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NIR Spectroscopy and Partial Least-Squares Regression for Determination of Natural α-Tocopherol in Vegetable Oils

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Near-infrared (NIR) spectroscopy and partial least-square regression were used for determination of α-tocopherol in edible oils after extraction with ethanol. The standard error of calibration and the standard error of prediction were calculated for evaluation of the calibration models. The chemometric calibration model was prepared in spectral region 6500−4500 cm^{-1} for standard α-tocopherol solutions (0.54−53.54 mg/mL). Obtained mean concentrations of natural α-tocopherol in different types of oils varied from 17.53 to 57.10 mg/100 g. Net analyte signal calculation was used to estimate detection limit (DL = 0.12 mg/mL), quantification limit (QL = 0.40 mg/mL), sensitivity (SEN = 0.045 mg/mL), and selectivity (SEL ranged between 0.24 and 0.54% of the measured reflectance signal) of the proposed NIR method. The comparable precision (RSD = 0.68−2.80% and 0.79−3.06%) and accuracy (recovery, 97.2−102.4% and 96.8−103.2%) for the proposed NIR and standard HPLC methods, demonstrate the benefit of the NIR method in the routine analysis of α-tocopherol in vegetable oils.

KEYWORDS: α-Tocopherol determinations; near-infrared spectroscopy; PLS regression

INTRODUCTION

α-Tocopherol (α-TOH), 5,7,8-trimethyltocol (**Figure 1**), is the most active form of vitamin E and an important natural antioxidant of lipids present in vegetable oils.

It is one of the best chain-breaking phenolic antioxidants that reacts rapidly with alkylperoxyl radicals (LOO•) (a). However, the tocopheryl radicals (α-TO•) react with other peroxyl (b) or α-TO• (c), forming more stable adducts and protecting lipids from peroxidation (*1*).

$$LOO^{\bullet} + \alpha\text{-TOH} \rightarrow LOOH + \alpha\text{-TO}^{\bullet} \quad \text{(a)}$$

$$LOO^{\bullet} + \alpha\text{-TO}^{\bullet} \rightarrow (\alpha\text{-TO})OOL \quad \text{(b)}$$

$$\alpha\text{-TO}^{\bullet} + \alpha\text{-TO}^{\bullet} \rightarrow (\alpha\text{-TO})_2 \quad \text{(c)}$$

That is why α-TOH prevents the rancidity of oils during storage and thus prolongs its shelf life (*2*). Therefore, control of the vitamin E level in edible oils is of great importance in determining the oxidative stability of fats.

Tocopherols in vegetable oils most often are analyzed by normal-phase (NP-HPLC) or reversed-phase high-performance liquid chromatography (RP-HPLC) with electrochemical (*3*, *4*), spectrophotometric (*5*, *6*), and fluorimetric detectors (*7*, *8*). NP-HPLC was suitable for the direct analysis of tocopherols in fats only after diluting the oils in organic solvents, e.g., hexane, 2-propanol, methanol, tetrahydrofuran (*5*, *9*). However, RP-HPLC is preferred in quantitative analysis of tocopherols, due to the reproducibility of the retention time, higher column stability, fast equilibration, and shorter analysis time (*4*, *6*−*11*). Also gas chromatography (GC) or gas−liquid chromatography (GLC) were used for tocopherol determination in edible oils and fats with flame ionization (FID) or mass spectrometry (MS) detection (*9*, *12*−*14*) with different methods of sample preparation [on-line transesterification (*13*), by solid-phase extraction (SPE) (*14*), saponification of fats (*12*)]. Data obtained from GC and HPLC methods revealed comparable accuracies (recovery ranged between 75 and 102% and 80−103%) and somewhat lower precisions (RSD ranged between 1.5 and 4.6% and 2.96−6.65%) for α-TOH determination in different types of vegetable oils (*3*, *10*, *11*, *13*, *14*). However, the detection limit obtained for the GC technique (0.7 μg mL^{-1}) (*13*) was one order higher than for HPLC analysis (3.75×10^{-8} mol L^{-1}, 28 and 11.5 ng/mL) (*3*, *7*, *11*). The chromatographic determinations of tocopherols in fats and oils require often tedious and time-consuming procedures of sample preparation (saponification, solvent extraction, and purification); therefore, electrochemical methods, e.g., chronopotentiometry (*15*), galvanostatic coulometry (*16*) and voltammetry (*17*, *18*), were applied as well for

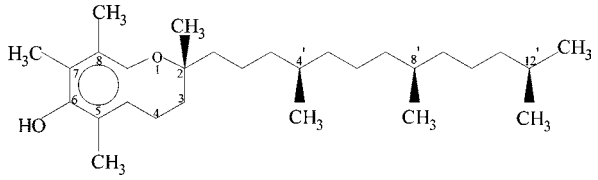

Figure 1. Chemical structure of the α-tocopherol.

