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Determination of Lycopene and β-Carotene Content in Tomato Fruits and Related Products: Comparison of FT-Raman, ATR-IR, and NIR Spectroscopy

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Tomatoes and various products derived from thermally processed tomatoes are major sources of lycopene, but apart from this micronutrient, other carotenoids such as β-carotene also are present in the fruit. They occur in tomato fruits and various tomato products in amounts of 2.62−629.00 (lycopene) and 0.23−2.83 mg/100 g (β-carotene). Standard methods for determining the carotenoid content require the extraction of the analyte as well as other cleanup steps. In this work, FT-Raman, ATR-IR, and NIR spectroscopy are applied in order to establish new, fast, and nondestructive calibration methods for quantification of lycopene and β-carotene content in tomato fruits and related products. The best prediction quality was achieved using a model based on IR spectroscopy (R^2 = 0.98 and 0.97, SECV = 33.20 and 0.16 for lycopene and β-carotene, respectively). In spite of the fact that Raman spectra of tomato products show characteristic key bands of the investigated carotenoids, this method gives slightly lower reliability (R^2 = 0.91 and 0.89, SECV = 74.34 and 0.34 for lycopene and β-carotene, respectively). NIR spectroscopy, which has been used for quantification purposes in the agricultural sector for several decades, in this study shows the worse prediction quality (R^2 = 0.85 and 0.80, SECV = 91.19 and 0.41 for lycopene and β-carotene, respectively).

Tomatoes and tomato products are an integral part of the human diet worldwide. It is assumed that ∼85% of the dietary lycopene, a polyenic chromophore with 11 double bonds, is provided by tomatoes and related products such as tomato juice, ketchup, or tomato paste. Although this acyclic carotene is not considered as an essential nutrient, it possesses high antioxidant activity in quenching singlet oxygen as well as in reacting with $ABTS^+$ and the phenoxyl radicals.[1−3] Besides this micronutrient, β-carotene is also present in tomato fruits. Animals and human beings are incapable of carotenoid biosynthesis, but they can modify some of them when absorbed from plant food, as for example β-carotene, which can be converted to retinol (vitamin A).

Depending on the individual tomato cultivar, mean levels of lycopene and β-carotene were measured in the range between 4.5 and 6.3, and 0.8 and 1.6 mg/100 g of fresh matter, respectively.[4] The lycopene content varies significantly with the ripening stage and the individual tomato variety and is mainly responsible for the red color of the fruit and its derived products.[5] It was found that cherry tomatoes (cv. Naomi F1) harvested at full ripeness exhibited the highest level of carotenoids and antioxidant activity in the water-insoluble fraction.[6]

During tomato processing, an increase of carotenoid content on a wet weight basis due to the loss of water was observed.[7] On a dry weight basis, the content of lycopene increased or decreased depending on the origin of the tomatoes, whereas the β-carotene content decreased or maintained stable. Additionally, it was observed that β-carotene isomerized during thermal processing, in contrast to lycopene. However, generally, processed tomatoes still contain high amounts of carotenoids and they are therefore recommended for healthy human nutrition.

Colorimetric estimation using chromaticity values has been proposed as a rapid method for analysis of the total carotenoid content (mainly lycopene) in tomatoes and related products.[8] Today, the content of individual carotenoids occurring in tomatoes is predominantly analyzed by reversed-phase high-performance liquid chromatography (HPLC); usually a sharp resolution of cis and trans isomers of lycopene can be obtained within a separation time of ∼25 min.[9]

