Quantitative Analysis of Palm Carotene Using Fourier Transform Infrared and Near Infrared Spectroscopy

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ABSTRACT: β-Carotene content is usually determined by using ultraviolet (UV)-visible spectrophotometry at 446 nm. In this study, two spectroscopic techniques, namely, Fourier transform infrared (FTIR) and near infrared (NIR) spectroscopy, have been investigated and compared to UV-visible spectrophotometry to measure the β-carotene content of crude palm oil (CPO). Calibration curves ranging from 200 to 800 ppm were prepared by extracting β-carotene from original CPO using open-column chromatography. Separate partial least squares calibration models were developed for predicting β-carotene based on the spectral region from 976 to 926 cm^{-1} for FTIR spectroscopy and 546 to 819 nm for NIR spectroscopy. The correlation coefficient (R^2) and standard error of calibration obtained were 0.972 and 25.2 for FTIR and 0.952 and 23.6 for NIR techniques, respectively. The validation set gave R^2 of 0.951 with standard error of performance (SEP) of 25.78 for FTIR technique and R^2 of 0.979 with SEP of 19.96 for NIR technique. The overall reproducibility and accuracy did not give comparable results to that of spectrophotometric method; however, the standard deviation of prediction was still within ±5% β-carotene content over the range tested. Because of their rapidness and simplicity, both FTIR and NIR techniques provide alternative means of measuring β-carotene content in CPO. In addition, these two spectroscopic techniques are environmentally friendly since no solvent is involved.

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KEY WORDS: β-carotene, FTIR, NIR, palm oil, spectroscopy.

Crude palm oil (CPO), from *Elaeis guineensis*, grown in Malaysia contains about 500–700 ppm of carotenoids (1). These deep red-orange pigments are also known for their provitamin A (retinol) activities. The predominant one, β-carotene, is composed of two molecules of retinol joined tail to tail. Thus the compound possess maximal provitamin A activity. The structures of all other provitamins A contribute 50% of the biological value of β-carotene. It was reported that β-carotene has anticancer effects (2). In fact, numerous studies have proved that β-carotene prevented or delayed carcinogenesis induced by viruses (3) and chemicals (4). A study conducted by Murakoshi *et al.* (5) found that α-carotene also inhibited the proliferation of human malignant tumor cells.

Analytically, the complex composition of the palm carotenoids is readily separable by high-performance liquid chromatography (HPLC). Reversed-phase columns also have been shown to give better resolution of standard carotenoids (6). Others reported using solvent extraction procedures for carotenoids before HPLC analysis (7). However, for quantitative analysis of carotenoids, ultraviolet (UV)-visible spectrophotometry is still the most convenient method (8–9), by measuring the absorbent at different wavelengths. In addition, nuclear magnetic resonance (10) and mass spectroscopy (11) were proved to be useful in identification of carotenoids.

The use of infrared spectroscopy in the study of fats and oils has been reviewed elsewhere (12). The development of Fourier transform infrared (FTIR) spectroscopy that operates in the mid-infrared region (4000–400 cm^{-1}) has been proven to be a powerful tool for quantitative analysis of fats and oils (13). FTIR spectroscopy using interferometers provides greater energy at the sample, can scan very much faster, and has the capability to co-add data so that within a reasonably short time spectra can be produced from poorly transmitting samples with acceptable signal-to-noise ratios. On the other hand, because of the absence of well-resolved peaks and loss of resolution due to overtones and combination bands in the near infrared region (700–2500 nm), near infrared (NIR) spectroscopy has traditionally been avoided by many classical spectroscopists. However, with the development of chemometric softwares that are capable of performing multivariate statistics, NIR spectroscopy has received better attention, especially in grain analysis (14). Compared to mid-infrared analysis, NIR spectroscopy in fats and oils analyses is a rather new technique.

Although numerous analytical techniques have been developed for routine carotenoid analysis, infrared spectroscopy does not play a major role. This work was undertaken to develop a foundation for the rapid determination of carotene content in CPO by FTIR and NIR spectroscopy.

MATERIALS AND METHODS

Samples and chemicals. The CPO purchased from Ngo Chew Hong Oils and Fats (M) Sdn. Bhd. (Selangor, Malaysia) con-

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tained ~550 ppm of carotene. All chemicals and reagents used in this study were of either analytical or HPLC grade.