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tocopherol analysis in fats. The electrochemical methods proved to be less precise [RSD ~ 10% (*18*) or between 3.5 and 6.5% (*15*)] and accurate (expressed as recovery = 104.3 μg g^{-1}) (*17*) than chromatographic procedures (RSD and recovery ranged between 1.5 and 6.65% and 75−103%, respectively) (*3*, *10*, *11*, *13*, *14*) for vitamin E determination in edible oils. Although, the obtained detection limit (DL = 1 × 10^{-8} mol L^{-1}) (*17*) was comparable with HPLC (3.75 × 10^{-8} mol L^{-1}) (*3*) and electrochemical methods appeared to be faster (3−5 min vs14 min for HPLC) and less expensive, these techniques are rarely used for determination of vitamin E in fats. Among disadvantages of electrochemical methods, the following should be mentioned: (a) the toxicity of solvents, e.g., benzene, heptane (*17*), toluene (*15*), and (b) insufficient resolution of tocopherols' oxidation peaks (peak potential 1.065, 1.140, 1.170, and 1.245 V for α-, β-, γ-, and δ-tocopherols, respectively) (*18*). Therefore new techniques that will reduce or eliminate such disadvantages are the subject of studies. Vibrational spectroscopy, such as Fourier transform mid-infrared (FT-MIR) and near-infrared (NIR), has been applied in oil analyses using the rapid screen procedures (1−4 min). Oxidative stability (*19*, *20*), peroxide value (PV), (*21*, *22*), iodine value (IV) (*23*, *24*), anisidine value (AV) (*20*), and free fatty acids (FFA) (*25*−*27*) in edible oils and fats were studied by FT-MIR and FT-NIR. Moreover, FTIR was applied for the quantitative analysis of moisture content (*28*), unsaturation grade, trans and cis isomers, conjugated linoleic acids (CLA) percentages (*24*, *29*, *30*), phospholipids (*31*), gossypol (*32*), and aflatoxin contents (*33*). IR spectroscopic techniques have also been used for testing authenticity of vegetable oils (*34*). However, only a few reports on applications of NIR/FTIR in analysis of vitamins in food were noted (*35*−*37*). Furthermore, to the best of our knowledge, there was no reference on determination of vitamin E in edible oils by NIR technique. Only, Shi et al. (*36*) used near-infrared spectrometry for determination of vitamin E (93−97.4%) in vitamin premixes, applying the partial least-squares method (PLS) in the concentration ranges of 93−97.4% and 80−97%. Recently, Che Man et al. (*37*) have reported the use of FTIR spectroscopy for α-TOH determination in refined bleached and deodorized (RBD) palm olein. The PLS regressions with SEC = 53.54 ppm, SEP = 63.59 ppm, and R^2 = 0.9922 were applied to spectral region 3100−2750 cm^{-1}. The accuracy (standard deviation of difference, SDD_a = 1.52) and repeatability (SDD_r = −1.50 and −1.78 for FTIR and HPLC methods, respectively) of the FTIR method was comparable to that for the HPLC method.

In the presented work, NIR spectroscopy was applied for quantitative determination of natural α-TOH in vegetable oils, and PLS regression was used for the calibration and validation of the proposed method. The aim of this work was to compare the new NIR method and the standard HPLC method for precision and accuracy.

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade. Absolute ethanol (99.9%) and methanol (99.9%) were of HPLC-grade and purchased from POCH (Gliwice, Poland). Deionized water (DW) was used for the preparation of solutions and samples. α-Tocopherol (95%) was supplied by Sigma-Aldrich. Standard solutions of α-TOH (3.11 × 10^{-4} mol/L, 0.1243 mol/L) were prepared in absolute ethanol and stored at 4 °C in the dark bottles.

Samples Preparation. Five commercial edible oils, sunflower (SFO), soybean (SO), corn (CO), rapeseed (RO), vegetable oil (MSO) (mix of rapeseed, soybean, and sunflower oils), were manufactured in Poland, and grapeseed oil (GO) (Spain), extra virgin olive oil (OO1) (Greece), olive oil (OO2) (mix of virgin plus refined olive oils) (Italy) were purchased from local markets in Toruń, Poland. All oils in the original packing (poly(ethylene terephthalate) (PET) or glass bottles) were stored below 10 °C in the dark.

Tocopherols were extracted from the oils by the method of Tasioula-Margari et al. (*10*) with some modifications: methanol was replaced by ethanol. The conical flasks with oils (20.0000−40.0000 g) and ethanol (3 mL) were shaken for 60 min at room temperature in the dark. The unsaponifiable fraction of tocopherol was extracted successively with three 3-mL portions of ethanol. After separation of the phases, the ethanol extracts were transferred quantitatively into 10-mL volumetric flasks. Prior to NIR and HPLC analysis, extracts were filtered through a 0.45 mm filter.

Instrumentation and Software. The NIR spectra were measured using a Perkin-Elmer Spectrum 2000 FT-IR spectrometer at 8 cm^{-1} resolution with a SPECAC temperature-variable cell at 294 ± 1 K. NIR absorbance spectra were registered in the range 4000−10 000 cm^{-1} using 2-mm glass cells, with a 1 cm^{-1} spectra resolution. Background spectrum (100 scans) was recorded daily, whereas for samples 50 scans were taken in three repetitions, to reduce the instrument noise.

The calibration methods were developed by means PLS regression available in MATLAB software from MathWorks Inc. The normalization and derivatization of spectra by the Savitsky−Golay method were performed with Spectrum for Windows V 1.5 (Perkin-Elmer) software.

HPLC analysis of α-TOH was performed with a Shimadzu chromatograph SPD-10A, UV−vis detector, and a column [Discovery C18, 150 × 4.6 mm, 5 μm particles (Supelco)]. HPLC chromatograms were obtained under the following conditions: mobile phase, methanol/water (97:3 v/v) solution; the flow-rate, 1 mL/min; UV detection at 292 nm.

NIR Method. Partial least squares (PLS) regression was employed to extract relevant information from the complex NIR spectra of α-TOH. The optimum number of PLS factors used for prediction was determined by full cross-validation. The accuracy of the calibration models is described by the squared correlation coefficient (R^2), also called coefficient of determination, and standard error of calibration (SEC) and standard error of prediction (SEP), both of which can be interpreted as the average modeling/prediction error, expressed in the same units as concentrations of α-TOH in oil samples. They represent the average difference between predicted and measured response values at the calibration/validation stage (*38*).