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Table 1. Lycopene and β-Carotene Content of Tomatoes and Various Tomato Products Determined by HPLC

tomato products (no. of samples)	concn (mg/100 g of fresh matter)			
	lycopene		β-carotene	
	range	mean ± SD	range	mean ± SD
tomatoes (6)	2.62−60.40	12.98 ± 12.01	0.23−0.78	0.44 ± 0.29
ketchup (5)	18.80−100.87	50.74 ± 33.89	0.46−1.11	0.76 ± 0.32
tomato puree (9)	53.36−128.60	97.80 ± 29.13	0.40−2.80	0.89 ± 0.78
tomato mash (8)	372.00−629.00	538.63 ± 85.04	1.63−2.83	2.61 ± 0.20
all samples	2.62−629.00	206.68 ± 232.94	0.23−2.83	1.18 ± 0.93

In this paper, we demonstrate the application of Fourier transform-Raman (FT-Raman), attenuated total reflection infrared (ATR-IR), and near infrared (NIR) spectroscopy for fast and direct measurements of lycopene and β-carotene in tomato products. Nondestructive determination of carotenoids in tomato products has been already reported by using NIR spectroscopy and multivariate calibration.[10] However, in this work, a broad range of tomato samples containing also very low amounts of carotenoids is investigated with the assistance of three vibrational spectroscopy techniques. Results obtained by using FT-Raman, ATR-IR, and NIR spectroscopy are compared, and the best calibration models are presented.

EXPERIMENTAL SECTION

Plant Material. Fresh tomatoes and various tomato products (28) were obtained from local supermarkets (see Table 1). Each sample was analyzed first by spectroscopic measurements and subsequently by HPLC. Reference analysis and spectra acquisition were performed just after the packages' opening. Pure carotenoid standards were purchased from Phytoplan (Heidelberg, Germany) and Sigma-Aldrich (Taufkirchen, Germany), respectively.

Fourier Transform-Raman Spectroscopy Measurements. FT-Raman spectra were recorded using a Bruker 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 a horizontal *xy* stage, a mirror objective, and a prism slide for redirection of the laser beam.

Spectral data of mashed tomato fruits and tomato products (sample amount, ∼1 mg) were accumulated from 512 scans with a spectral resolution of 4 cm^{-1} in the range of 100−4000 cm^{-1} with a laser power of 150 mW supplied by an unfocused laser beam. For each sample, three spectra were collected and subsequently averaged.

Attenuated Total Reflection Infrared Spectroscopy. The mid-infrared spectra were recorded in the range between 650 and 4000 cm^{-1} on an Equinox spectrometer (Bruker) in a nine-reflection configuration using a diamond−ZnSe ATR crystal. About 2−3 mg of tomato samples was placed on the surface of the ATR crystal (diameter, 0.5 mm^2). Three spectra for each sample were accumulated from 30 scans with a spectral resolution of 4 cm^{-1}. For further analyses, averaged spectra were used.

Near Infrared Spectroscopy. Measurement of tomato samples were performed using a FT-IR spectrometer system IFS 55 Equinox (Bruker) equipped with a light source, beam splitter, detector, and y-shaped fused-silica fiber suitable for the NIR region. Approximately 1−2 g of mashed tomatoes was transferred into an open 30-mm-diameter spinning sample cup. Such a rotating system reduces the inhomogeneity of the sample to a minimum. The fiber-optic probe was placed with a constant distance above the sample material, so the measuring spot had a diameter of ∼4 mm. In all cases, three spectra were collected, each one using 12 scans in the 4000−15000-cm^{-1} range, with 4 cm^{-1} of spectral resolution.

High-Performance Liquid Chromatography Measurements. Carotenoids were extracted and quantified according to a slightly modified procedure described earlier.[11] Approximately 2 g of the individual tomato product, exactly weighed, was dissolved in 100 mL of distilled water, and the solution obtained was applied to a 3-mL Chromabond C18 SPE cartridge. In order to remove interfering substances such as sugars and organic acids, the charged SPE column was washed with 2 × 1 mL of water and 0.5 mL of methanol. The carotenoid fraction was eluted with 2 × 1 mL of a methanol/dichloromethane mixture (45:55 v/v). Then, the eluate was evaporated to dryness, the residue was redissolved in a mixture of acetonitrile/dichloromethane (50:50 v/v), and 3 μL of the solution was injected in a HP 1100 series HPLC system (Agilent) hyphenated with a UV−visible detector. A 3-μm RP-Aqueous C30 Phenomenex Develosil column (150 × 3.0 mm i.d.) was used for separation. Compounds were identified by comparison of retention times and coinjection spiking and by comparing their UV−visible spectra with authentic standards. Quantification was performed by integrating the peak areas of the HPLC results using the Agilent HPLC software.