Column chromatographic extraction. A water-jacketed glass chromatographic column (3 cm i.d. × 33 cm) operated at 60°C and packed with 70 g adsorption resins was used to extract carotenoids from CPO. Types of adsorbent used were ion exchange resin (Diaion SK104 H) and separation resin (Sepabeads SP825) purchased from Mitsubishi Kasei Corp., Tokyo, Japan. Oil (50 ± 0.2 g) was loaded onto the column and eluted with 550 mL solvent. The eluent was collected in a 1-L round-bottom flask. The solvent was removed by rotary vacuum evaporator (NAJ-100 EYELA, Tokyo Rikakikai Co., Japan). The carotene adsorbed onto the adsorbents was eluted with excess hexane to ensure no residual was left in the column. This eluent, carotene concentrate, was evaporated to dryness in a separate flask and spiked into a normal CPO. The range of carotene obtained from this method was from 200 to 800 ppm.

UV-visible spectrophotometry. Carotenoids were quantified by UV-visible spectrophotometer (Model UV-160IPC; Shimadzu Scientific Instrument, Columbia, MD) based on the Palm Oil Research Institute of Malaysia Test Methods (15). Homogenized oil (0.1 g) was weighed into a 2-mL volumetric flask. The test sample was then dissolved with *n*-hexane to the mark. The absorbance was measured at 446 nm as carotene in ppm. The standard error (SE) obtained over the analytical range of 200–800 ppm of β-carotene was 7.81. The regression line showed an excellent linearity with a correlation coefficient (R^2) of 0.997. The small SE indicates that these calibration standards are good, in terms of accuracy, for secondary analysis.

FTIR. The FTIR instrument. used in this study was a Perkin-Elmer model 1000 Paragon spectrometer (Perkin-Elmer Instrument Corporation, Norwalk, CT) capable of covering the spectral range of 4000–400 cm^{-1}. The instrument was controlled by a Pentium PC run under Windows-based (Microsoft, Redmond, WA) Perkin-Elmer Spectrum Light Software (Version 1.5). An automated flow-through transmission cell (0.1 mm BaF_2) was used to handle samples. The temperature of the flow cell was set at 80°C (±2°C) and controlled by attaching strips of heating tape on the cell window and the stainless steel tubing, so all components of the accessory were heated to prevent crystallization of oil in the lines or cell. An outlet line from the flow cell emptied into a collection vessel, which was attached to a vacuum pump unit. Prior to analysis, the sample compartment was purged with pure N_2 gas at slow rate for 30 min to minimize water vapor and CO_2 interference.

NIR. Forty samples used in FTIR analysis for calibration development were analyzed in four replications by NIR. Log (1/reflectance) spectra were recorded at 2-nm intervals from 400 to 2500 nm with an NIR scanning monochromator (Model 6500; NIRSystem, Inc., Silver Spring, MD). A quartz cuvette (NIRSystem) with a 10-mm pathlength, suitable for oil analysis, was used for sample presentation. All spectra were recorded at 65°C. A Deltron 486 PC equipped with NSAS software (NIRSystem) was used to collect and store data after the reading of 32 scans per sample.

Data treatments. All the spectral data were converted and saved under JCAMP-DX format to diskettes. These spectral data were then downloaded to Nicolet Turbo Quant-IR Calibration and Prediction Package (Nicolet Instrument Co., Madison, WI) for subsequent partial least squares (PLS) calibration development. The selection of spectral regions for calibration development was assisted by examination of correlation and variance spectra. Each calibration was assessed by using the leave-one-out cross validation procedure. The optimization of calibration factors was guided by the predicted residual error sum of squares (PRESS) test when the minimal number of factors was obtained. The calibration was further refined by using the mean difference (MD) and standard deviation of the difference (SDD) between the predicted and actual carotene as a measure of improved performance of the calibration. The correlation coefficient (R^2) was used to measure the strength of the linear relationship between the predicted and actual values, and the standard error (SE) generated during analysis. Prediction was carried out using an independent set of samples to test the applicability of the calibration model developed.