Single PLS calibration set (n = 20) was applied for working solutions (0.54−53.54 mg/mL) prepared from the stock standard solution of α-TOH in absolute ethanol. PLS regressions were used to analyze the content of α-TOH in the oil samples.

HPLC Method. Standard HPLC method was applied to quantitative determination of α-TOH in the spiked oil samples (Polish Standard Method PN-EN 12822) (*39*). α-TOH peak was identified by comparing its retention time, 11.74 min, with that of the standard solution, which was similar to the value reported by Lavedrine (*40*) for determination of α-TOH by HPLC in walnuts.

The calibration curve was prepared using working solutions of α-TOH between 2.68 and 26.78 μg/mL in ethanol. Five calibration curves were constructed by plotting the peak area of each analyte versus concentration using the least-squares method. The representative linear regression equation for this method was $y = 343.5 \pm 8.3x - 62.0 \pm 137.8$ with a correlation coefficient of 0.9991, and the relative standard deviation of the slope was 1.1% (n = 5). In comparison, Rodas Mendoza et al. (*41*) found a similar linear regression equation (y = 249.86x − 43.806) with R^2 = 0.999 for analysis of α-TOH (5−50 μg/mL) in infant formulas by an HPLC method.

Precision and Accuracy of the Analytical Procedures. The content of α-TOH in the oil samples determined (five portions of each oil were extracted and each solution analyzed three times within 1 day) by the studied method were compared with the official standard HPLC method (*39*). In each single measurement the extract was introduced into the cell and the spectrum was recorded. After collection of each spectrum, a new solution of α-TOH was placed into the cell for the next measurement. The reproducibility of the NIR method was checked by five replicate determinations of α-TOH in the same oil samples over a period of 3 days. Both methods were compared for within-day (repeatability) and between-day (reproducibility) precision using the F-test and accuracy, expressed as recovery values. The recovery

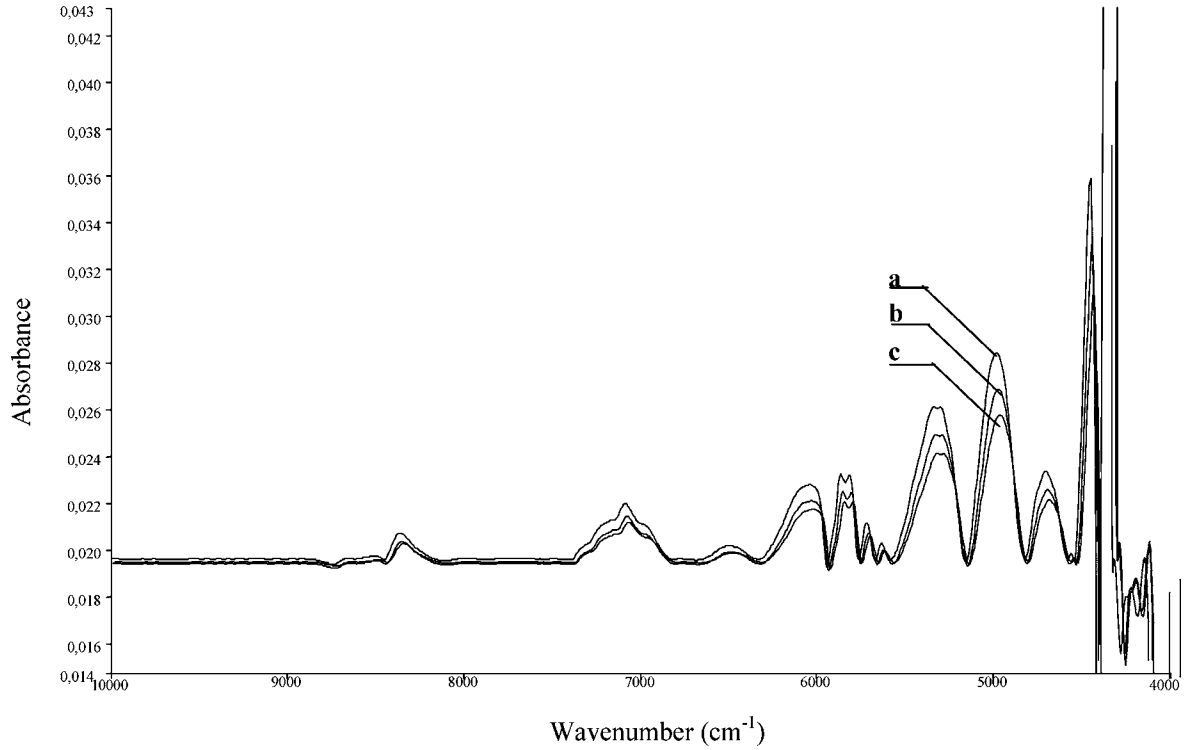

Figure 2. NIR spectra of α-tocopherol in ethanol after baseline correction and normalization scale (0–2 au) for the following samples concentrations: (**a**) 32.13 mg/mL, (**b**) 10.72 mg/mL, and (**c**) 1.072 mg/mL.

