Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1179–1187
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

MIR spectroscopy and partial least-squares regression for determination of phospholipids in rapeseed oils at various stages of technological process

Aleksandra Szydłowska-Czerniak *

Faculty of Chemistry, Nicholas Copernicus University, ul. Gagarina 7, 87-100 Toruń, Poland

Received 18 October 2006; received in revised form 29 January 2007; accepted 16 February 2007

Abstract

Fourier transform-mid infrared spectroscopy (FT-MIR) and partial least-square (PLS) regression were used for determination of phospholipids (PL) in rapeseed oils at various stages of technological process. The standard error of calibration (SEC) and the standard error of prediction (SEP) were calculated for evaluation of the calibration models. The chemometric calibration model was prepared in spectral region 1760–860 cm^{-1} for standard PL solutions (1.5–120 mg/mL). Obtained mean concentrations of PL in rapeseed oils at different stages of conventional technological operations varied from 22,710 to 224.6 mg/kg. Satisfactory values of precision (RSD = 0.23–0.73%) and accuracy (recovery – 96.1–101.9%), demonstrate the benefit of the proposed MIR-PLS method in the routine analysis of PL in vegetable oils.

Keywords: Phospholipids determinations; Fourier transform-mid infrared spectroscopy; Partial least-square regression; Rapeseed oils

1. Introduction

The quality of fully refined rapeseed oil is influenced by the quality of crude oil and rapeseeds from which the oil is pressed or extracted. Crude rapeseed oils are constituted mainly of triacylglycerols (TAG) but contain some minor components such as phospholipids (PL), free fatty acids (FFA), sterols, tocopherols, pigments. Besides, trace amounts of metals, flavonoids, and glycolipids may also be present (Gunstone, Harwood, & Padley, 1994). PL have moderate antioxidant activity especially in the presence of phenolic antioxidants and/or acidic synergists. They improve the oxidative stability by chelating trace of heavy metals and by decomposing hydroperoxides (Kourimska, Pokorny, & Reblova, 1994). Moreover, PL have therapeutic properties, and they were used to improve human physiological and mental performance, lowering cholesterol levels, and treating neurological disorders (Hidalgo & Zamora, 2006). Nevertheless, PL in rapeseed oil are considered as undesirable impurities causing refining problems, oil losses due to the formation of emulsions during the alkali treatment. Therefore, they must be removed from the crude oil at various steps (degumming, neutralization, washing, drying, bleaching, filtration and deodorization) in conventional chemical refining process. There are two chemically different sources of phosphorus: inorganic phosphates and PL in edible oils. Crude rapeseed oils contain two types of PL: hydratable (HPL) and non-hydratable (NHPL). NHPL, unlike HPL, are phospholipids which during the degumming of crude oil do not hydrate with water, swell, form gels or precipitate from oil and are not separated by centrifugation. Therefore, the PL amounts in crude and refined rapeseed oils must be determined.

PL in vegetable oils most often were analyzed by the chromatographic techniques with different methods of sample preparation by solid-phase extraction (SPE) or pre-concentration achieved by column chromatography

* Tel.: +48 56 6114786; fax: +48 56 6542477.
E-mail address: olasz@umk.pl

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.02.038

(Carelli, Brevedan, & Crapiste, 1997; Chapman, 1980; Kang & Row, 2001; Nomikos, Karantonis, Fragopoulou, & Demopoulos, 2002; Nzai & Proctor, 1998a; Przybylski & Eskin, 1991; Racicot & Handel, 1983; Zhang, Yang, Ren, & Jiang, 2005). Generally PL are separated from edible oils by either normal phase or reversed phase high performance liquid chromatography (HPLC) with evaporative light scattering (ELS) (Zhang et al., 2005) or UV detection at 206 nm (Carelli et al., 1997; Nomikos et al., 2002). Also thin-layer chromatography (TLC) was applied for rapid separation, identification and analysis of PL in vegetable oils (Chapman, 1980; Nzai & Proctor, 1998a). This technique does not require often tedious and time consuming procedure of sample preparation – purification, but quantitative determinations can be affected by errors. Two-dimensional TLC (Chapman, 1980), TLC-imaging densitometry (Nzai & Proctor, 1998a) and TLC-flame ionization detector (FID) (Przybylski & Eskin, 1991) were used for PL identification and determination in soybean, sunflower and canola oils.

Chromatographic techniques appear to be useful for isolation and identification of PL mixtures. However, chromatographic determinations of PL in oils require often tedious and time consuming procedures of sample preparation. Moreover, application of these techniques is not necessary, when the total PL content in studied material is in the order of few hundred mg/kg. Therefore, spectroscopic methods, e.g., UV–vis spectrophotometry (Goh, Tong, & Gee, 1984; PN-88/A-86930, 1988; Szydłowska-Czerniak & Szłyk, 2003; Tosi, Cazzoli, & Tapiz, 1998; Totani, Pretorius, & du Plessis, 1982), FTIR (Nzai & Proctor, 1998b; Nzai & Proctor, 1999) and ^{31}P NMR (Bosco, Culeddu, Toffanin, & Pollesello, 1997; Glonek, 1998) were applied as well for PL analysis in vegetable oils and lecithin. So far, colorimetric methods were used for determination of total content of PL in edible oils after digestion or extraction (PN-88/A-86930, 1988). The PL can be extracted from the vegetable oils and quantified by the phospholipid–prussian blue complex in glacial acetic acid (Totani et al., 1982) and phospholipid–molybdenum blue complex in hexane (Goh et al., 1984), respectively. Also, spectrophotometric method based on formation of the ion-associate of molybdophosphate with Malachite Green (MG) was applied for total phosphorus determination in rapeseed oils after ashing (Szydłowska-Czerniak & Szłyk, 2003). Although these methods are not expensive there are some disadvantages: (a) troublesome because pre-extractions of molybdophosphate are needed to avoid large reagent blanks, (b) not directly applicable, mainly due to low levels of PL in the refined oil and time-consuming procedures of sample preparation (c) the interference caused by pigments present in vegetable oils.

