SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Print: 1304-7582, Online: 2146-9377
http://www.academicfoodjournal.com

Akademik Gıda 12(2) (2014) 6-10

Research Paper / Araştırma Makalesi

Determination of Polar Compound Contents of Vegetable Oils by Differential Scanning Calorimetry and Fourier Transformed Mid-Infrared Spectroscopy

Magdalena Reder ✉, Joanna Bryś, Ewa Ostrowska Ligęza, Hanna Ciemniewska-Żytkiewicz, Katarzyna Sujka, Piotr Koczoń

Department of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska St. 166, 02-787 Warsaw, Poland

Received (Geliş Tarihi): 18.05.2014, Accepted (Kabul Tarihi): 25.06.2014
✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): magdalena_reder@sggw.pl (Magdalena Reder)*
✆ +48 22 59 37 607 🖷 +48 22 59 37 635

ABSTRACT

This study evaluates two different approaches (application of equation given in literature using data from DSC coupled with GC data as well as FT-MIR spectroscopy data) to determine total polar compound (TPC) contents of 20 vegetable oil samples. Moreover, FT-MIR spectroscopy exclusively was tested as an alternative approach for estimating PUFA contents of oil samples. Column chromatography was used as a reference method for determining TPCs. Enthalpy of crystallization was determined with DSC while polyunsaturated fatty acids content with GC. Infrared spectra were registered in the range of 4000 – 370 cm^{-1}. Advanced statistical models using principal components to describe relationship between spectral data and the data on content of TPC and PUFA were calibrated and validated. Results indicated the DSC method to be not accurate enough for the determination of TPCs of oils. On the other hand, FT-MIR method was suitable for the determination of both total polar compounds and polyunsaturated fatty acids contents of vegetable oil samples.

Key Words: Total polar compounds, DSC, FT-IR, GC, vegetable oils.

Bitkisel Yağlarda Polar Bileşik İçeriğinin Diferansiyel Taramalı Kalorimetre ve Fourier Dönüşümlü Orta-Kızılötesi Spektroskopi ile Belirlenmesi

Bu çalışmada 20 bitkisel yağ örneğindeki toplam polar bileşiklerin (TPC) belirlenmesi için gaz kromatografisi (GC) verileri ile birleşik diferansiyel taramalı kalorimetre (DSC) ve Fourier dönüşümlü orta-kızılötesi spektroskopi (FT-MIR) verileri kullanılarak literatürde verilen eşitliğin uygulanması olarak iki farklı yaklaşım değerlendirilmiştir. Ayrıca örneklerdeki çoklu doymamış yağ asitleri (PUFA) içeriğinin tahmin edilmesi için alternatif bir yaklaşım olarak FT-MIR spektroskopisinin kullanılabilirliği test edilmiştir. Toplam polar bileşik miktarının belirlenmesi için referans metot olarak kolon kromatografisi kullanılmıştır. Kristalizasyon entalpisi DSC, PUFA içeriği ise GC ile belirlenmiştir. Kızılötesi spektrum 4000 – 370 cm^{-1} arasında belirlenmiştir. Spektral veriler ile TPC ve PUFA içeriği verileri arasındaki ilişkiyi tanımlamak için temel bileşenler kullanılarak ileri istatistiksel modeller kalibre ve valide edilmiştir. Elde edilen veriler çalışmada kullanılan bitkisel yağlardaki TPC içeriğinin belirlenmesi için DSC yönteminin yeterince hassas olmadığını göstermiştir. Diğer taraftan FT-MIR yönteminin hem TPC hem de PUFA içeriğinin belirlenmesi için uygun bir metot olduğu bulunmuştur.

Anahtar Kelimeler: Toplam polar bileşik, Diferansiyel taramalı kalorimetre, Fourier dönüşümlü kızılötesi, Gaz kromatografisi, Bitkisel yağ.

INTRODUCTION

The content of total polar compounds (TPC) is recognized and commonly used quality measure of oils. The standard method used for TPC level determination bases on separation of a sample by column chromatography, followed by gravimetric measurement. Although accurate it is time consuming, laborious and requires high amounts of organic solvents [1]. Due to mentioned disadvantages, several attempts have been made to apply rapid, accurate and environmentally friendly technique for determination of TPC content in oils as reported in literature [2-5]. Methodology that bases on solid-phase extraction and size exclusion chromatography reduces the usage of chemicals, however is not considered rapid since it takes up to one hour to analyze one sample [2]. High performance liquid chromatography with evaporative light scattering detection has been proposed as accurate and less time consuming than standard method [3]. Various additional rapid techniques have been also investigated, e.g. methods based on dielectric properties of oils, nuclear magnetic resonance or spectrophotometric measurements [4, 5].