Table 1. Absorption Bands for α-Tocopherol in the Range 8400–4100 cm^{-1}

observed bands, cm^{-1}	assignments
8391	$-CH_3$ second overtone, symmetric stretching
7126	$-CH_2$ 2xCH stretching + CH bending
6162	CH aromatic first overtone
5886	$-CH_3$ first overtone, asymmetric stretching
5742	$-CH_2$ first overtone, asymmetric stretching
5142	combination OH stretching + OH bending
4703	aromatic CH stretching + ring C═C stretching
4426	$-CH_3$ combination C–H stretching and C–H bending
4230	–OH bending second overtone
4120	combination C–H stretching + C–C stretching

experiments were performed as follows: α-TOH was extracted from the oils as described in the section Sample Preparation, standard solutions of α-TOH (10 mg/mL) were added to ethanol extracts, and the obtained mixtures were shaken and brought up to 10 mL with ethanol.

RESULTS AND DISCUSSION

NIR Calibration Using Partial Least-Squares Regression. NIR transmittance spectra and their first derivatives were examined to identify spectral features that could be correlated with α-TOH concentration. Spectra of the studied solutions of α-TOH in ethanol are presented at **Figure 2**.

Wavenumber selection (in 10 000−4000 cm^{-1} region) was performed in order to include characteristic spectral features of α-TOH identified in the study. In the range 10 000−9000 cm^{-1} only noise was observed. Detected bands in the NIR spectrum of α-TOH were assigned according to Siesler (*42*) and are listed in **Table 1**.

The absorbance spectra were treated by the Savitsky−Golay first-derivative method followed by the scale normalization procedure to enhance the resolution by removing the overlapping peaks and correcting the baseline. First-derivative spectra of the α-TOH in ethanol presented in **Figure 3** were used for calibration models construction.

The calibration models for α-TOH determination were developed using 11 absorption bands at wavelengths that are characteristic for α-TOH functional groups (**Table 2**). The distinct combinations of the selected wavenumbers to construction the PLS calibration models with varying wavenumbers from 2 to 11 were used. The standard error of calibrations (SEC) and the standard error of prediction (SEP) were calculated from each PLS model. Absorption bands at wavelengths for which the calculated SEC and SEP values were below 0.5% are listed in **Table 2**.

PLS Calibration Models Using Cross-Validation. Due to overlapping of the overtones from the different groups, the PLS method was applied to convert the complex spectral data into analytical parameters. The number of significant PLS factors was chosen by using the predicted residual error sum of squares (PRESS) value for every possible factor. The PRESS value was the sum of the squared difference between the predicted and the known concentrations. It was calculated by building calibration models with different number of factors and then predicting some samples of known concentration against the model. The number of factors where the PRESS plot reached a minimum was three for α-TOH and were chosen as the optimum number of factors for the calibration model described in this study.

The quality of this model was checked by calculating the SEC and SEP. The calibration model description and performance results are presented in **Table 2**. It was found that the NIR spectral range between 6500 and 4500 cm^{-1} (overtones and combination bands at 6162, 5886, 5742, and 4703 cm^{-1} from CH aromatic, CH_3, CH_2, and C═C groups vibrations) was the best for determining α-TOH in the studied system with the lowest SEC = 0.17% and SEP = 0.20% and the highest R^2 = 0.9931. In comparison, Che Man et al. (*37*) found a somewhat lower correlation coefficient (R^2 = 0.9900) for data from oil