Therefore, new techniques, which will reduce or eliminate such disadvantages, are the subject of studies. The vibrational spectroscopy, such as Fourier transform-mid infrared (FT-MIR) and near infrared (NIR) has been applied in oil analyses for screening procedures (1–4 min). Oxidative stability (Yildiz, Wehling, & Cuppett, 2001), peroxide value (PV) (Van de Voort, Ismail, Sedman, Dubois, & Nicodemo, 1994), iodine value (IV) (Hendl, Howell, Lowery, & Jones, 2001), anisidine value (AV) (Yildiz et al., 2001), free fatty acids (FFA) (Alawi, van de Voort, & Sedman, 2004), unsaturation grade, *trans* and *cis* isomers, conjugated linoleic acids (CLA) percentages (Christy, Egeberg, & Østensen, 2003), tocopherols (Szłyk, Szydłowska-Czerniak, & Kowalczyk-Marzec, 2005) and moisture contents (Che Man & Mirghani, 2000) in edible oils and fats were determined by FT-MIR and FT-NIR. However, only a few reports on applications of MIR in analysis of PL in food were encountered (Nzai & Proctor, 1998b; Nzai & Proctor, 1999; Villé et al., 1995). Only, Nzai and Proctor (1998b) used mid-infrared spectrometry for determination of PL (74–18,200 mg/kg) in crude soybean oil, applying the linear regression equation ($y = 0.0453x + 0.312$) and correlation coefficient ($R^2 = 0.968$) for PL band area (1200-970 cm^{-1}) vs. concentration (0.13–4.0 mg/mL). The proposed FTIR method was comparable to the TLC-ID method ($R^2 = 0.959$). However, Authors did not provide any detail about the multivariate calibration approach expect that analysis was performed using SAS Inc. computer program.

Therefore, in the presented work MIR spectroscopy associated with partial least-squares (PLS) regression, standard error of calibration (SEC) and standard error of prediction (SEP) used for determination of total PL content in rapeseed oils at different stages of conventional technological operations. The developed PLS model was able to improve the selectivity and quantify PL in the studied oils. The validation of the applied PLS model was performed. Moreover, the aim of this work was to compare results of PL determination in rapeseed oils by the proposed MIR-PLS method to those obtained using the official standard vanadomolybdate – VM method (PN-88/A-86930, 1988) and previously described Malachite Green – MG method (Szydłowska-Czerniak & Szłyk, 2003).

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade. *n*-Hexane ($\geqslant$99%) was of GC-grade and purchased from Aldrich. The phospholipids standards: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA) were supplied by Sigma and used without further purification. Mixed phospholipids standard solution (34% PC, 18% PE, 19% PI, 5% PS and 20% PA) was prepared in *n*-hexane. The composition of the standard mixture is representative of the PL content in rapeseed oils. Working solutions of PL (120, 60, 30, 15, 7.5, 3 and 1.5 mg/mL hexane) were obtained using the standard phospholipids mixture.

Ammonium phosphate(V) (5.0×10^{-5} mol/L), ammonium molybdate(VI) (0.1 and 4.05×10^{-2} mol/L), Malachite Green (MG) (2.0×10^{-3} mol/L), polyvinyl alcohol

(PVA) (1%), ammonium metavanadate(V) (2.14×10^{-2} mol/L), potassium dihydrogen ortophosphate (3.23×10^{-2} mol/L), water solutions were prepared from reagents purchased from POCH, Gliwice, Poland. Deionized water (DW) was used for the preparation of solutions and samples for spectrophotometric methods (VM and MG).

2.2. Samples

Six rapeseed oils at various stages of industrial processes: extracted oil after last evaporator (EOLE), extracted oil after lecithin removal (EOLR), crude pressed oil (CPO), neutral pressed oil (NPO), bleached pressed oil (BPO), refined mixed oil (RMO), and refined bleached deodorized rapeseed oil (RBDO) were kindly provided by a local vegetable oil factory. All oils in the original packing (poly(ethylene terephthalate) (PET) or glass bottles) were stored below 10 °C in the dark.

2.3. Instrumentation and software

The MIR spectra were measured using a Perkin Elmer Spectrum 2000 FT-IR Spectrometer at resolution 4 cm^{-1} with a SPECAC temperature variable cell at 294 ± 1 K. MIR absorbance spectra were registered in the range 400–4000 cm^{-1} using a potassium bromide stainless steel sealed precision pathlength 0.12 mm cell. Background spectrum (100 scans) was recorded daily, whereas for samples 50 scans were taken in three repetitions, in order to reduce the instrument noise. The working solutions of standard phospholipids mixture and oil samples dissolved in hexane were loaded onto cell with a microsyringe. The spectrum of solvent was subtracted from each spectrum of sample to eliminate the functional groups common to the oil sample and hexane. In each single measurement the solution of studied oil was introduced onto the cell and the spectrum was recorded. After collection of each spectrum, a new solution was placed onto the cell for the next measurement. The cell was cleaned five times with hexane after each sample analysis.

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

Spectrophotometric determination of total phosphorus (VM and MG methods) was performed with a Helios α – UNICAM spectrophotometer in a 1-cm quartz cell. Total phospholipids contents of the studied oils were calculated basing of the elemental phosphorus contents using factor 26 for conversion of P [mg/kg] to PL [mg/kg] (PN-88/A-86930, 1988).

2.4. FTIR method

The phospholipids content in studied rapeseed oils was determined by measuring of MIR spectra solutions containing 20 mg EOLE, 50 mg EOLR, 50 mg CPO, 700 mg NPO, 700 mg BPO and 700 mg RMO in 1 mL hexane. The obtained spectra were subtracted from a spectra of 20, 50 and 700 mg RBDO/mL hexane to remove the C–H and C–O–C stretching, bending and wagging vibrations due to triglycerides (TAG).

2.4.1. PLS calibration models with using cross-validation

PLS regression was employed to extract relevant information from the complex MIR spectra of PL. The optimum number of PLS factors used for prediction was determined by full cross-validation.

The number of significant PLS factors was chosen by using predicted residual error sum of squares (PRESS) value for every possible factor. The PRESS value was the sum of 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 quality of the calibration models is described by the squared correlation coefficient (R^2), standard error of calibration (SEC), and standard error of prediction (SEP), which both can be interpreted as the average modeling/prediction error, expressed in the same units as concentrations of PL in oil samples. They represent the average difference between predicted and measured response values at the calibration/validation stage (Miller & Miller, 2000).