RESULTS AND DISCUSSION

Sample characteristics. Figure 1 illustrates the effects of flow rate and solvent types with the adsorbents during the extraction processes. It was obvious that low flow rate did not improve the extraction. Carotene adsorption on the adsorbents at 8 mL/min was not significantly different ($P > 0.05$) from 20 mL/min. However, there were some differences between the types of eluent and the adsorbents used. In terms of the eluent, both hexane and acetone were poor solvents. There was almost no carotene retained in the column. On the other hand, isopropanol showed the greatest effect. However, the ability to extract carotene from the oil was also dependent on the type of adsorbent. With SK104H as adsorbent, there was only ~150 ppm reduction of carotene after extraction compared to the original (~550 ppm). However, reduction was nearly doubled in the SP825 system. The results showed that isopropanol with SP825 as adsorbent reduced the carotene in the original CPO by ~50%. From this method, the analytical range was broadened from ~200 to ~550. To prepare the CPO with higher carotene content, residual carotene adsorbed on the adsorbant was eluted out with hexane and spiked with the original CPO. The highest carotene content obtained through spiking was ~800 ppm. Therefore, the final range obtained for calibration development was from ~200 to ~800 ppm.

FTIR spectroscopy. The FTIR spectrum of pure β-carotene (4000–400 cm^{-1}) is shown in Figure 2. Naturally, unless isomerization occurs, most β-carotene exists in *trans* form (16). The off-scale absorption bands from 2900 to 3050 cm^{-1} were the common asymmetric and symmetric stretching

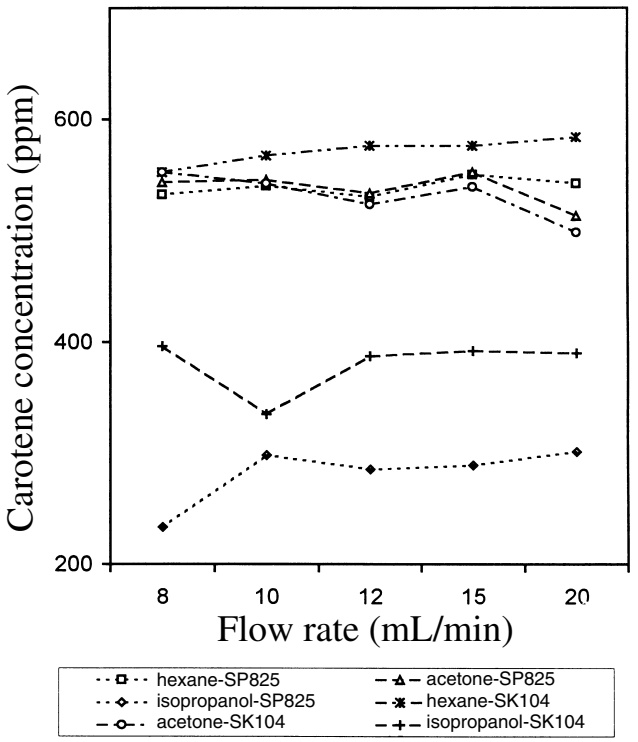

FIG. 1. Effect of different solvents and flow rates on adsorbents during the extraction of palm carotene.

modes of the C-H groups. The weak absorption band observed at 3005 cm^{-1} is due to the *trans*-CH=CH- of the β-carotene. The sharp band at 1455 cm^{-1} arose from the asymmetric deformation mode, whereas the band at 1373 cm^{-1} was the symmetric deformation mode of the C-H group. At lower frequencies, the band at 956 cm^{-1} corresponded to the *trans* conjugated alkene -CH=CH- out-of-plane deformation mode (17), whereas the weak band near 727 cm^{-1} was assigned to the CH_2 group in rocking mode.

Figure 3 represents overlaid spectra of CPO, containing ~200–800 ppm β-carotene, centered on the *trans*-CH=CH- of the β-carotene. A PLS calibration was constructed and optimized based on the FTIR spectral region from 976 to 926 cm^{-1} referenced to a single-point baseline at 926 cm^{-1}. The selection and optimization of the wavelength range, type of baseline configuration, and number of factors were guided by the PRESS test. Figure 4 illustrates the calibration plot obtained from the PLS calibration model using seven factors in terms of FTIR predicted vs. actual spectrophotometric β-carotene for 30 samples (Table 1). The plot shows overall linear regression with R^2 of 0.972 and SE of 25.8 ppm. When the calibration was examined with the leave-one-out cross validation, the overall SE was 29.6 ppm.