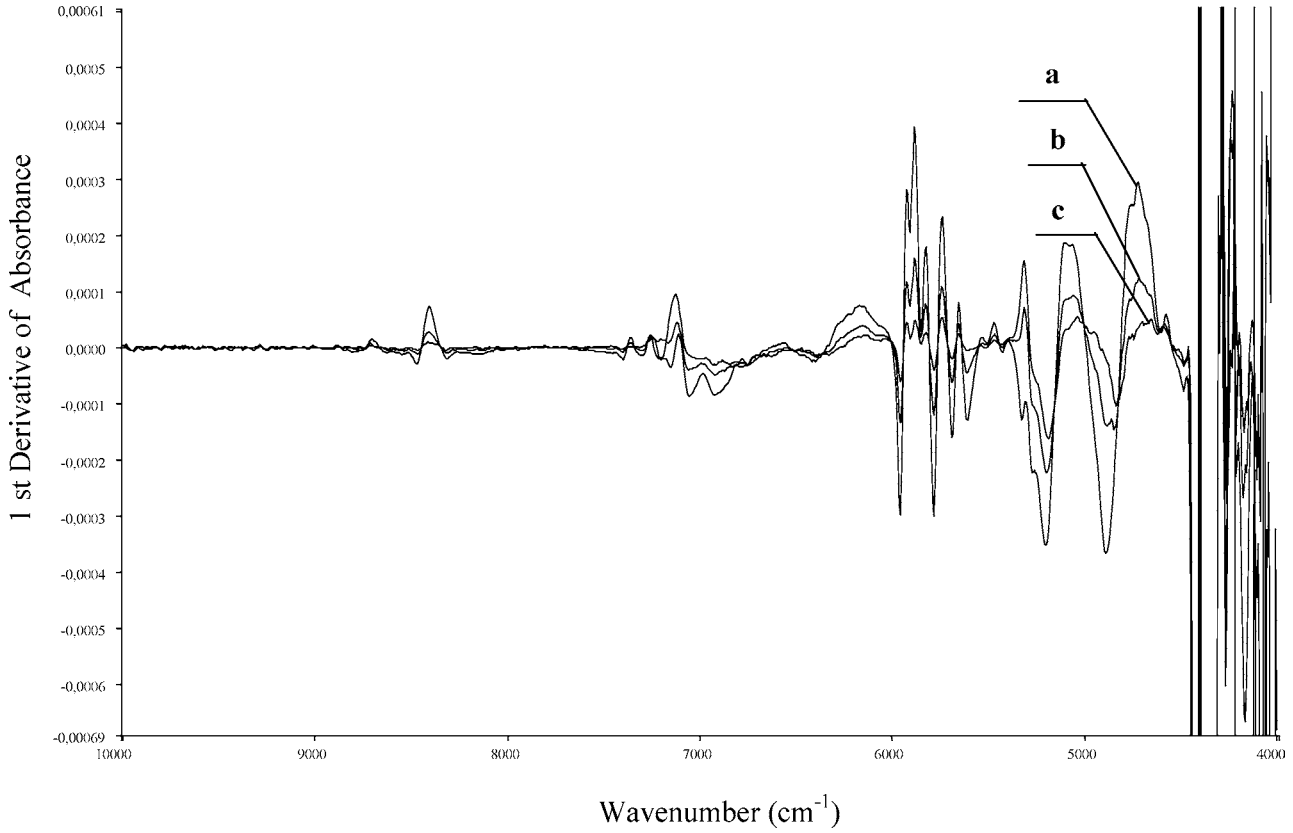

Figure 3. First derivative of α-tocopherol spectra for three samples: (**a**) 32.13 mg/mL, (**b**) 10.72 mg/mL, and (**c**) 1.072 mg/mL in calibration models.

Table 2. Results of PLS Calibration Model for α-Tocopherol Determination in Different Spectral Regions

wavelengths, cm^{-1}	SEC, %	SEP, %
8391, 7126, 6162, 5886, 5742, 5326, 5045, 4703, 4426, 4230, 4120	0.26	0.40
7126, 6162, 5886, 5045, 4230	0.19	0.36
6162, 5886, 5742, 4703	0.17	0.20
8391, 5886, 4703	0.20	0.37
6162, 4703	0.24	0.29

samples versus the PLS−FTIR predicted values. The calibration was represented graphically by plotting the theoretical concentration of the reference α-TOH samples (used for the calibration model) versus the predicted values by the model based on the NIR spectra (**Figure 4**). Because the correlation points were located along straight lines, the normal distribution of the data can be suggested. The within-day precision (repeatability) was found by regression analysis of the (NIR-predicted concentrations of α-TOH) = *f*(NIR-actual concentrations of α-TOH) curve and expressed as the relative standard deviation of the slope, RSD = 1.96% ($n = 5$) (*38*). The regression line was calculated by the method of least squares. The confidence limits ($P = 0.05$) for the slope and the intercept of the line were given by $b \pm t_{(n-2)}s_b$ and $a \pm t_{(n-2)}s_a$, where s_b and s_a are the standard deviations of slope and intercept, respectively. The calibration plot was linear ($y = 1.0236 \pm 0.04217x + 0.0678 \pm 1.0511$ and $R^2 = 0.9931$) in the concentration range between 0.54 and 53.54 mg/mL for α-TOH determination.

Analytical Figures of Merit. Net analyte signal (NAS) calculation was used to estimate the figures of merit in multivariate calibration models, such as limits of detection (DL) and quantification (QL), sensitivity (SEN), and selectivity (SEL). The NAS for analyte (*k*) is defined as the part of the spectrum

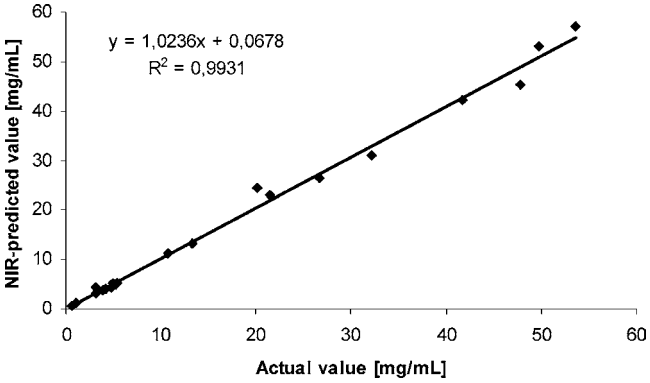

Figure 4. Full cross-validation model plotting the theoretical concentration of reference α-tocopherol solutions vs the predicted values using PLS–NIR regression.

that is orthogonal to the space spanned by the spectra of all other analytes. The NAS was computed using the regression coefficients vector generated by PLS models (*43*). The DL was calculated from the following equation: $DL_{k,un} = \Delta(\alpha,\beta)\delta r$, where the factor $\Delta(\alpha,\beta)$ is the noncentrality parameter of a noncentral *t*-distribution, which depends on the α and β probabilities, as well as on the number of degrees of freedom, ν, given by δr. $\Delta(\alpha,\beta)$ was obtained numerically according to the procedure presented by Boqué and Rius (*44*). Estimated standard deviation (δr) is calculated for the predicted concentration under the null hypothesis (*43*, *44*). In the presented work, the detection and quantification limits of α-TOH were 0.12 and 0.40 mg/mL, respectively. It is noteworthy that DL and QL of the reported NIR method for concentration range 0.54−3.21 mg/mL were about 4 orders higher than those obtained by the HPLC method (DL = 28 ng/mL, QL = 84 ng/mL (*7*) and DL =11.5 ng/mL, QL= 23 ng/mL (*11*)) for three orders lower

Table 3. Determination of α-Tocopherol in Edible Oils (mg/100 g)