Differential scanning calorimetry (DSC) has been used extensively for studying thermal oxidation of vegetable oils. Studies done previously by other authors reported, that the content of compounds formed during thermal processing, such as polar compounds, influence the crystallization process of oils and thus crystallization parameters [6-8]. According to literature data, the content of total polar compounds and the enthalpy of crystallization are statistically related by specific exponential equation [1].

Fourier Transformed Infrared Spectroscopy (FT-IR) enables fast and accurate evaluation of vegetable oils quality without use of organic solvents or specific sample preparation and with use of minimal amount of sample, by rapid determination of specific oil characteristics such as: peroxide value, anisidine value, acid value or iodine value [9-12]. Limited number of papers report on the use of FT-IR spectroscopy for determination of TPC content in oils, however data refer exclusively to near infrared region (NIR) and consider samples subjected previously to thermal processing [3].

The purpose of this study was to evaluate ability of both DSC and Fourier Transformed Infrared Spectroscopy in middle region (MID) as rapid and convenient methods for the determination of total polar compounds content in fresh, expired and used frying oils. Additionally attempt was made to relate FT-IR data with GC data on content of PUFA.

MATERIALS and METHODS

Materials

Twenty samples of studied oils included: 6 fresh rapeseed (R1-R4) and frying oils (F1, F2) obtained in Warsaw, Poland 4 expired rapeseed oils (P1-P4) and 10 used frying oils collected from restaurants located in central Poland (S1-S13). As declared by the owners of gastronomy points those oils were rapeseed oils as well. All chemicals used were of analytical grade and purchased from Sigma Aldrich.

PUFA Content by Gas Chromatography

The composition of fatty acids was determined by gas capillary chromatography according to procedure described by Reder et al. [13]. Oil samples were converted into fatty acid methyl esters (FAME) during methylation process with potassium methoxide as a catalyst. Samples of FAME dissolved in hexane were subjected to gas chromatography analysis.

Gas chromatography analysis was performed using GC–17A Shimadzu apparatus (Shimadzu, Japan) equipped with flame ionization detector and BPX70 capillary column. The following temperature programming was used: 60°C was maintained for 1 min, then it was increased to 170°C with the rate of 10°C min^{-1}; from 170 to 230°C it was increased by 3°C min^{-1}; then kept at 230°C for 15 min. The temperature of the split injector was 225°C, with a split ratio of 1:100, and the detector temperature was 250°C, nitrogen was used as the carrier gas. The identification of fatty acids was carried out using the lipid standard purchased from Sigma Aldrich. Peak areas corresponding to each fatty acid were measured. The percentage of each fatty acid was computed in GC Clarity software, those results were used for calculating the PUFA content.

TPC Content by Column Chromatography

2.5 g of sample was weighted with the accuracy of 0.001 and diluted with the 20 mL of petroleum ether:diethyl ether solution (eluent solvent, 80:20 v/v). Samples were separated into triacylglycerols (nonpolar fraction) and polar fraction in glass column filled with silica gel and eluent solvent. Nonpolar fraction was eluted for 50 minutes with 150 mL of eluent mixture. After evaporation of the solvent the percentage of TPC was determined gravimetrically, the result was calculated according to the following equation (1):

$$TPC\% = \frac{m_s - m_n}{m_s} \times 100\% \quad (1)$$

TPC – total polar compounds, m_s – initial sample mass, m_n – nonpolar fraction mass

Heat of Crystallization by Measurements of Differential Scanning Calorimetry

Q200 differential scanning calorimeter (TA Instruments) was used to obtain presented beneath data. 3 – 4 mg of the sample was weighted in the aluminium pan and sealed non-hermetically, while empty pan was used as a reference. During the measurement the temperature decreased from 10°C to -80°C at the rate of 1°C/min, the DSC cell was purged with nitrogen at 50 mL/min. The instrument was calibrated with standard pure indium. The enthalpies of crystallizations were obtained by calculating crystallization peak areas with the use of

TA Universal Analysis 2000 software. Every measurement was done in three replicates.