NIR spectroscopy. Figure 5 represents a mean spectrum of CPO (30 samples) in the NIR region, which was more difficult to interpret mainly because of the extensive band overlapping due to the overtone and combination of the fundamental vibrations involving hydrogenic stretching modes. However, it was possible to predict the positions of a corresponding overtone band in reference to the fundamental band in the mid-IR. The absorption bands from 1700 to 1900 nm were due to the first overtone bands of C-H stretching modes. The second overtones can be observed from 1300 to 1500 nm. The aromatic C-H group had the first and second overtones at 1685 and 1143 nm (18). The distinct absorption bands at 2140 and 2190 nm were undoubtedly due to *cis*-unsaturation of fatty acids (19). Other stretching and deformation modes of the C-H group occurred between 2000 and 2500 nm.

Quantitative analysis of β-carotene by FTIR was based on

TABLE 1
Comparison of Mean Duplicate Samples[a]

UV-visible reference method	FTIR method	NIR method
528	528	535
530	527	542
535	546	537
535	528	532
526	530	513
527	528	530
526	543	477
537	533	511
503	519	500
542	549	479
503	519	500
539	500	518
553	554	535
532	528	517
794	751	782
733	NA	755
692	651	699
574	564	547
343	365	355
360	411	380
301	334	260
222	294	321
261	300	301
304	333	347
257	245	306
248	NA	301
272	288	285
266	243	268
531	528	546
520	NA	543
559	567	537
546	568	533
562	580	586
565	587	567
563	576	556
395	379	316
393	379	426
346	361	315
388	379	353
388	362	379
282	249	311
299	278	319
287	NA	310
239	NA	310
281	249	318

[a]UV, ultraviolet; FTIR, Fourier transform infrared spectroscopy; NIR, near infrared spectroscopy; NA, not available.

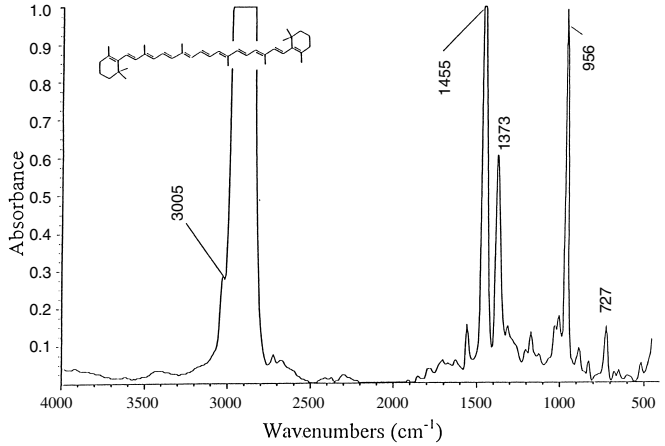

FIG. 2. A pure β-carotene spectrum by Fourier transform infrared (FTIR) spectroscopy.

the *trans*-CH=CH- functional group. However, this characteristic band did not exist in the NIR region (20). The correlation spectrum (Fig. 6) also revealed that there was a mathematical correlation between spectral changes with the changes in the β-carotene content. However, there was strong correlation between 500 and 700 nm in the visible region.