Single PLS calibration set ($n = 35$) was applied for working solutions (1.5–120 mg/mL) prepared from the mixed standards solutions of PL in hexane. PLS regressions were used to analyze the content of PL in the oil samples.

2.5. Spectrophotometric methods

Samples of rapeseed oils were digested as follows: 0.100 g of MgO and samples (0.1000–10.000 g) were weighed in quartz crucible, placed in a furnace and ashed at 800 °C. Blank digestions were also carried out in the same way.

2.5.1. Polish standard method (vanadomolybdate VM) (PN-88/A-86930, 1988)

Five milliliters of HNO_3 (6.0 mol/L), 10 mL of ammonium molybdate (4.05×10^{-2} mol/L), 10 mL of ammonium metavanadate were added to ashed samples, and the absorbance was measured at 460 nm against a reagent blank, after 20 min.

2.5.2. Malachite Green method (MG) (Szydłowska-Czerniak & Szłyk, 2003)

The ashed samples were dissolved in 20 mL of sulphuric acid (0.5 mol/L), transferred into 50 mL calibrated flask, and made up to the mark with DW. Then 4 mL of the obtained solution, 6.8 mL of the ammonium molybdate (0.1 mol/L), 1.5 mL the Malachite Green and 1 mL of the

PVA solutions were placed in a 50 mL calibrated flask and made up to volume with DW. After 20 min, the absorbance of solution was measured at 640 nm against a reagent blank.

Calibration curves were prepared using working solutions of KH_2PO_4 and $(NH_4)_2HPO_4$ between 0.05–0.4 mg P/mL and 0.0155–0.248 μg P/mL for VM and MG methods, respectively. Five calibration curves were constructed by plotting the absorbance of each analysis versus concentration using the least-squares method. The representative linear regression equations were $y = 0.8661 \pm 0.013x + 0.0128 \pm 0.003$ and $y = 1.990 \pm 0.033x + 0.1121 \pm 0.0022$ with a correlation coefficient of 0.9996 and 0.9992 for VM and MG methods, respectively. The relative standard deviations of the slopes were 2.24% ($n = 5$) for VM and 1.51% ($n = 5$) for MG methods.

2.6. Statistical analysis

The content of PL in the oil samples determined (five portions of each oil were dissolved in hexane and each solution analyzed within 1 day) by the proposed method and were compared with the official standard VM method (PN-88/A-86930, 1988) and MG method (Szydłowska-Czerniak & Szłyk, 2003). The within-day precision of analytical methods, expressed as the relative standard deviation (RSD, %) were assessed. The between-day precision (RSD, %) of the proposed MIR method was checked by determination of PL in the same oils over the period of three days. Five replicate analyses were made at each oils.

The recovery experiments were performed as follows: oil samples were dissolved in hexane, standard phospholipids mixture were added to solutions, and the obtained mixtures shaken and made up to 10 mL with hexane. Accuracy of the proposed MIR method was calculated as the mean percentage of PL recovered in the assay (Recovery [%] = [Found PL − Content PL]/[Added PL] × 100%).

The *F*-test, *t*-test and regression lines for comparing the analytical methods (MIR-VM and MIR–MG) were used. 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 – standard deviations of slope and intercept, respectively. The slope (b) and intercept (a) values were evaluated with the ideal ones (1 for slope and 0 for intercept) using the elliptic joint confidence region (EJCR) test. The equation describing the joint region is $n(a-\alpha)^2 + 2(\Sigma x_i)(a-\alpha)(b-\beta) + (\Sigma x_i)^2(b-\beta)^2 = 2s^2F_{2,n-2}$, where n is the number of data points, s^2 is the regression variance, and $F_{2,n-2}$ is the statistical F value with 2 and $n-2$ degrees of freedom at a confidence level $P = 0.05$, α and β are the true intercept and slope parameters (Mandel & Linnig, 1956).

The detection limit (DL) and the quantification limit (QL) expressed as $DL = (3S_{y/x})/b$ and $QL = (10S_{y/x})/b$, where $S_{y/x}$ – the estimated standard deviation of y-residuals (y – MIR method and x – VM method) and b – the slope of the final calibration model (Miller & Miller, 2000).

3. Results and discussion

3.1. MIR calibration using partial-lest squares regression

FT-MIR absorbance spectra and their first derivatives were examined to identify spectral features that could be correlated with PL concentrations. The absorbance spectra were treated by 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 standard PL mixture in hexane with hexane subtracted (Fig. 1) were used for calibration models construction.

Moreover, first derivative spectra of the RBDO, EOLE in hexane and the different EOLE spectrum subtracted from the RBDO spectrum were presented in Fig. 2.

The calibration models for PL determination were developed using 14 absorption bands at wavelengths which are characteristic for PL functional groups (Table 1).

The distinct combinations of the selected wavenumbers to construction the PLS calibration models with varying wavenumbers from 5 to 14 were used. The standard error of calibration (SEC = 0.77–2.35%) and the standard error of prediction (SEP = 0.85–2.79%) were calculated from each PLS model. The number of factors where the PRESS plot reached a minimum was three for PL and were chosen as the optimum number of factors for the calibration model described in this study.

It was found that MIR spectral range between 1760 and 860 cm^{-1} (bands at 1756, 1305, 1287, 1267, 1245, 1123, 1107, 880 and 864 cm^{-1} from C=O, P=O, PO_2 and C–O–C groups vibrations) was the best for determining PL in the studied system with the lowest SEC = 0.77%, and the highest $R^2 = 0.9947$. The calibration plot was linear, $y = 0.9805 \pm 0.0253x - 0.2413 \pm 1.3244$ in the concentration range between 1.5 and 120 mg/mL for PL determination. Because the correlation points were located along a straight lines, hence the normal distribution of the data can be suggested. The within day precision was found by regressions analysis of (MIR-predicted concentrations of PL) = f(MIR-actual concentrations of the standard solutions of PL) curve, and expressed as the relative standard deviation of the slope, RSD = 0.81% ($n = 5$) (Miller & Miller, 2000).