	NIR method				HPLC method					
oil	$c_{\alpha-\text{tocopherol}}$,[a] mg/100 g	SD,[a] mg/100 g	RSD,[a] %	confidence limit[b]	$c_{\alpha-\text{tocopherol}}$,[a] mg/100 g	SD,[a] mg/100 g	RSD,[a] %	confidence limit[b]	F_{calcd}[c]	t_{calcd}
sunflower (SFO)	46.02	0.62	1.35	46.02 ± 0.77	46.69	1.43	3.06	46.69 ± 1.77	5.25	0.81
soybean (SO)	45.35	0.91	2.01	45.35 ± 1.45	45.04	0.86	1.91	45.04 ± 1.06	1.18	0.46
corn (CO)	57.10	0.82	1.44	57.10 ± 1.01	57.51	0.68	1.18	57.51 ± 0.84	1.44	0.70
mixed seed oils[d] (MSO)	33.39	0.38	1.14	33.39 ± 0.48	34.64	0.67	1.93	34.64 ± 0.83	3.02	4.50
rapeseed (RO)	23.54	0.66	2.80	23.54 ± 0.82	23.37	0.37	1.58	23.37 ± 0.46	3.13	0.75
grapeseed (GO)	17.53	0.15	0.86	17.53 ± 0.18	17.05	0.23	1.35	17.05 ± 0.29	2.41	4.72
extra virgin olive oil (OO1)	19.11	0.13	0.68	19.11 ± 0.16	19.02	0.15	0.79	19.02 ± 0.18	1.35	0.86
olive oil[e] (OO2)	17.98	0.19	1.06	17.98 ± 0.24	17.96	0.15	0.84	17.96 ± 0.19	1.66	0.20

[a] $n = 5$. $c_{\alpha-\text{tocopherol}}$, mean concentration of α-tocopherol; SD, standard deviation; RSD, relative standard deviation. [b] Probability level, $P = 0.05$. [c] $F_{calcd} = s_2^2/s_1^2$ ($n = 5$). s_1^2, s_2^2 are variance of results of α-tocopherol determinations for NIR and HPLC methods. $F_{theoretical} = 6.39$ ($P = 0.05$); $t_{theoretical} = 2.78$ ($P = 0.05$); $t_{theoretical} = 8.61$ ($P = 0.001$). [d] Mix of rapeseed, soybean and sunflower oils. [e] Mix of virgin plus refined olive oils.

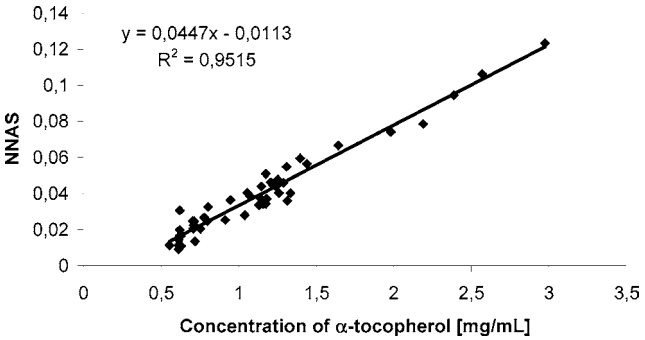

Figure 5. Norm of NAS of the 50 validation samples as a function of the actual concentration of α-tocopherol. Pseudo-univariate calibration plot for α-tocopherol determination in oil samples.

concentrations of analytes (0.5−100 μg/mL and 1−25 μg/mL, respectively) (*7*, *11*).

Moreover, SEN and SEL of the proposed method were estimated using NAS calculations. SEN in the context of univariate calibration is defined as the slope of the calibration curve (*43*). The relationship between the norm of the NAS (NNAS) for each sample (standard solution of α-TOH, $n = 10$ and oil extracts, $n = 40$) and the concentration of α-TOH in samples is presented in **Figure 5**. The SEN of the PLS model for the prediction of α-TOH concentration was evaluated from the slope of the regression line between the NNAS and the actual concentration of α-TOH, yielding a value of 0.045 mg/mL (**Figure 5**).

SEL is a measure of the degree of overlap aimed to indicate what part of the total signal is lost due to spectral overlap (*43*). The linear relationship between selectivity values and concentration of α-TOH in studied samples (standard solution of α-TOH, $n = 10$ and oil extracts, $n = 40$) was noted. The equation obtained can be written as SEL = 0.0043 mg/mL α-TOH − 0.00022, with correlation coefficient $R^2 = 0.9924$. For the studied oil samples containing 0.62−1.31 mg/mL of α-TOH, the selectivity ranged between 0.24 and 0.54% of the measured reflectance signal. The obtained values of SEL were relatively low, indicating that there was a significant loss of signal due to overlap with signals from interferents. The selectivity values obtained for the α-TOH determination in oil samples were somewhat higher than those reported by Iñón et al. (SEL = 0.28%) for olive oil acidity determination by the PLS−ATR−FTIR method (*27*).

Validation of the Proposed NIR Method. The concentrations of α-TOH in the analyzed oils determined by the NIR method were compared with those obtained by HPLC (**Table 3**).