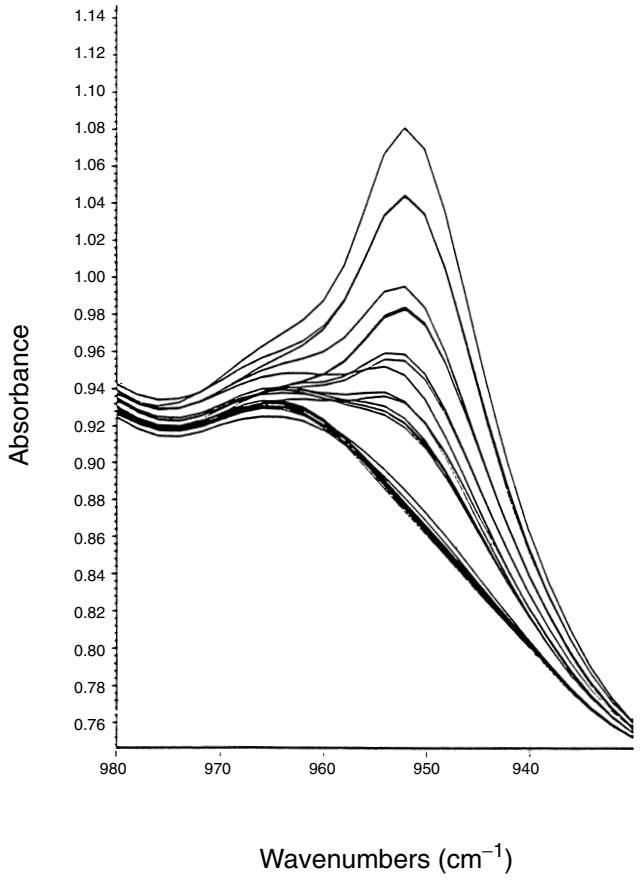

FIG. 3. Overlaid spectra of crude palm oil samples showing different concentrations of carotene using FTIR spectroscopy. See Figure 2 for abbreviation.

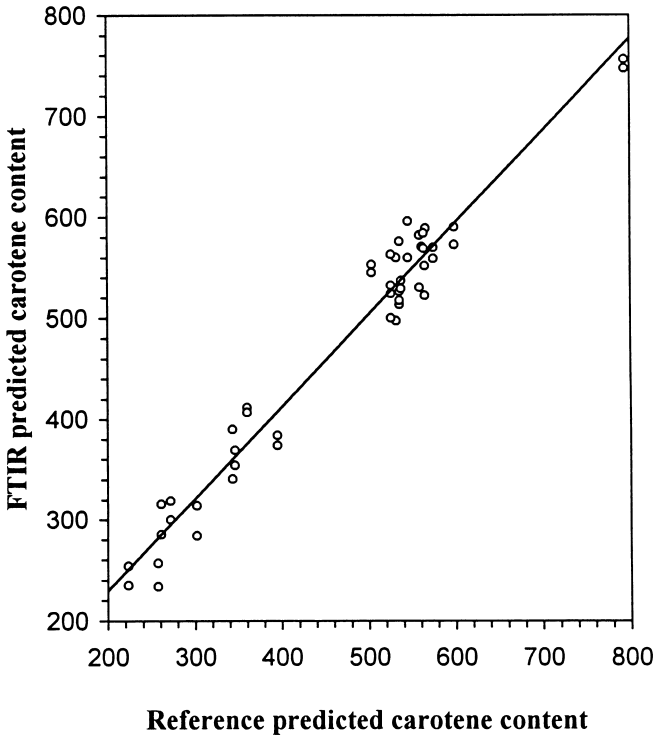

FIG. 4. Plot of mean carotene content obtained from calibration standards by ultraviolet (UV)-visible spectrophotometry reference method vs. FTIR method. See Figure 2 for abbreviation.

When the calibration model was developed using PLS in this region, a linear plot (Fig. 7) was obtained in terms of NIR predicted vs. actual spectrophotometric β-carotene for 30 standards (Table 1), with overall SE of 23.6 and R^2 of 0.951. The optimal spectral range used to predict the β-carotene was 546–819 nm, with 800 nm as a single-point baseline as guided by the PRESS test.

Validation of equations. Figures 8 and 9 show the plots of duplicate IR spectroscopic estimated vs. actual spectrophotometric (UV-visible) β-carotene in terms of FTIR and NIR, respectively. The standard error of performance (SEP) obtained

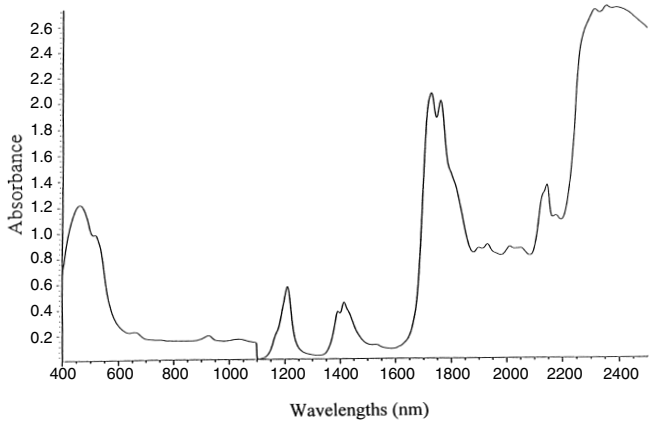

FIG. 5. Mean spectrum obtained by near infrared (NIR) spectroscopy based on 30 crude palm oil samples.