Moreover, the correlation between the MIR and the standard spectrophotometric method VM values for the calibration set ($n = 15$, the concentration range 1.5–7.5 mg/mL) and validation oil samples ($n = 20$) for studied PL was found. The correlation points were located along a straight lines, hence the linearity of the validation and calibration data can be analyzed. The equation for the MIR predicted values (y, mg/mL) of PL versus the VM values (x, mg/mL) was $y = 0.9863 \pm 0.01984x + 0.0218 \pm 0.09676$ with correlation coefficient $R^2 = 0.9968$. In comparison, Nzai

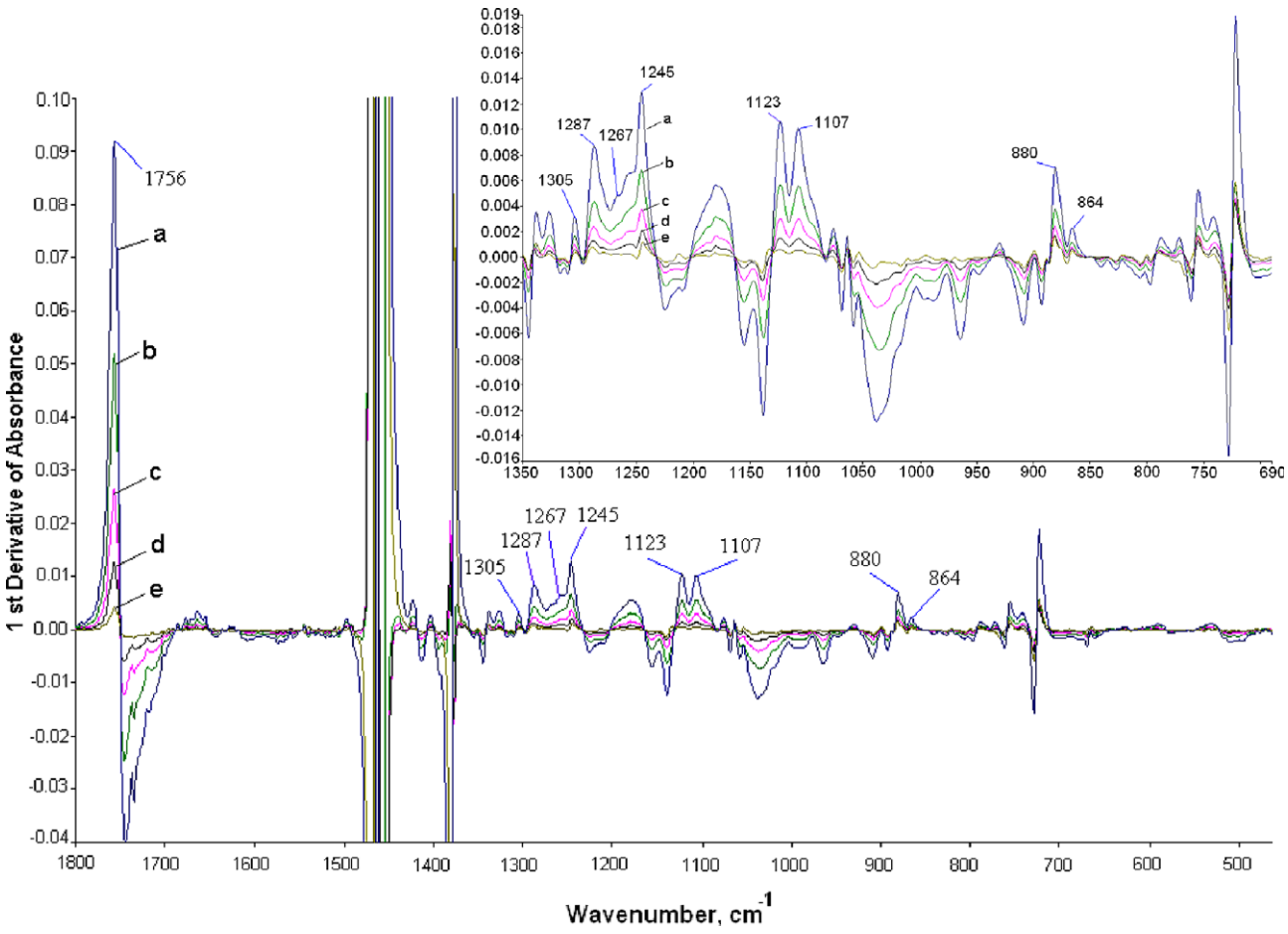

Fig. 1. First derivative of PL spectra for standard solutions: (a) 120 mg/mL, (b) 60 mg/mL, (c) 30 mg/mL, (d) 15 mg/mL, and (e) 1.5 mg/mL.

and Proctor (1998b) found a somewhat lower correlation coefficient ($R^2 = 0.968$) for data from phospholipids standard concentrations (0.13–4.0 mg/mL) versus the band areas between 1200 and 970 cm^{-1}.

Besides, the confidence limits of the slopes $b = 0.9863 \pm 0.01984$ for PL determination include the model value of 1, whereas intercepts $a = 0.0218 \pm 0.09676$ embraces 0. After, the parameters b and a are obtained from the linear fit they are compared with ideal values of 1 and 0 using the elliptic joint confidence region (EJCR) for true slope and intercept according to Mandel and Linnig equation (Mandel & Linnig, 1956). If the point (0, 1) lies inside the ellipse drawn, it can be concluded that proportional and constant biases are absent. As can be seen in Fig. 3, the point (0, 1) lies inside the EJCR (at a confidence level of $P = 0.05$) for regression line.

The detection (DL) and quantification limits (QL) for MIR method were evaluated from 35 independent determinations of PL for standard solutions ($n = 15$) and oil samples ($n = 20$) in the concentration range 1.5–7.5 mg/mL. The calculated DL and QL were 0.42 and 1.40 mg/mL for PL determination, respectively. The obtained results confirm linearity concentrations range (1.5–7.5 mg/mL) for PL determinations in studied oil samples.

3.2. Validation of the proposed MIR method

The total PL contents in the rapeseed oils at various stages of industrial processes were determined by MIR method and compared with these obtained by spectrophotometric methods: VM and MG (Table 2).