The content of the initial α-TOH in olive oils and grape seed samples obtained by NIR method (19.11, 17.98 and 17.53 mg/100 g for OO1, OO2 and GO, respectively) was fairly constant and significantly lower when compared to studied vegetable oils (46.02, 45.35, 57.10, 23.54, and 33.39 mg/100 g for SFO, SO, CO, RO, and MSO, respectively). For comparison, the concentration of the α-isomer of vitamin E in olive oils reported by other authors was in the same range between 11.5 and 19.1 mg/100 g (*3*, *7*, *45*). Besides, grapeseed and rapeseed oils contain somewhat higher amounts of α-TOH (17.53 and 23.54 mg/100 g, respectively) in comparison with the reported results (10.06 and 19.51 mg/100 g) (*7*). It can be noted that α-TOH concentration in CO (57.10 mg/100 g) was the highest among analyzed oils. Similar α-TOH content in corn oil was determined by Suturovic (53.10 mg/100 g) (*15*). However, the amount of α-TOH in the discussed oil was about 2 and 4 times higher, when compared to results obtained by Sánchez-Pérez et al. (25.4 and 15.5 mg/100 g) (*3*), Gliszczyńska-Świgło et al. (20.38 mg/100 g) (*7*), and Ribarova et al. (14.30 mg/100 g) (*45*), respectively. Moreover, the results listed in **Table 3** indicate similar amounts of α-TOH in SFO and SO oils: 46.02 and 45.35 mg/100 g, respectively. For comparison, in Ribarova's work, the concentration of α-TOH in sunflower oil was at the same level (44.88 mg/100 g) (*45*), although, higher amounts of α-TOH in sunflower oils (59.1−72.8 mg/100 g) were determined by Sánchez-Pérez et al. (*3*), Gliszczyńska-Świgło et al. (*7*), and Suturovic et al. (*15*). It is noteworthy that the content of the α-isomer of vitamin E in mixed seed oil (33.39 mg/100 g) was similar in comparison with the reported results (33.4 mg/100 g) (*3*).

The high variability in the amount of α-TOH in vegetable oils has been widely reported and depends on several factors, such as genetic, agronomic, environmental, and extraction procedural (*46*).

The intraday precision (repeatability) of proposed NIR and standard HPLC methods was tested by analyses of all commercial oils in five replicates. The values of RSD were below 3.5% for α-TOH determination, indicating reasonable repeatability of the used methods (**Table 3**). In comparison, Suturovic et al. (*15*) and Gliszczyńska-Świgło et al. (*7*) found similar values of RSD = 3.5% and 2.45% for α-TOH analysis in vegetable oils by chronopotentiometric and HPLC methods, respectively. Also, Che Man et al. (*37*) obtained the comparable standard deviation of difference for repeatability ($SDD_{FTIR} = -1.50$ and $SDD_{HPLC} = -1.78$) for α-TOH determined in palm olein by FTIR and HPLC methods. In addition, the confidence

Table 4. Recovery Tests

	α-tocopherol content,[a] mg/100 g (RSD, %)		α-tocopherol added,[b]	α-tocopherol found,[a] mg/100 g (RSD, %)			
oil	NIR method	HPLC method	mg/mL	NIR method	% recovery	HPLC method	% recovery
sunflower	46.02 (1.35)	46.69 (3.06)	0.6000	66.75 (0.94)	99.6	66.88 (0.85)	98.8
soybean	45.35 (2.01)	45.04 (1.91)	0.6000	67.56 (2.11)	100.5	66.07 (1.78)	98.7
corn	57.10 (1.44)	57.51 (1.18)	0.6000	86.04 (0.74)	99.0	86.42 (1.46)	99.0
mixed seed oils[c]	33.39 (1.14)	34.64 (1.93)	0.6000	49.42 (0.86)	98.4	52.05 (0.94)	101.1
rapeseed	23.54 (2.80)	23.37 (1.58)	0.3000	33.14 (1.15)	101.7	33.32 (0.71)	102.7
grapeseed	17.53 (0.86)	17.05 (1.35)	0.3000	26.66 (1.42)	102.4	26.37 (1.66)	103.2
extra virgin olive oil	19.11 (0.68)	19.02 (0.79)	0.3000	26.41 (0.73)	97.2	26.21 (1.69)	96.8
olive oil[d]	17.98 (1.06)	17.96 (0.84)	0.3000	26.01 (0.97)	97.5	26.90 (1.87)	100.9

[a] $n = 5$. [b] Standard solution of α-tocopherol (10 mg/mL). [c] Mix of rapeseed, soybean, and sunflower oils. [d] Mix of virgin plus refined olive oils.

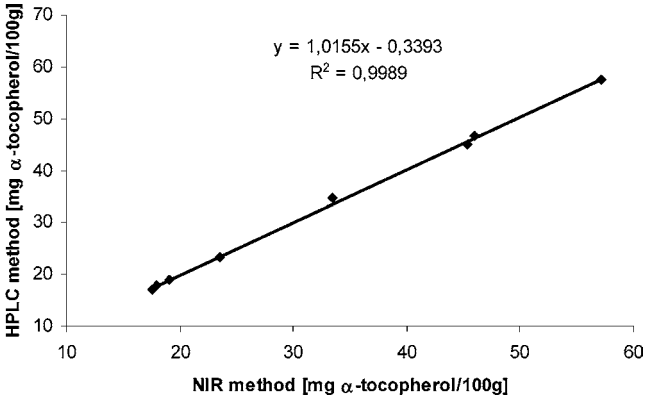

Figure 6. Correlation between HPLC and PLS–NIR methods for the determination of α-tocopherol in oils samples.

limits for the proposed NIR and standard HPLC methods were comparable (**Table 3**).

Statistical analysis of the results obtained by NIR and HPLC methods using the F-test revealed no significant difference between the variances (squares of the standard deviations) of the applied methods at the probability level $P = 0.05$. The calculated F values (the variance ratio of standard HPLC and proposed NIR methods for α-TOH determinations), ranging from 1.18 to 5.25, are below $F_{\text{theoretical}} = 6.39$ (**Table 3**). Therefore, the proposed NIR and standard HPLC methods do not significantly differ in their precision. However, the experimental t-values for the studied α-TOH content in two oil samples, MSO and GO, were higher than the critical value $t_4 = 2.78$ ($P = 0.05$). The results collected in **Table 3** (t-values and confidence intervals) indicate that there are significant differences between the mean concentration of α-TOH in MSO and GO samples, assayed by both analytical methods. Comparison of two experimental means of α-TOH content ($n = 5$) in studied oils indicated that the proposed method in two cases is affected by systematic errors. Although, the results of α-TOH determination in all oils obtained by the two analytical methods do not differ significantly at $P = 0.001$, because t_{calcd} values are below $t_{\text{theoretical}} = 8.61$ (**Table 3**). Therefore, the proposed NIR method gives accurate results for α-TOH determination in edible oils except the two mentioned above cases.