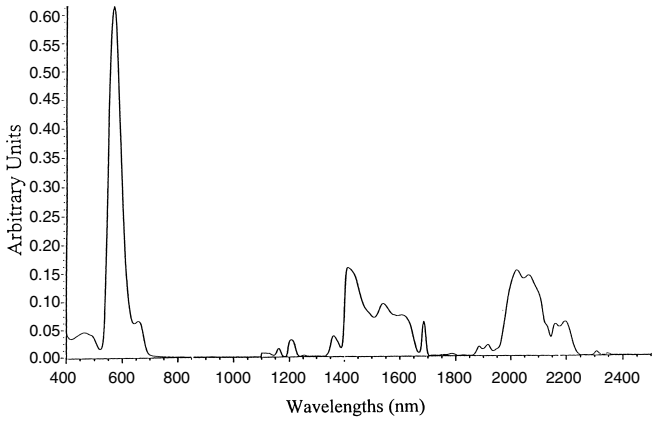

FIG. 6. Correlation spectrum used in developing partial least squares calibration model.

from the validation sets are 25.78 for FTIR method and 19.96 for NIR method. These plots are linear, with R^2 of 0.951 for FTIR technique and 0.979 for NIR technique, respectively. The regression lines were:

$$\text{FTIR } \beta\text{-carotene} = 30.401 + 0.9144 \text{ UV-vis } \beta\text{-carotene} \quad [1]$$

$$\text{NIR } \beta\text{-carotene} = 26.983 + 0.9505 \text{ UV-vis } \beta\text{-carotene} \quad [2]$$

Table 2 provides an assessment of analytical reproducibility in terms of the mean difference (MD_r) and the standard deviation of the difference (SDD_r) for β-carotene predictions for 20 sample pairs used in the validation set. Both FTIR and NIR instruments gave larger MD_r and SDD_r compared to the spectrophotometer. Even so, it was still possible to estimate β-carotene content as closely as ±5% by both instruments. In terms of accuracy (MD_a and SDD_a), the results indicated that, on average, the predictions matched the performance results. The overall results indicate that the best prediction for both instruments used to predict β-carotene content was within 3–5% deviation.

TABLE 2
Statistical Comparison[a] of Carotene Data Obtained by UV-Visible Spectrophotometer with FTIR and NIR Methods

Statistic	UV-visible reference method	FTIR method	NIR method
Max. value	795	751	783
Min. value	223	243	260
Mean	452	456	448
MD_r	2.67	6.45	9.11
SDD_r	1.67	2.37	8.95
MD_a	–3.78	–6.44	
SDD_a	–0.69	–7.27	

[a]MD_r, mean difference of reproducibility; SDD_r, standard deviation of the difference of reproducibility; MD_a, mean difference of accuracy; SDD_a, standard deviation of the difference of accuracy. For other abbreviations, see Table 1.