FT-IR Measurements

Infrared spectra were registered in the spectral range of 4000 – 370 cm^{-1}, with the use of Perkin Elmer System 2000 spectrometer. 25 scans for each oils sample were conducted in transmission mode, with resolution set to 4 cm^{-1} and shift velocity 2 cm/s. Resulting spectrum of every sample was average of three measurements. Spectral data was collected and processed with PEGRAMS software, baseline and offset corrections were applied.

Statistical Analysis

Multiple Range Test was performed at the confidence level of 95%, in Statgraphics Plus 5.1 software. Tukey's method was used for estimating the honestly significant difference. Statistical model describing the relation between spectral data and the content of total polar compounds was created with the use of TQ ANALYST 8 software. The values of coefficients of determination (R^2) and root mean square errors (RMSE) were computed in Microsoft Excel 2012 software.

RESULTS and DISCUSSION

Reference analysis and DSC results

Results of polyunsaturated fatty acids content, the content of total polar compounds and enthalpy of crystallization are presented in Table 1. TPC content in studied oils ranged from 0.84 to 7.77%, being statistically greater for used frying oils than for fresh oils ($p<0.05$). The example of cooling DSC curve is presented in Fig. 1. Enthalpy of crystallization was statistically smaller for samples with greater content of TPC ($p<0.05$), similar tendency was observed by Gloria and Aguilera [7], Tan and Che Man [8], Cuvelier et al. [1]. The content of polyunsaturated acids ranged from 10.0 to 39.9%.

Table 1. Enthalpy of crystallization and the content of total polar compounds and polyunsaturated fatty acids in studied oils*

Sample	PUFA [%]	TPC [%]	E_c [J/g]
S1	25.8±0.3^{b}	4.7±0.6^{cd}	39.3±1.6^{abc}
S2	28.8±0.4^{ef}	4.2±0.5^{bcd}	41.8±2.04^{c}
S4	27.4±0.1^{cd}	7.8±0.7^{f}	37.1±1.4^{abc}
S5	26.6±0.0^{bc}	4.7±0.5^{cd}	34.5±1.1^{a}
S7	28.2±0.3^{de}	3.7±0.6^{abc}	35.3±1.1^{ab}
S8	25.8±0.0^{b}	7.1±0.5^{f}	40.4±1.1^{bc}
S9	27.3±0.4^{cd}	4.2±0.6^{bcd}	37.2±2.6^{abc}
S10	28.3±0.3^{def}	5.7±0.6^{ef}	40.5±1.4^{bc}
S11	30.3±0.1^{h}	6.1±0.4^{ef}	38.8±1.6^{abc}
S13	29.1±0.0^{efg}	3.8±0.8^{abc}	42.6±2.3^{c}
R1	26.9±0.4^{c}	1.1±0.2^{a}	48.0±1.8^{d}
R2	29.2±0.5^{fg}	0.9±0.7^{a}	49.5±2.1^{d}
R3	29.1±0.3^{efg}	1.5±0.6^{a}	48.8±1.5^{d}
R4	28.3±0.1^{ef}	0.8±0.6^{a}	48.9±2.4^{d}
F1	39.9±0.1^{i}	1.6±0.4^{a}	47.6±1.3^{d}
F2	10.0±0.0^{a}	1.7±0.4^{a}	62.7±3.4^{e}
P1	28.4±0.2^{ef}	2.4±0.8^{ab}	46.8±2.1^{d}
P2	30.0±0.2^{gh}	2.8±0.6^{abc}	49.3±2.6^{d}
P3	29.2±0.3^{efg}	1.5±0.4^{a}	47.6±2.1^{d}
P4	29.1±0.0^{ef}	1.8±0.5^{a}	48.3±1.2^{d}

*: PUFA – polyunsaturated fatty acids, TPC – total polar compounds, E_c – enthalpy of crystallization; homogenous groups are labeled with letters (a-i).

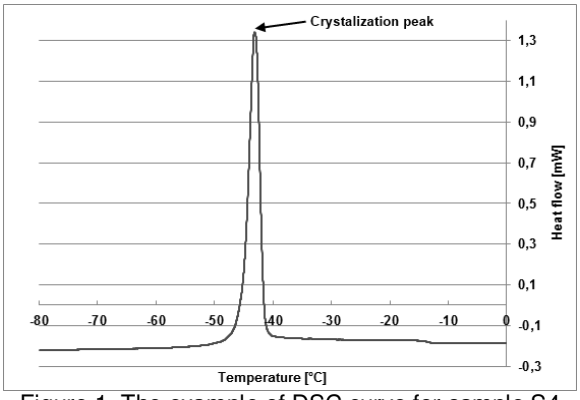

Figure 1. The example of DSC curve for sample S4

Determination of TPC by Differential Scanning Calorimetry

The content of TPC was determined according to the methodology described by Cuvelier et al. [1], with following equation (2):

$$TPC\,(\%) = (35.27 - 0.7492 \cdot \Delta H) + 5.004 \cdot PUFA \cdot e^{\frac{-PUFA}{10.10}} \quad (2)$$

*: TPC – total polar compounds, E_c – enthalpy of crystallization, PUFA – polyunsaturated fatty acids content.