The concentrations of total PL in crude rapeseed oils obtained by MIR method (22,710, 6854 and 5656 mg/kg for EOLE, EOLR and CPO, respectively) were significantly higher when compared to studied oils at different steps of the refining process (398.7, 224.6, 240.1 mg/kg for NPO, BPO and RMO, respectively). For comparison, the concentrations of PL in crude and degummed sunflower and soybean oils reported by other authors were in the similar range between 2480–18,200 mg/kg and 74–237 mg/kg, respectively (Nzai & Proctor, 1998b; Totani et al., 1982). It can be noted that, crude oil obtained by pressing has lower PL content (5656 mg/kg) than these obtained by solvent extraction (22,710 and 6854 mg/kg for EOLE, EOLR, respectively). Therefore, the PL amounts in crude oils depend on the method of extraction. Similar PL contents in crude pressed and extracted sunflower and rapeseed oils were determined by Carelli et al. (1997) and Płatek (1998) (7370 and 11,950 mg/kg for

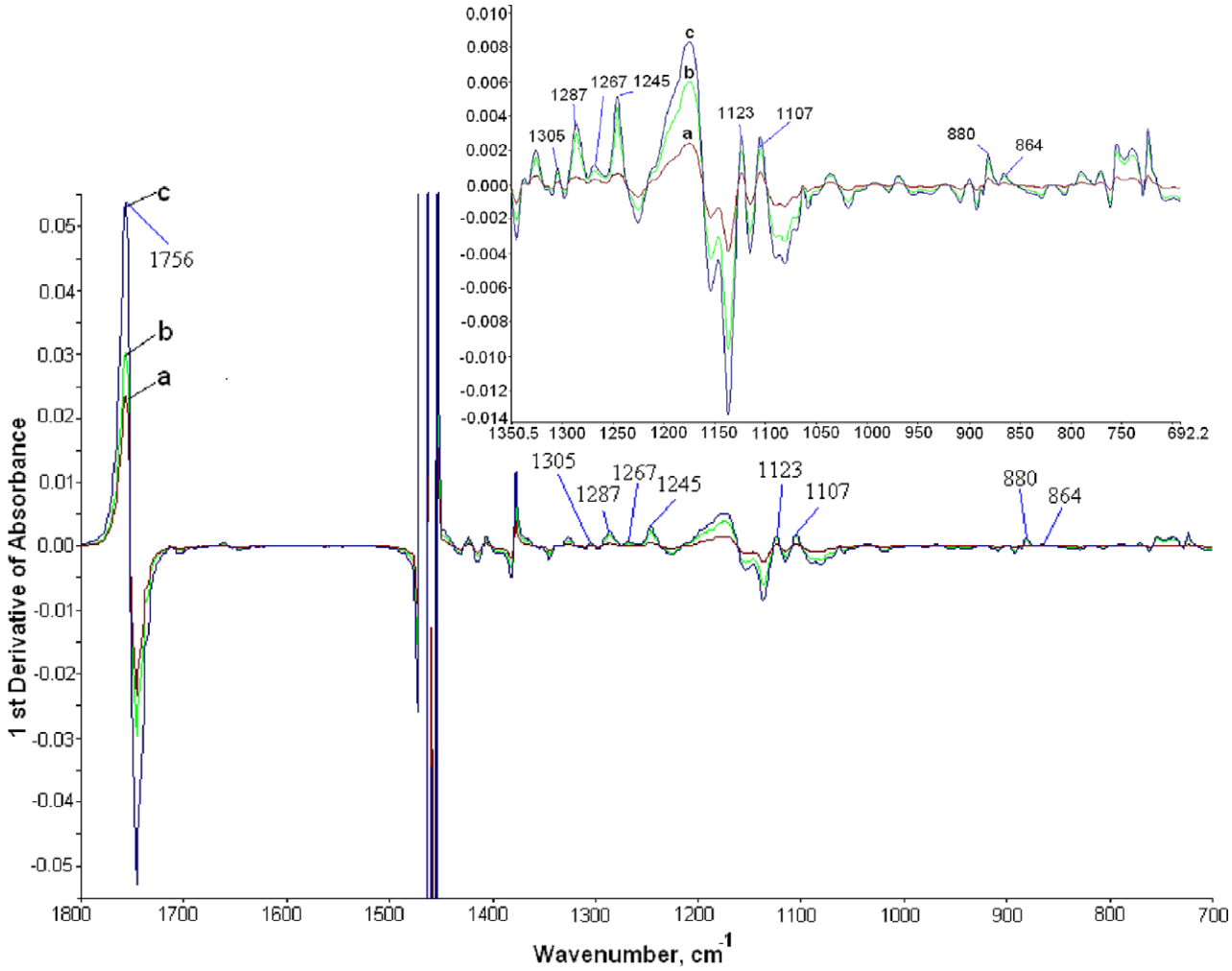

Fig. 2. First derivative of rapeseed oils spectra in hexane: (a) RBDO, (b) EOLE subtracted from the RBDO, and (c) EOLE.

pressed and extracted sunflower oils and 4706 and 12,090 mg/kg for pressed and extracted rapeseed oils, respectively). However, the amounts of PL in EOLE (22,710 mg/kg) and EOLR (6854 mg/kg) oils were about two and three times higher, when compared to results (11,950 and 2070 mg/kg for extracted and extracted–degummed sunflower oil) obtained by Carelli et al. (1997). The lowest concentrations of PL in BPO (224.6 mg/kg) and RMO (240.1 mg/kg) oils were observed. This fact indicated that the refining process of crude rapeseed oil ensures decrease of PL content in refined oil. For comparison, the concentration of total PL in bleached rapeseed oil reported by other authors was in the same range between 208 and 325 mg/kg (Drozdowski, Nowak-Połomska, & Datta, 1994; Płatek, 1998).

Table 1
The results of PLS calibration models for PL determination in different spectral regions

Wavelengths (cm^{-1})	SEC (%)
1756, 1305, 1287, 1267, 1178, 1123, 1107, 1075, 930, 880, 788, 771, 754, 741	0.83
1756, 1305, 1287, 1267, 1245, 1123, 1107, 880, 864	0.77
1756, 1305, 1287, 1267, 1245, 1123, 1107, 930, 771	1.49
1305, 1287, 930, 864, 771	1.28
1305, 1267, 1123, 1107, 930, 880, 788, 741	1.59
1756, 1287, 1267, 1245, 1178, 930, 771	2.35

Assignments of bands: ν(C=O) – 1756 cm^{-1}; ν(P=O) – 1305, 1287, 1267; $\nu(PO_2)_{as}$ – 1245 cm^{-1}; ν(P=O) associated – 1178 cm^{-1}; $\nu(C–O–C)_{as}$ – 1123, 1107, 880, 864 cm^{-1}; $\nu(P–O–C)_{as}$ – 1075 cm^{-1}; $\nu(C–N^{+}–C)_{as}$ – 972 cm^{-1}; δ(C–H) out-of-plane bend – 930 cm^{-1}; $\nu(PO_2)$ – 788, 771, 754, 741 cm^{-1}; (Coates, 2000); ν – stretching vibration, δ – bending vibration; as – asymmetric.

