ICNTC-2019 Special Issue

J. Indian Chem. Soc.,
Vol. 96, September 2019, pp. 1239-1244

The influence of chemical composition on peroxidation kinetics of palm and nut oils

Temel kan Bakır

Faculty of Science and Letters, Department of Chemistry, Kastamonu University, Kastamonu, Turkey

E-mail: temelkan@kastamonu.edu.tr

Manuscript received online 26 April 2019, revised and accepted 20 July 2019

In this study, the fatty-acid content of peanut, hazelnut, coconut and palm oils were determined by gas chromatography (GS-MS), and correlated to the kinetics of lipid oxidation. The peroxidation of oil emulsions was performed at in a ventilated incubation environment at 37°C and pH 7. Primary products (hydroperoxides) and the secondary products (malondialdehyde) were monitored by ferric thiocyanate method (Fe(III) SCN) and thiobarbituric acid reactive substances (TBARS) analytical methods, respectively. Pseudo-first order kinetics of hydroperoxides and aldehydes were observed in copper-catalysed oil emulsions, and the absorbance values obtained as a function of the incubation period resulted in sigmoidal curves. The rate constants found via Fe(III) SCN method were calculated to be in the following descending order: peanut oil > hazelnut oil > palm oil > coconut oil, whereas that via TBARS method yielded peanut oil > hazelnut oil > coconut oil > palm oil. This study showed that the fatty acid content and peroxidation kinetics data can be used together in the selection of more nutritious fats with a longer shelf life.

Keywords: Ferric thiocyanate method, palm oil, hazelnut oil, lipid peroxidation, fatty acids.

Introduction

Determination of saturated and unsaturated fatty acid compositions which may vary according to genetic, ecological and morphological properties and their effects on oil quality are important in clarifying the physicochemical properties of oils[1,2]. In addition to these, the usage times, oxidation resistance, antioxidant capacity should be known. These parameters are closely related to fatty acid contents, iodine indices and refractive indices in oils[3].

Fatty acid profiles that give information about the nutritional value and quality of the oil have different biochemical properties[4,5]. For example, unsaturated fatty acids have been shown to prevent atherosclerosis and coronary heart disease[6]. Although unsaturated fatty acids are healthier than saturated fatty acids, saturated fatty acids undergo less peroxidation than their unsaturated equivalents[1]. Because of this oxidative imbalance, polyunsaturated fatty acids are limited in their use as nutritionally useful lipids in functional foods[7,8]. Partial hydrogenation is often applied to reduce the oxidation potential of unsaturated fatty acids, however, this can lead to the formation of harmful *trans* unsaturated fatty acids. Many studies have shown that *trans* unsaturated fatty acids are more atherogenic than saturated fatty acids[9].

Understanding how the physical structure of fats affects lipid oxidation reactions would allow for the development of new antioxidant technologies and more efficient use of the existing antioxidant components. An important step to this understanding of lipid oxidation is determining the kinetics of the peroxidation reaction in fats. The development of new antioxidant technologies that arise from this understanding could allow for increased use of nutritionally valuable polyunsaturated lipids in foods, as well as increase the shelf-life of certain foods and allow food producers to have more nutritionally useful fatty acids in their products[10].

The aim of this study was to investigate the effect the physical properties of lipids have on their oxidation chemistry by examining the kinetics of the peroxidation reaction. The oxidation of hazelnut, peanut, palm and coconut oil emulsions were studied at 37°C in aerated acetate buffered solution (NH_4Ac) (ionic strength, I = 0.9, pH = 7). Iodine indices and refractive indices of hazelnut, peanut, coconut and palm oils were calculated and fatty acids were examined chromatographically.

Experimental

Chemicals and instruments:

Reagent grade analytical chemicals were supplied by Sigma-Aldrich Co. LLC. The absorption measurements were recorded with a Shimadzu UVM-1240 UV-Visible spectrophotometer (Shimadzu Corp., Kyoto, Japan manufacture). All the experiments were carried out with a thermostated system (NUVE BM 30 Circulation Water Bank, Ankara, Turkey). The oils used were obtained fresh from Doğavita Pharmaceutical Food Industry and Trade Co. (Çiftcizade, Turkey).

Iodine index and refractive index calculations:

The iodine indices of the oils were examined by sodium thiosulfate titration using the standard AOCS official method Cd 1-25[11]. For this, peanut, hazelnut, palm oils (0.3 g) and coconut oil (1.0 g) were separately weighed into 250 mL erlenmeyer flask. The iodine index values were calculated after titration.

The refractive index was determined by HANNA (HI 96801-Refractometer, 0–85% Brix) refractometer. Samples from oil emulsions were compared to a standard of pure water.

Preparation of oil emulsions:

Oil (0.3 g) was added to a 100 mL volumetric flask. Stock solution (3 mL) was added as an emulsifier (Tween 80 + Span 80, HLB: 10, 0.3 g in 3 mL ethanol)[12]. Ethanol (2 mL) and NH_4Ac (1 *M*, pH 7) buffer solution (90 mL) was slowly added to the mixture and emulsified by stirring by using a magnetic stirrer. The emulsion was then homogenised in the brand homeogeniser (VELP-OV5, Homogenizator). $CuCl_2$ stock solution at a concentration of 0.01 *M* was used as initiator.

Measurements by GC-MS method:

The oils were analysed by GC-MS fatty acid methyl ester analysis (FAME) according to the IUPAC (International Union of Pure and Applied Chemistry) standard method[13]. Chromotographic measurements were performed using a Shimadzu GCMS QP 2010 ULTRA instrument on a RTX-5MS Capillary column (30 m; 0.25 mm; 0.25 μm) using an ion source temperature of 200°C.