Chemometrics. Development of appropriate chemometric methods for calibration of lycopene and β-carotene was carried out with the commercial statistic program GRAMS (Galactic Ind., Salem, MA). Several pretreatments, evaluated to transform the spectral data, were tested, e.g., multiplicative scatter correction (MSC), standard normal variate, vector normalization (VN), and baseline correction (BC). The data were subsequently mean centered. Selected wavenumber ranges, as well as the full recorded spectral range, were evaluated for development of partial least-squares (PLS) calibration models for the individual carotenoids, i.e., lycopene and β-carotene. A PLS algorithm was used with an optimum number of PLS factors, and outlier samples were eliminated from the data set. The calibration accuracy was described by the multiple coefficients of determination (R^2) and

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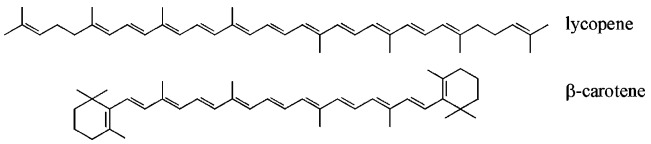

Figure 1. Molecular structure of lycopene and β-carotene.

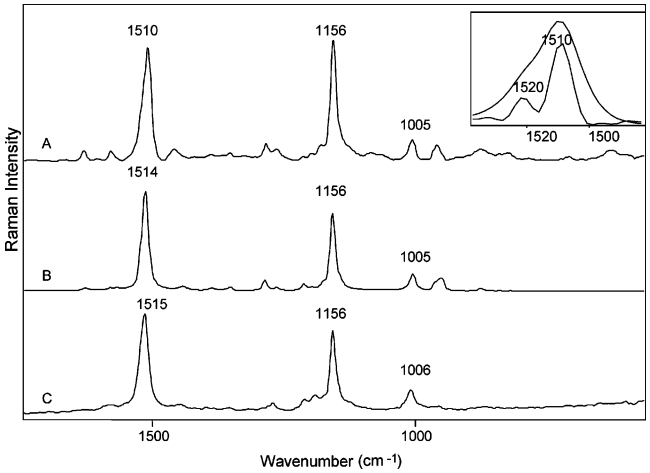

Figure 2. FT-Raman spectra of tomato puree (A), lycopene (B), and β-carotene (C). The inset presents a deconvoluted band of tomato puree.

the overall error between modeled and reference HPLC values (standard error of cross-validation, SECV).

RESULTS AND DISCUSSION

Vibrational Spectra of Tomato Fruits and Related Products. Three vibrational spectroscopic methods, FT-Raman, ATR-IR, and NIR, have been used for in situ analysis of naturally occurring carotenoids in tomato fruits and tomato products. Principal pigments of red tomato fruits are lycopene and β-carotene, which are 11- and 9-conjugated carotenes, respectively (see Figure 1). All measurements have been performed directly from fresh and processed tomatoes as well as from pure, isolated carotene standards.