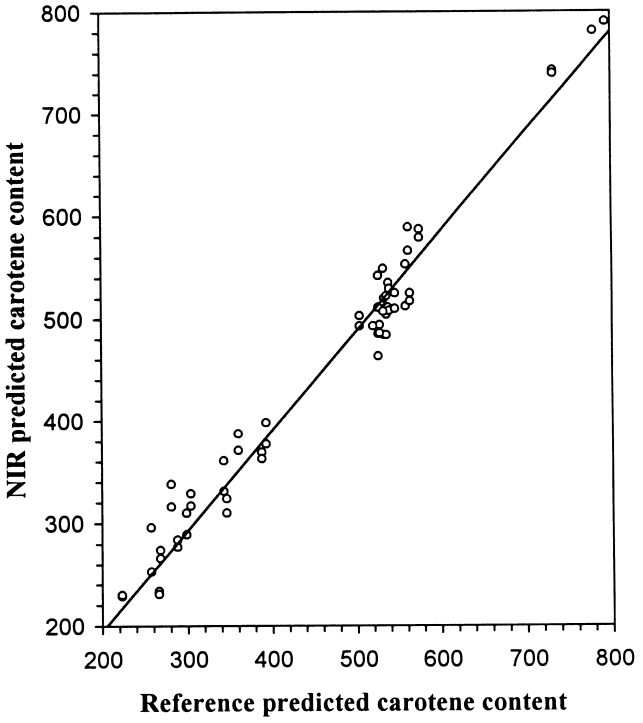

FIG. 7. Plot of mean carotene content obtained from calibration standards by UV-visible spectrophotometry reference method vs. NIR method. For abbreviations see Figures 4 and 5.

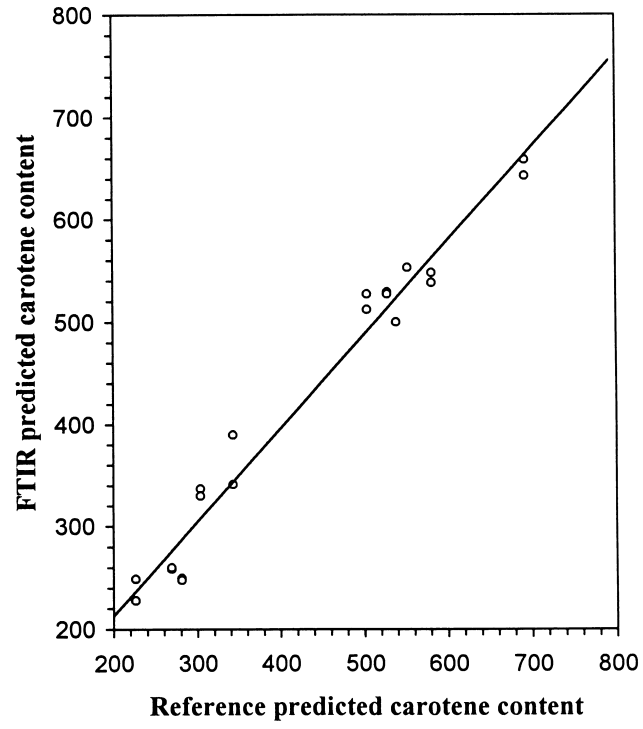

FIG. 8. Plot of mean FTIR carotene content vs. mean reference carotene content of validation samples. For abbreviation see Figure 2.

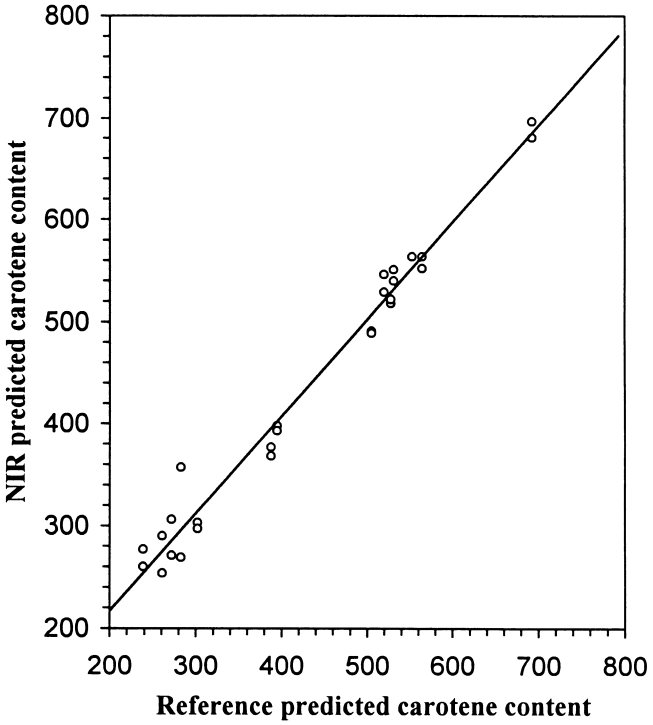

FIG. 9. Plot of mean NIR carotene content vs. mean reference carotene content of validation samples. For abbreviation see Figure 5.

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