On the other hand, the regression lines for comparing the two analytical methods were used.

The correlation plots between the obtained results of α-TOH determination in different oil samples using proposed NIR and standard HPLC methods are presented in **Figure 6**. A relatively high correlation coefficient of $R^2 = 0.9989$ ($n = 8$) for α-TOH determinations in all studied oils indicates a good agreement between both methods. Moreover, the slope of the regression lines $b = 1.02 \pm 0.034$ was close to the model value of 1. Besides, the confidence limit of the intercept $a = -0.34 \pm 1.23$ includes the ideal value of 0. Therefore, the comparison between the results obtained by standard HPLC and the proposed NIR methods indicates that the two procedures give statistically comparable values of α-TOH concentration in oil samples.

The accuracy of the proposed and standard methods was expressed also as a recovery study, and the results are presented in **Table 4**.

The recoveries of α-TOH added to real sample solutions ranged between 97.2 and 102.4% for the studied NIR method and 96.8−103.2% for the HPLC method, respectively (**Table 4**). Moreover repeatability (calculated using RSD, $n = 5$) for α-TOH determination by both methods did not exceed 3%. Also, the standard deviation of difference for accuracy ($SDD_a = 1.52$) calculated by Ch Man et al. (*37*) indicated that α-TOH in RBD palm olein could be measured with good accuracy by the FTIR technique. Furthermore, similar mean recovery for α-TOH (102.98%) determination in vegetable oils was detected using the HPLC method (*11*). However, Tasioula-Margari et al. reported significantly lower recovery values for α-TOH (77−85%) determination in virgin olive oils by HPLC after four extraction steps (*10*). Furthermore, the standard deviation of difference for accuracy ($SDD_a = 1.52$) calculated by Ch Man et al. (*37*) indicated that α-TOH in RBD palm olein could be measured with good accuracy by the FTIR technique. As can be expected, statistically significant differences between the two techniques were not detected for the examined samples.

Reproducibility (interday precision) of the proposed method was evaluated by performing the determination within 3 days on all oil samples ($n = 5$), and results are reported in **Table 5**.

The reproducibility of the NIR method is satisfactory with RSD ranging between 3.03 and 6.27% for α-TOH concentrations 17.25−56.70 mg/100 g in studied oils. The results were in agreement with those obtained by Sánchez-Pérez et al. (RSD = 6.65%) (*3*) and Tasioula-Margari et al. (RSD < 6%) (*10*). Comparison of the RSD values for repeatability (1.35, 2.01 1.44, 2.80, 1.14, 0.68, 0.86, and 1.06% for α-TOH in SFO, SO, CO, RO, MSO, OO1, GO, and OO2, respectively) with the reproducibility data (3.41, 4.17, 3.03, 6.01, 5.64, 3.22, 5.45 and 6.27%) revealed that the interday precision was approximately 2, 5, and 6 times higher than the intraday precision of the proposed method.

The proposed NIR method is relatively simple, precise, accurate, and convenient for the determination of α-TOH in vegetable oils after direct extraction. The NIR measurements are generally rapid (ca. 1−2 min), but the extraction of tocopherol (60 min., ethanol) from oil samples is required. The application of NIR spectroscopy and the PLS multivariate calibration model allowed for prediction of α-TOH content in

Table 5. Reproducibility Test

oil	statistical parameters: $c_{\alpha-\text{tocopherol}}$,[a] mg/100 g	SD,[a] mg/100 g	RSD,[a] %	confidence limit,[b] mg/100 g
sunflower	45.52	1.55	3.41	45.52 ± 0.86
soybean	45.05	1.88	4.17	45.05 ± 1.04
corn	56.70	1.72	3.03	56.70 ± 0.95
mixed seed oils[c]	33.33	1.88	5.64	33.33 ± 1.04
rapeseed	23.30	1.40	6.01	23.30 ± 0.77
grapeseed	17.25	0.94	5.45	17.25 ± 0.52
extra virgin olive oil	18.96	0.61	3.22	18.96 ± 0.34
olive oil[d]	17.54	1.10	6.27	17.54 ± 0.61

[a] $n = 15$; each value is the mean of five determinations, and each determination was repeated three times. $c_{\alpha-\text{tocopherol}}$, mean concentration of α-tocopherol; SD, standard deviation; RSD, relative standard deviation. [b] Probability level, $P = 0.05$. [c] Mix of rapeseed, soybean, and sunflower oils. [d] Mix of virgin plus refined olive oils.

oil samples. The mean concentrations of α-TOH in studied oils determined by the proposed NIR method agreed with those obtained by the standard HPLC method. Therefore, it can be applied for α-TOH determination in the concentration range between 0.54 and 53.54 mg/mL. It is noteworthy that our NIR method does not require toxic solutions and reagents. Moreover, the cost of the instrumentation is considerably lower than in the case of the standard HPLC method.

The proposed NIR−PLS method can be applied in quality control of edible oils and fats to monitoring their oxidative stability in routine analyses.

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