Figure 2 presents the FT-Raman spectrum of tomato puree (A), with three intense bands to be seen at 1510 (ν_1), 1156 (ν_2), and 1005 cm^{-1} (ν_3). The signal at 1510 cm^{-1}, which can be assigned to lycopene, is asymmetric and it can be assumed that the shoulder registered at higher wavenumbers at ~1520 cm^{-1} (see the inset in Figure 2) is due to β-carotene, which is also present in tomatoes but in lower amounts.[12,13] However, the spectra obtained from the pure carotenoid compounds (Figure 2B and C) show characteristic signals at positions slightly different from those observed for tomato products. The most intense C=C stretching vibration of lycopene standard is observed at 1514 cm^{-1} (B) whereas for β-carotene the same mode is measured at 1515 cm^{-1} (C). The reason for that shift may be an interaction of naturally occurring carotenoids with plant matrixes changing their chemical form or physical structure and influencing the wavenumber position of their characteristic Raman bands. The band registered at 1510 cm^{-1} is the strongest in the spectrum of red

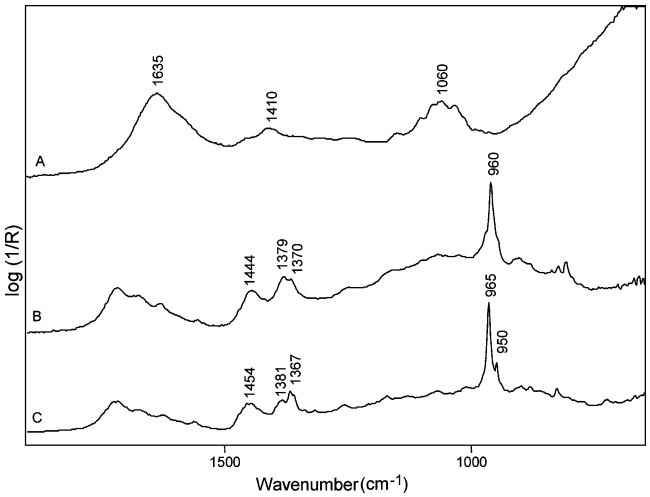

Figure 3. ATR-IR spectra of tomato puree (A), lycopene (B), and β-carotene (C).

tomato puree, which contains the highest amount of lycopene among the investigated products. It has been reported before[12] that spectra measured in orange tomatoes show higher intensity of the band near 1520 cm^{-1} with a shoulder at 1510 cm^{-1} that corresponds to higher amounts of β-carotene (1520 cm^{-1}) in comparison to lycopene (1510 cm^{-1}), which is reflected also in the orange color of this vegetable. The wavenumber of the ν_1 band decreases with the length of the conjugated central polyene chain due to an electron−phonon coupling.[12,14] Also, the observed ν_1 shift toward red is correlated with an increased number of conjugated double bonds in the carotenoid chain, i.e., 1520 (9) → 1510 cm^{-1} (11).

Raman spectroscopy has been already applied for noninvasive and simultaneous determination of these compounds occurring in human skin[15,16] as well as in tomato fruits[17] by using laser excitation in the visible range (488 or 514.5 nm). Such experiments offer strong resonance enhancement of carotenoid signals in the registered Raman spectrum but do not allow discrimination between lycopene and β-carotene. All bands of both carotenoids were observed at the same wavenumber positions, i.e., 1525, 1159, and 1008 cm^{-1}.[15−17] However, the application of FT-Raman spectroscopy with excitation in the near-infrared range (1064 nm) provides preresonance enhancement and offers the option to discriminate between lycopene (1510 cm^{-1}) and β-carotene (1520 cm^{-1}) signals.[12,13] Thus, FT-Raman spectroscopy seems to be better suited for simultaneous determination of both carotenoids in tomato products.