The within-day precisions of the proposed MIR and spectrophotometric VM standard method and MG method were tested by analyses of all oils in five replicates. The values of RSD were below 1.60% for PL determination, indicating reasonable repeatability of used methods (Table 2). In comparison, Nzai and Proctor (1998b) found a similar values of RSD ranging between 0.24% and 2.97% for PL analysis in soybean oils by FTIR method. However, Carelli et al. (1997) and Totani et al. (1982) obtained the somewhat higher relative standard deviations (0.67–8.8%) for PL determined in

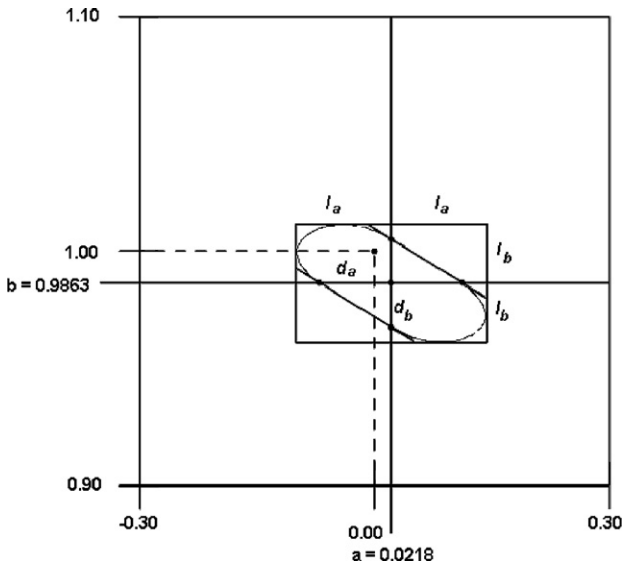

Fig. 3. Elliptic joint confidence region for the slope and intercept of regression line ($y = 0.9863x + 0.0218$) for the PL determination in rapeseed oils, where $l_b = \sqrt{2Fs_b^2} = 0.0251$, $l_a = \sqrt{2Fs_a^2} = 0.1222$, $d_b = \sqrt{4zs_{y/x}^2 F/\sum x \sum x^2 + z} = 0.0187$, $d_a = d_b l_a / l_b = 0.0912$, $z = \sqrt{n\sum x^2}$, $n = 35$; $s_b = 0.0097$, $s_a = 0.0474$ – the standard deviations of slope b and intercept a; $s_{y/x} = 0.1382$ – the standard deviation of y-residuals, $F_{2,33} = 3.32$ ($P = 0.05$).

soybean and sunflower oils at various stages of technological process by SPE-HPLC and colorimetric methods. In addition, the confidence limits for the proposed MIR and the standard VM method and spectrophotometric MG method were comparable (Table 2).

Statistical analysis of the results obtained by MIR and spectrophotometric VM and MG methods using F-test ($P > 0.05$) revealed no significant difference between the variances (squares of the standard deviations) of the applied methods. The calculated F values (the variances ratio of spectrophotometric VM or MG methods and the proposed MIR method for PL determinations), ranging from 1.13 to 5.00, are below $F_{\text{theoretical}} = 6.39$ (Table 2). Therefore, the proposed MIR and the standard VM (PN-88/A-86930, 1988) and MG (Szydłowska-Czerniak & Szłyk, 2003) methods do not significantly differ in their precisions.

However, the experimental t_1-value (4.10) for the studied PL content in EOLR oil was higher than the critical value $t = 2.78$ ($P = 0.05$). The results collected in Table 2 (t-values and confidence intervals) indicate that there are significant differences between the mean concentration of PL in EOLR sample, assayed by both analytical methods: MIR–VM. Comparison of two experimental means of PL content ($n = 5$) in studied oil indicated, that the proposed method MIR in this case is affected by systematic error. Although, the results of PL determination in all oils obtained by the two analytical methods: MIR–MG do not differ significantly at $P = 0.05$, because $t_{\text{calculated}}$ (0.058–2.45) are below $t_{\text{theoretical}} = 2.78$ (Table 2). Therefore, the proposed MIR method gives accurate results of PL determination in rapeseed oils at various stages of technological process except one mentioned above case.

On the other hand, the regression lines for comparing the two analytical methods (MIR–VM and MIR–MG) were used. The calculated slopes of the regression lines $b = 0.9957 \pm 0.0046$ and $b = 1.0039 \pm 0.0047$ for MIR–VM and MIR–MG correlations were close to model value of 1. Besides, the confidence limits of the intercepts $a = -19.64 \pm 46.34$ and $a = -17.04 \pm 46.18$ for MIR–VM and MIR–MG correlations, include the ideal value of 0. Moreover, a relatively high correlation coefficient $R^2 = 1.000$ ($n = 6$) for PL determinations in oils at different stages of technological process, indicates a good agreement between both methods. Therefore, the comparisons between the results obtained by the proposed MIR and the standard VM method (PN-88/A-86930, 1988) or spectrophotometric MG (Szydłowska-Czerniak & Szłyk, 2003) method indicate that the two procedures give statistically comparable values of PL concentration in oil samples.

The accuracy of the proposed MIR were expressed as a recovery study, and the results presented in Table 3.

