of MIR spectra of juice samples

component	range [g/100g]	R^2	RMSECV [g/100g]
fructose	0.00 - 7.72	0.94	0.39
glucose	0.00 - 6.90	0.94	0.38
sucrose	0.07 - 2.60	0.62	0.30
total sugar	0.74 - 16.17	0.91	0.95

Carotenoids occur in carrot as minor components in amounts of several mg kg^{-1}, but their sensitive detection can be achieved by Raman spectroscopy (WITHNALL et al., 2003). In the Raman spectrum taken directly from carrot root, presented in Fig. 4, three carotenoid signals can be observed. Bands at 1520 cm^{-1} and 1156 cm^{-1} can be assigned to in-phase -C=C- (ν_1) and -C-C- (ν_2) stretching vibrations of the polyene chain of the carotenoids, respectively. Additionally, in-plane rocking modes of CH_3 groups attached to the polyene chain and coupled with -C-C- bonds are seen as a peak of medium intensity at 1007 cm^{-1}.

The spectral position of -C=C- stretching vibrations provide information about the structure of investigated carotenoids, i.e. about the length as well as the terminal substituents of their polyene chain. We have already reported before that the wavenumber localisation of ν_1 mode is different for the main carotenoids occurring in carrots (SCHULZ et al., 2005). For the pure, isolated compounds, this band has been observed at 1515 cm^{-1} for β-carotene, at 1521 cm^{-1} for α-carotene and at 1522 cm^{-1} for lutein. In spectra taken from carrot roots the position of this band is shifted to higher wavenumbers, and for β-carotene it is observed at 1520 cm^{-1}. So the Raman spectrum demonstrated in Fig. 4 reveals principally the presence of β-carotene in the investigated carrot.

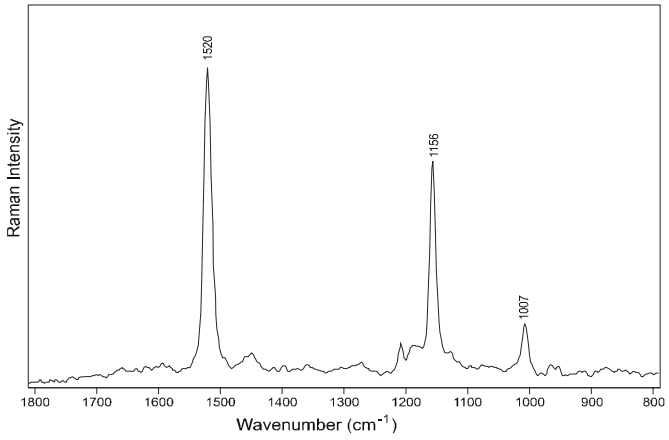

Fig. 4: Raman spectrum of carrot root slice

The occurrence of strong carotenoid signals in the Raman spectrum of carrot gives a good precondition to apply mapping technique to investigate the distribution of these compounds (BARANSKI et al., 2005). In the investigated carrot, carotenoids are not uniformly distributed over the whole root section. The highest accumulation can be found in the phloem and xylem parenchyma whereas periderm/ pericyclic parenchyma and the tissue close to the secondary cambium exhibit a reduction of these compounds.

In conclusion, NIR-FT-Raman spectroscopy can be used to analyse carotenoids *in situ* in intact plant material in a single point as well as in some area. Raman spectra provide reliable information with regard to the structure of the analysed carotenoids whereas Raman mapping technique gives deeper knowledge of the carotenoid distribution in various intact plant tissues.

Conclusions

Spectroscopic measurements have the advantage of fast, simultaneous and partly nondestructive determination of several quality parameters of carrot roots. However only one spectroscopy method may not supply all parameters with high accuracy or determination coefficients. But the combination of different spectroscopic methods in UV-VIS, NIR and IR wavelength ranges supplies a better precondition to obtain results with high accuracy or determination coefficients.

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