IR spectra obtained from tomato samples show no visual evidence of lycopene and β-carotene occurrence (see Figure 3A). Most intense signals observed in the spectra of isolated carotenoids (Figure 3B and C) due to wagging vibration ((RH)-C=C-(RH)) can be seen at 965 and 960 cm^{-1} for β-carotene and lycopene, respectively.[18] An additional satellite band at 950 cm^{-1} indicates that the standard of β-carotene used for measurement

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Table 2. FT-Raman, ATR-IR, and NIR Spectroscopy Calibration Results for Lycopene and β-Carotene Content of Tomatoes Fruits and Various Tomato Products

	FT-Raman		ATR-IR		NIR	
	lycopene	β-carotene	lycopene	β-carotene	lycopene	β-carotene
R^2	0.91	0.89	0.98	0.97	0.85	0.80
SECV	74.34	0.34	33.20	0.16	91.19	0.41
no. of PLS factors	11	11	5	5	7	7
no. of outliers	2	2	2	2	2	2

was not pure and contained small amounts of another carotenoid. At ~1370 cm^{-1}, both investigated pigments show a vibrational mode due to symmetric deformation δ_{sym} (CH_3) whereas near 1450 cm^{-1} a deformation vibration δ (CH_2) is observed. None of these bands can be noticed in the spectrum obtained from tomato puree (Figure 3A) dominated by signals to be seen at 1635 and 1060 cm^{-1}, which are due to vibrational modes of water. It is not surprising, since water molecules have high dipole moment and their IR bands overlay the signals from other plant constituents. Contrary to that, in Raman spectroscopy, water has only a low response due to its low polarizability.[19] Therefore, quality analysis of both dried and fresh samples can be successfully performed by using Raman techniques whereas IR spectroscopy is generally better suited for dehydrated samples.

A similar statement can be formulated for the NIR spectrum of tomato purée, which is also dominated by water signals. Generally, NIR spectra consist of broad overlapped bands that are overtones and combinations of the fundamental vibrational modes occurring in the mid-infrared range. It has been proven that NIR spectroscopy can be successfully applied for quality analyses[20−22] but, in most cases, is not useful for an identification of the individual analytes.

Quantification. Tomato fruits and various tomato products (28 samples) containing lycopene in amounts of 2.62−629 mg/100 g and β-carotene in the range from 0.23 to 2.83 mg/100 g of fresh matter were used to develop linear calibration models (Table 1). For each spectra set (Raman, IR and NIR), several methods of preprocessing data were applied in the whole and reduced wavenumber ranges. In this paper, only the best models are presented.

For IR spectra, vector normalization and baseline correction followed by mean centering was applied. A reasonable model was obtained by using the full wavenumber range (R^2 = 0.95, SECV = 0.21 for β-carotene and R^2 = 0.97, SECV = 37.23 for lycopene); however, the best model was obtained when the spectral range was limited to 650−1800 cm^{-1} (R^2 = 0.97, SECV = 0.16 for β-carotene and R^2 = 0.98, SECV = 33.20 for lycopene) (see Table 2 and Figure 4). This narrow wavenumber range was more convenient since above 1800 cm^{-1} IR spectra are disturbed by CO_2 absorption. Some other methods of preprocessing data, such as MSC,

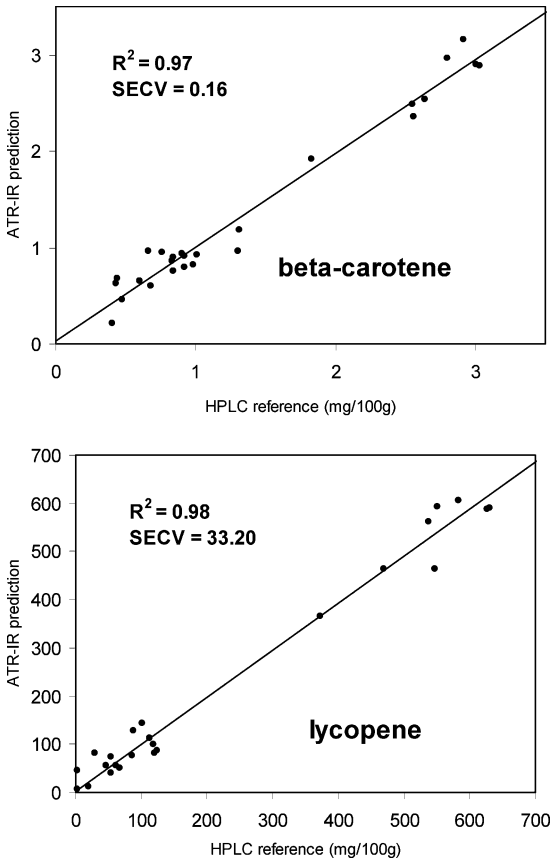

Figure 4. Reference HPLC values vs ATR-IR predictions of the lycopene and β-carotene content in tomato products.