In order to validate the results the coefficient of determination and root mean square error were calculated. Relatively small value of R^2 (0.62) and great value of RMSE (9.70) indicated that the results on content of TPC obtained by DSC did not correlate with values determined by column chromatography at statistically significant level. Cuvelier et. al [1] using developed equation obtained significantly better results with R^2=0.94 and RMSE below 2. Similarly other authors evidenced for good agreement of DSC and CC data [6-8].

To improve constants of equation applied, recalibration was carried out using nonlinear regression technique with use of Statgraphics Plus 5.1 software. Accordingly function constants used for the estimation of the TPC content (2) have been modified as follows (3):

$$TPC(\%) = (18.2859 - 0.3443 \cdot \Delta H) + 52.2576 \cdot PUFA \cdot e^{\frac{-PUFA}{21.543}} \quad (3)$$

*: TPC – total polar compounds, E_c – enthalpy of crystallization, PUFA – polyunsaturated fatty acids content.

Application of improved mathematical model resulted in lower value of RMSE (1.21) while R^2 remained at the same level (0.62).

Obtained results indicated that proposed model could not be used for the accurate determination of TPC content in studied oils. Research conducted previously by Cuvelier et al. [1] indicate however, that presented model yields correct results for samples of oils heated in laboratory conditions. The reason for these discrepancies could be that the used frying oils generated by food industry have much more complex composition than oils heated without foodstuff (which was investigated in previous research). In order to apply the DSC method for the evaluation of the content of total polar compounds in food industry further research is needed, focused on investigation of different types of functions correctly describing TPC and DSC data relation.

Determination of TPC and PUFA Content by Fourier Transformed Mid-Infrared Spectroscopy

Statistical parameters of models obtained are given in Table 2. Spectral regions used for estimating TPC and PUFA content were selected with use of Statistical Spectra diagnostic available in TQ ANALYST software. For the content of total polar compounds best results were obtained with use of following spectral regions for the calibration process: 2988 – 2847 cm^{-1}, 1766 – 1722 cm^{-1}, 1479 – 1439 cm^{-1}, and 1217 – 1128 cm^{-1}, respectively. Great value of R^2 (Table 2) and relatively small value of RMSE (Table 2) indicated that there is strong, statistically significant correlation between spectral data and both GC data on PUFA content and CC data on TPC content. This statement is further confirmed and visually presented by Actual vs. Predicted scatter plots, describing the good agreement between data obtained by FT-MIR and standard methods (see TPC – Figure 2, GC - Figure 3, respectively).

Table 2. Statistical parameters of PLS models§

Parameter	Mean	Number of factors	Number of calibration samples	Number of validation samples	R^2	RMSE
The content of polar compounds	3.40	6	16	4	0.90	0.67
The content of polyunsaturated fatty acids	27.9	8	15*	3*	0.91	0.40

§: R^2 – coefficient of determination, RMSE – root mean square error
*: Samples F1 and F2 were identified as an outliers

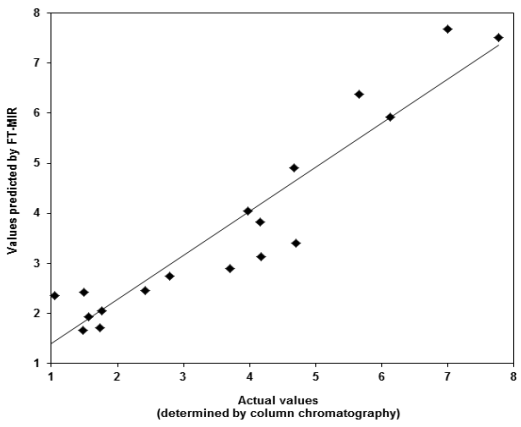

Figure 2. Actual vs. predicted plot for the total polar compounds content

Data from spectral regions: 3077 – 2973 cm^{-1}, 1821 – 1663 cm^{-1}, 1232 – 1123 cm^{-1}, 1008 – 936 cm^{-1}, 775 – 643 cm^{-1} were found to be highly appropriate for the estimation of polyunsaturated fatty acids content. The great R^2 and small RMSE followed by graphic interpretation of correlation (Table 2, Figure 3, respectively) evidence correlation between FT-IR data and reference data (GC) at statistically significant level, therefore strong ability of FT-IR to be applied for indirect PUFA determination.