Table 2
Determination of PL in rapeseed oils at various stages of technological process

Rapeseed oils	MIR method		Spectrophotometric methods				F_1	F_2	t_1	t_2
			VM		MG					
	c_{PL}[a] ± confidence limit[b] (mg/kg)	RSD (%)	c_{PL}[a] ± confidence limit[b] (mg/kg)	RSD (%)	c_{PL}[a] ± confidence limit[b] (mg/kg)	RSD (%)				
EOLE	22710 ± 184	0.65	22811 ± 258	0.91	22624 ± 204	0.73	1.95	1.23	1.04	1.03
EOLR	6854 ± 34	0.40	6926 ± 55	0.64	6852 ± 70	0.82	2.66	4.26	4.10	0.058
CPO	5656 ± 51	0.73	5750 ± 114	1.60	5707 ± 24	0.34	5.00	4.36	2.11	2.45
NPO	398.7 ± 2.8	0.56	403.7 ± 2.9	0.59	401.5 ± 4.4	0.87	1.13	2.44	2.67	2.28
BPO	224.6 ± 1.6	0.57	227.0 ± 1.9	0.67	222.8 ± 1.2	0.40	1.45	1.79	2.20	2.04
RMO	240.1 ± 0.7	0.23	242.1 ± 1.4	0.47	238.8 ± 1.5	0.50	4.25	4.73	2.78	2.41

$F_1^a = s_1^2/s_2^2$ or $F_1^a = s_2^2/s_1^2$; $F_2^a = s_1^2/s_3^2$ or $F_2^a = s_3^2/s_1^2, s_1^2, s_2^2, s_3^2$ – variance of results of PL determinations for MIR, VM and MG methods; F^b theoretical = 6.39; t^b theoretical = 2.78.

[a] c_{PL} – content of phospholipids (mg/kg), $n = 5$.

[b] Probability level, $P = 0.05$.

Table 3
Recovery test

Rapeseed oils	Sample weight (g)	Content PL[a] (mg/mL) (RSD (%))	Added[b] PL (mg/mL)	Found PL[a] (mg/mL) (RSD (%))	Recovery (%)
EOLE	0.200	4.542 (0.65)	2.000	6.522 (0.43)	99.0
EOLR	0.500	3.427 (0.40)	2.000	5.405 (1.06)	98.9
CPO	0.500	2.828 (0.73)	2.000	4.833 (0.57)	100.3
NPO	7.000	2.791 (0.56)	1.000	3.810 (0.41)	101.9
BPO	7.000	1.572 (0.57)	1.000	2.533 (0.75)	96.1
RMO	7.000	1.681 (0.23)	1.000	2.670 (1.11)	98.9

[a] $n = 5$.
[b] Standard solution of PL mixture (10 mg/mL).

Moreover, the standards errors of prediction (SEP) were calculated, for accuracy evaluation of the studied MIR-PLS method, resulting in 0.45% and 0.88% for PL determination in rapeseed oils at different stages of technological process.

The recoveries of PL added to real samples solutions ranged between 96.1 and 101.9% for the proposed MIR method (Table 3). Moreover repeatability (calculated using RSD, $n = 5$) for PL determination by using MIR-PLS method did not exceed 1.5%. Also, the similar recoveries (>90%, RSD = 4.4–7.2%) calculated by Przybylski and Eskin (1991) indicated that PL in canola oils at different stages of processing could be measured with good accuracy by TLC-FID technique. Furthermore, somewhat lower mean recoveries ($86 \pm 2.1\%$ to $98 \pm 1.2\%$) for total PL determination in soybean oils were detected using TLC-ID method (Nzai & Proctor (1998a)). However, Carelli et al. (1997) reported significantly higher recovery values (94.3–107.4%) for PL determination in sunflower oil by SPE-HPLC.

The between-day precision of the proposed MIR method was evaluated by performing the determination within three days on all oil samples ($n = 5$) and the obtained results were satisfactory with RSD ranged between 2.01% and 6.18% for PL concentrations: 22,691–221.1 mg/kg. The RSD values were lower than those calculated by Nzai and Proctor (1998b) (3.07–29.14%) for the standard phospholipids. Comparison of the RSD values for within-day precision (0.65%, 0.40%, 0.73%, 0.56%, 0.57% and 0.23% for PL content in rapeseed oils at different stages of processing: EOLE, EOLR, CPO, NPO, BPO and RMO, respectively) with the between-day precision data (2.73%, 2.01%, 2.45%, 3.53%, 6.18% and 3.54%) revealed that the between-day precision was approximately from three to fifteen times higher than the within-day precision of the proposed MIR method.

4. Conclusions

The proposed MIR-PLS method is relatively simple, convenient and rapid (ca. 1–2 min, spectra collection), therefore can be useful for the nondestructive determination of PL in vegetable oils at various stages of technological process. The application of MIR spectroscopy and PLS multivariate calibration model allowed for prediction of PL content in edible oils. The mean concentrations of PL in studied rapeseed oils determined by the proposed method agreed with those obtained by the standard spectrophotometric VM and the previously described MG methods. Therefore, it can be applied for PL determination in the concentration range between 1.5 and 120 mg/mL. It is noteworthy that MIR-PLS method does not require a toxic solutions and reagents. The main advantage of the proposed MIR-PLS method is that it can be applied in the vegetable oil industry for on-line determination of phospholipids in rapeseed oils.

References

Alawi, A. A., van de Voort, F. R., & Sedman, J. (2004). New FTIR method for the determination of FFA in oils. *Journal of the American Oil Chemists' Society, 81*(5), 441–446.

Bosco, M., Culeddu, N., Toffanin, R., & Pollesello, P. (1997). Organic solvent system for ^{31}P nuclear magnetic resonance analysis of lecithin phospholipids: Applications to two-dimensional gradient-enhanced ^{1}H-detected heteronuclear multiple quantum coherence experiments. *Analytical Biochemistry, 245*(1), 38–47.

Carelli, A. A., Brevedan, M. I. V., & Crapiste, G. H. (1997). Quantitative determination of phospholipids in sunflower oil. *Journal of the American Oil Chemists' Society, 74*(5), 511–514.

Chapman, G. W. Jr., (1980). A conversion factor to determine phospholipid content in soybean and sunflower crude oils. *Journal of the American Oil Chemists' Society, 57*(9), 299–302.

Che Man, Y. B., & Mirghani, M. E. S. (2000). Rapid method for determining moisture content in crude palm oil by Fourier Transform Infrared Spectroscopy. *Journal of the American Oil Chemists' Society, 77*(6), 631–637.