resulted also in satisfactory prediction quality. Contrary to that, second-derivative preprocessing inserted considerable noise to the spectra and consequently gave very poor calibration models. The same observation for this method has been already reported when applied for calibration of NIR spectra of tomato products.[10]

FT-Raman spectra of tomatoes show several characteristic key bands of lycopene and β-carotene in the range between 1600 and 1000 cm^{-1}. The intensity of these bands is correlated with the carotenoid concentration in the measured samples. Evaluation of selected wavenumber ranges and the full spectrum indicated that the best PLS calibration models were obtained when the full spectrum was used between 100 and 4000 cm^{-1}. The calibration model for the prediction of carotenoid content was not improved when only specific carotenoid key bands were selected for development of the PLS model. This finding is in correspondence with studies performed earlier.[23,24] Generally, for the PLS regres-

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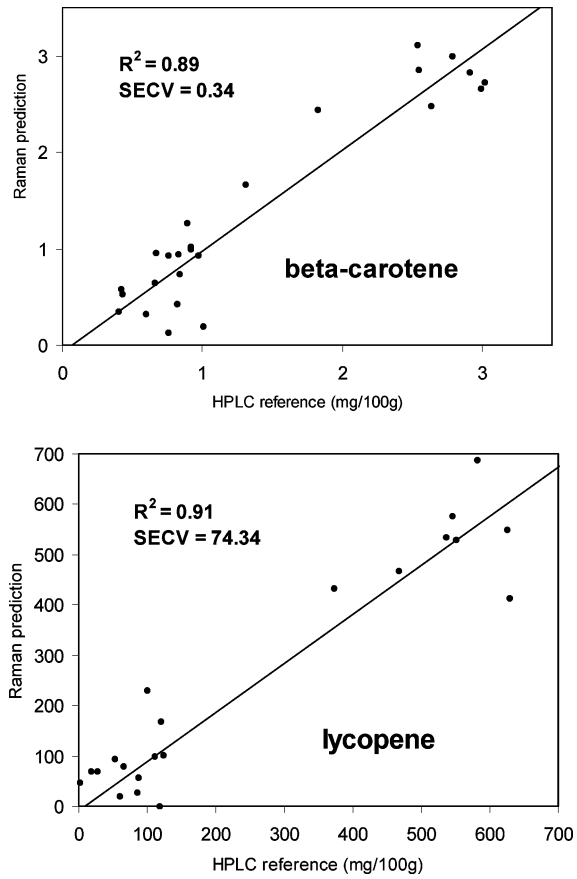

Figure 5. Reference HPLC values vs FT-Raman predictions of the lycopene and β-carotene content in tomato products.