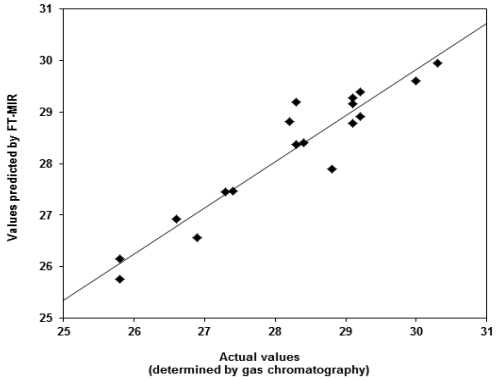

Figure 3. Actual vs. predicted plot for the polyunsaturated fatty acids content

CONCLUSIONS

Obtained results indicated that DSC method considered for determination of the content of total polar compounds in oils used at gastronomy points is not adequately accurate. It is in contrast to literature data, however former were obtained for samples of oils heated in laboratory conditions. Significantly better

results covering rapid and satisfactory accurate indirect measurement of TPC in oils were deduced from FT-MIR spectral data, as great correlations between them and data on both TPC and PUFA content were proved to exist. The chief point of replacing standard methods with FT-MIR spectroscopy is significant reduction of analysis time from one hour to several seconds, and reduced usage of organic solvents and sample amount.

REFERENCES

[1] Cuvelier M-E., Lacoste F., Courtois F., 2012. Application of a DSC model for the evaluation of TPC in thermo-oxidized oils. *Food Control* 28(2): 441-444.

[2] Marquez-Ruiz G., Jorge N., Martfn-Polvillo M., Dobarganes M.C., 1996. Rapid, quantitative determination of polar compounds in fats and oils by solid-phase extraction and size-exclusion chromatography using monostearin as internal standard. *A Journal of Chromatography* 749(1-2): 55-60.

[3] Anton Kaufmann A., Ryser B., Suter B., 2001. HPLC with evaporative light scattering detection for the determination of polar compounds in used frying oils. *European Food Research and Technology* 213(4-5): 372–376.

[4] Hein M., Henning H, Isengard H-D., 1998. Determination of total polar parts with new methods for the quality survey of frying fats and oils. *Talanta* 47(2): 447-454.

[5] Xu X-Q., 1999. A Modified VERI-FRY Quick Test for Measuring Total Polar Compounds in Deep-Frying Oils. *Journal of American Oils Chemists' Society* 76(9): 1087-1089.

[6] Chiavaro, E., Rodriguez-Estrada, M. T., Vittadini, E., & Pellegrini, N., 2010. Microwave heating of different vegetable oils: relation between chemical and thermal parameters. *LWT - Food Science and Technology* 43(7): 1104-1112.

[7] Gloria, H., Aguilera, J.M., 1998. Assessment of the quality of heated oils by differential scanning calorimetry. J. Agric. Food Chem. 46(4): 1363–1368.

[8] Tan C.P., Che Man Y.B., 1999. Quantitative differential scanning calorimetric analysis for determining total polar compounds in heated oils. *Journal of the American Oil Chemists' Society* 76(9): 1047-1057.

[9] 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 American Oils Chemists' Society* 71(9): 921-926.

[10] Dubois J., van de Voort F.R., Sedman J., Ismail A. A., Ramaswamy H.R.., 1996. Quantitative Fourier transform infrared analysis for anisidine value and aldehydes in thermally stressed oils. *Journal of American Oils Chemists' Society* 73(6): 787-794

[11] Hendl O., J.A. 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.

[12] Rao Y., Xiang B., Zhou X., Wang Z., Xie S., Xu J. Quantitative and qualitative determination of acid value of peanut oil using near-infrared spectrometry. *Journal of Food and Engineering* 93(2): 249-252.

[13] Reder M., Koczoń P., Wirkowska M., Sujka K., Ciemniewska-Żytkiewicz H. 2014. The application of FT-MIR spectroscopy for the evaluation of energy value, fat content, and fatty acid composition in selected organic oat products. *Food Analytical Methods* 7(3): 547-554.