Christy, A. A., Egeberg, P. K., & Østensen, E. T. (2003). Simultaneous quantitative determination of isolated trans fatty acids and conjugated linoleic acids in oils and fats by chemometric analysis of the infrared profiles. *Vibrational Spectroscopy, 33*(1–2), 37–48.

Coates, J. (2000). Interpretation of infrared spectra, a practical approach. In R. A. Meyers (Ed.), *Encyclopedia of analytical chemistry* (pp. 10815–10837). Chichester: John Wiley.

Drozdowski, B., Nowak-Połomska, G., & Datta, I. (1994). Effect of some minor compounds on the kinetics of oxidation of double improved rapeseed oil. *Polish Journal of Food and Nutrition Sciences, 3*(44), 63–72.

Glonek, T. (1998). ^{31}P NMR phospholipid analysis of anionic-enriched lecithins. *Journal of the American Oil Chemists' Society, 75*(5), 569–573.

Goh, S. H., Tong, S. L., & Gee, P. T. (1984). Total phospholipids in crude palm oil: Quantitative analysis and correlations with oil quality parameters. *Journal of the American Oil Chemists' Society, 61*(10), 1597–1600.

Gunstone, F. D., Harwood, J. L., & Padley, F. B. (1994). *The lipid handbook* (2nd ed.). London: Chapman & Hall, pp. 265–279.

Hendl, O., Howell, J. A., Lowery, J., & Jones, W. (2001). A rapid and simple method for the determination of iodine values using derivative Fourier Transform Infrared measurements. *Analytica Chimica Acta, 427*(1), 75–81.

Hidalgo, F. J., & Zamora, R. (2006). Peptides and proteins in edible oils: Stability, allergenicity, and new processing trends. *Trends in Food Science & Technology, 17*(2), 56–63.

Kang, D. H., & Row, K. H. (2001). High-purity separation of phospholipids by preparative high-performance liquid chromatography. *Journal of Industrial and Engineering Chemistry, 7*(3), 178–182.

Kourimska, L., Pokorny, J., & Reblova, Z. (1994). Phospholipids as inhibitors of oxidation during food storage and frying. *Prehrambenotehnol Biotehnol. Rev., 32*(2–3), 91–94.

Mandel, J., & Linnig, F. (1956). Statistical methods in chemistry. *Analytical Chemistry, 28*(4), 770–777.

Miller, J. N., & Miller, J. C. (2000). *Statistic and chemometrics for analytical chemistry* (4th ed.). Pearson Education, Harlow.

Nomikos, T., Karantonis, H. C., Fragopoulou, E., & Demopoulos, C. A. (2002). One-step separation system for the main phospholipids, glycolipids, and phenolics by normal phase HPLC Application to polar lipid extracts from olive and sunflower oils. *Journal of Liquid Chromatography & Related Technologies, 25*(1), 137–149.

Nzai, J. M., & Proctor, A. (1998a). Phospholipids determination in vegetable oil by thin-layer chromatography and imaging densitometry. *Food Chemistry, 63*(4), 571–576.

Nzai, J. M., & Proctor, A. (1998b). Determination of phospholipids in vegetable oil by Fourier Transform Infrared Spectroscopy. *Journal of the American Oil Chemists' Society, 75*(10), 1281–1289.

Nzai, J. M., & Proctor, A. (1999). Soy lecithin phospholipid determination by Fourier Transform Infrared Spectroscopy and the acid digest/arseno-molybdate method: A comparative study. *Journal of the American Oil Chemists' Society, 76*(1), 61–66.

Płatek, T. (1998). Fosfolipidy a skuteczność odszlamowania oleju rzepakowego. *Tłuszcze Jadalne, 33*(1–2), 44–55.

Przybylski, R., & Eskin, N. A. M. (1991). Phospholipid composition of canola oils during the early stages of processing as measured by TLC with flame ionization detector. *Journal of the American Oil Chemists' Society, 68*(4), 241–245.

Racicot, L. D., & Handel, A. P. (1983). Degumming of soybean oil: Quantitative analysis of phospholipids in crude and degummed oil. *Journal of the American Oil Chemists' Society, 60*(6), 1098–1101.

Standard Methods for the Analysis of Oils and Edible Vegetable Fats. (1988). Determination of the phosphorus content (PN-88/A-86930).

Szłyk, E., Szydłowska-Czerniak, A., & Kowalczyk-Marzec, A. (2005). NIR spectroscopy and partial least squares (PLS) regression for determination of natural α-tocopherol in vegetable oils. *Journal of Agricultural and Food Chemistry, 53*(18), 6980–6987.

Szydłowska-Czerniak, A., & Szłyk, E. (2003). Spectrophotometric determination of total phosphorus in rape seeds and oils at various stages of technological process: Calculation of phospholipids and non-hydratable phospholipids contents in rapeseed oil. *Food Chemistry, 81*(4), 613–619.

Tosi, E. A., Cazzoli, A. F., & Tapiz, L. M. (1998). Phosphorus in oil. Production of molybdenum blue derivative at ambient temperature using noncarcinogenic reagents. *Journal of the American Oil Chemists' Society, 75*(1), 41–44.

Totani, Y., Pretorius, H. E., & du Plessis, L. M. (1982). Extraction of phospholipids from plant oils and colorimetric determination of total phosphorus. *Journal of the American Oil Chemists' Society, 59*(4), 162–163.

Van de Voort, F. R., Ismail, A. A., Sedman, J., Dubois, J., & Nicodemo, T. (1994). The determination of peroxide value by Fourier Transform Infrared Spectroscopy. *Journal of the American Oil Chemists' Society, 71*(9), 921–926.

Villé, H., Maes, G., De Schrijver, R., Spincemaille, G., Rombouts, G., & Geers, R. (1995). Determination of phospholipid content of intramuscular fat by Fourier Transform Infrared Spectroscopy. *Meat Science, 41*(3), 283–291.

Yildiz, G., Wehling, R. L., & Cuppett, S. L. (2001). Method for determining oxidation of vegetable oils by near infrared spectroscopy. *Journal of the American Oil Chemists' Society, 78*(5), 495–502.

Zhang, Y., Yang, Y., Ren, Q., & Jiang, H. (2005). Quantification of soybean phospholipids in soybean degummed oil residue by HPLC with evaporative light scattering detection. *Journal of Liquid Chromatography and Related Technologies, 28*(9), 1333–1343.