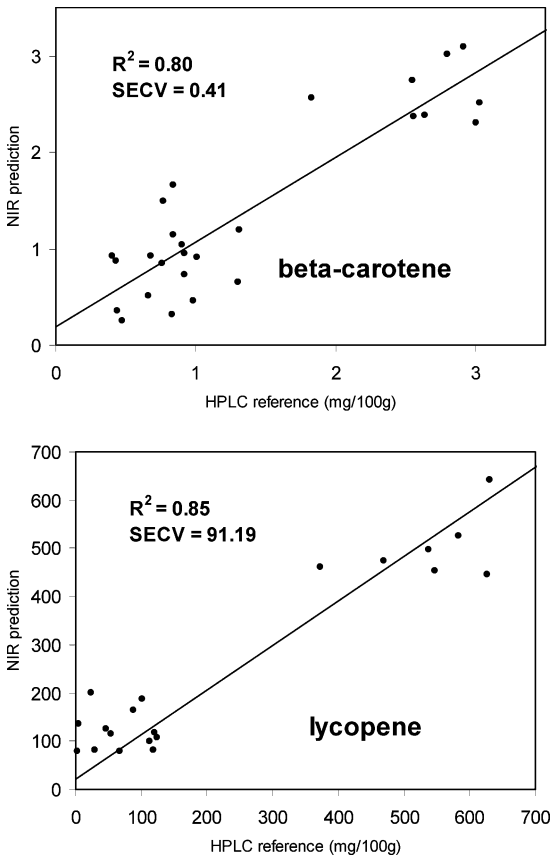

Figure 6. Reference HPLC values vs NIR predictions of the lycopene and the β-carotene content in tomato products.

sion method, the calibration model based on Raman spectra is improved with an increasing number of data points when the quality of the spectra determined by spectral noise is at the same level in the whole range. Thus, with vector normalization, baseline correction, and mean centering the following statistics has been obtained: $R^2 = 0.89$, SECV = 0.34 for β-carotene and $R^2 = 0.91$, SECV = 74.34 for lycopene. The R^2 values are still satisfactory in comparison to the IR model; however, the errors are larger and the dispersion of points near the calibration line is significant (see Figure 5A and B). Furthermore, the number of PLS factors required to built a decent Raman model was considerably higher (11) than used before for IR statistics (5), and in spite of the fact that the number was determined by calculating the PRESS function, there still exists the risk of overfitting. It was expected that the reason for lower Raman prediction quality is related to a noisy spectral response of the measured samples in comparison to IR spectra. However, smoothing of Raman spectra did not result in improved statistics, so the problem seems to be an insufficient sensitivity of the method. In spite of the characteristic key bands of carotenoids, which dominated the Raman spectra of tomato products, this method is less sufficient than IR spectroscopy for reliable determination of lycopene and β-carotene.

Results of the calibration statistics based on NIR spectra are presented in Table 2 and Figure 6. Data pretreatment consisted of vector normalization and baseline correction, which could remove the side effect of light scattering and variation in particle size. Other pretreatments, such as multiplicative scatter correction or standard normal variation, were also tested, but the final calibration results were not as good as for the combination of VN and BC. Two outliers were removed from the analyzed spectral set. Chemometric analyses of the NIR data in the whole range from 4000 to 15000 cm^{-1} allowed us to achieve a coefficient of correlation equal to 0.80 (SECV = 0.41) and 0.85 (SECV = 91.19) for β-carotene and lycopene, respectively. These models show the poorer prediction quality in comparison to IR and Raman calibration equations. The reason for that might be a high water content in all tomato samples, which dominate the NIR spectra. In the mid-infrared range, water bands are also intense; however, in the near-infrared range, these bands are particularly enhanced as a result of their multiplication (overtones). Probably, the sensitivity of the applied sampling technique was also not high enough.

CONCLUSIONS

It has been found that FT-Raman spectroscopy can be successfully applied for the identification of carotenoids directly in the plant tissue and food products without any preliminary sample preparation. Compared with the very intense carotenoid signals, the spectral impact of the surrounding biological matrix is weak, and therefore, it does not contribute significantly to the obtained

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results. On the other hand, ATR/FT-IR and NIR measurements show mainly strong water signals and no bands characteristic for carotenoids are observed. However, calibration models for determination of lycopene and β-carotene contents in tomato samples obtained on the basis of the three applied vibrational methods show the best statistics when IR spectroscopy is used. The prediction quality of Raman models is poorer and not improved when the wavenumber range limited to the specific carotenoid key bands is selected. NIR spectroscopy shows the worst prediction potential in the presented study.

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