Determination of lipid hydroperoxides:

The oxidation of the oils was determined spectrophotometrically at 500 nm using the Fe(III) SCN method based on the hydroperoxides oxidising Fe^{2+} to Fe^{3+}. The degree of oxidation was measured by sequentially adding ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL) and ferrous chloride (0.02 *M* in 3.5% HCl)[14].

Determination of malondialdehyde:

A common secondary oxidation product, malondialdehyde (MDA), was found in the oil emulsions, as determined by the TBARS method. 0.1 mL sample were transferred to test tubes containing trichloroacetic acid (TCA, 0.15 mL, 2.8%), thiobarbituric acid (TBA, 0.1 mL, 1%) and deionized water (2.65 mL), for a total volume of 3 mL. These tubes were stored for 15 min in a water bath at 95°C. The resulting pink-colored MDA-TBA adduct was cooled in an ice bath for 5 min, and the absorbance was measured against the control blanks at 532 nm[15].

Statistical analysis:

Descriptive statistical analyses for calculating the standard error of the mean were performed using Microcal Origin 8.0 (Origin Lab Corp., Northampton, MA). The results were evaluated using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows, version 13[16].

Results and discussion

The process of oxidation can be monitored in a number of ways, such as via a measurement of primary product hydroperoxides or secondary aldehyde or ketone products, iodine indices. In addition to these methods, the analysis, both qualitative and quantitative, of fatty acid methyl esters by gas chromatography gives important insight into the mechanism of oxidation and the correct interpretation of the results[17].

Iodine index and refractive index in oils:

Iodine index determinations were made of the degrees of unsaturation of the oils and found to be 101.52, 91.79, 6.98 and 50.76 gI_2/100 g for peanut oil, hazelnut oil, coconut oil and palm oil, respectively. The iodine indices may hint at the degree of oxidation present before the oxidation studies of the various oils were started. However, the ability of the oils to undergo oxidation is not only dependent on the degree of unsaturation, but also on the composition of the oil and on external factors. Although using only the iodine index is not enough to interpret the degree of oxidation or rate, this study has allowed us to predict that oxidation of peanut and hazelnut oil would be easier than coconut and palm oil. As can be seen from the iodine values, peanut and hazelnut oils (iodine values > 90) have more unsaturated fatty acids,

whereas coconut and palm oil with an iodine value of less than 51 have less unsaturated fatty acids.

In addition, the refractive indices of each emulsion solution in the incubation medium were measured at specific time intervals. The measured refractive index values are given in Fig. 1 against the oxidation time. It was found that the index of refraction decreased with the oxidation time for all oil samples. The decline in refractive indices over time was faster in hazelnut and peanut oil emulsions, and slower in coconut oil and palm oils. This was in agreement with our prediction based on the iodine values.

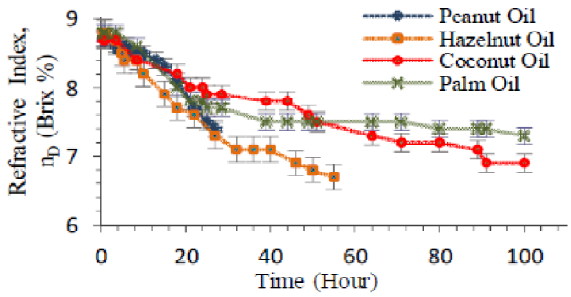

Fig. 1. Changes in the refractive-indices-versus-time curve of peanut-, hazelnut-, coconut- and palm-oil emulsions incubated at 37°C and pH 7.00. The calculated results (expressed as vertical bars) are given as mean ± SEM (standard error of the mean).

GC-MS measurements in fats:

Fats and oils that are primarily composed of unsaturated fatty acids have been shown to oxidise faster[18]. The rate of autoxidation is largely dependent on the rate of fatty acid or acylglycerol alkyl radical formation. The relative oxidation rate of oleic, linoleic and linolenic acids has been reported to be depending on oxygen uptake[19].

GC-MS analysis are given in Table 1 for peanut, hazelnut, coconut and palm oils respectively. Thus, in each oil, the 18-carbon fatty acids were found to be linoleic acid, oleic acid, stearic acid methyl ester (methyl stearate), and fatty acid chains generally observed between 16 and 23 carbons. Unlike the other oils, coconut oil and palm oil were also found to have fatty acids ranging from 8 to 14 carbon atoms.

The total saturated fatty acids (Σ SFA) in coconut and palm oils were found to be 93.09% and 99.62%, respectively, while peanut and hazelnut oils were found to be 11.93% and 10.21%, respectively. These percentages indicate that saturated fatty acids make up almost the entirety of coconut and palm oils.

However, it appeared that peanut and hazelnut oils have much larger percentages of monounsaturated fatty acids than coconut and palm oils. In decreasing order, the total percentages of monounsaturated fatty acids were: hazelnut oil > peanut oil > coconut oil > palm oil.

The peanut oil contained no polyunsaturated fatty acids but some were found in hazelnut, coconut, and palm oils in low percentages.

The percentage of total polyunsaturated fatty acids (Σ PUFA) in decreasing order is hazelnut oil > coconut oil > palm oil. *Trans* fatty acids (Σ Trans) were observed to be the highest in peanut oil, and there was a very low rate in the hazelnut oil. *Trans*-fats were not observed in coconut and palm oils.

Evaluation of kinetic parameters for primary and secondary oxidation product formation:

The experimental phase of this work was carried out using the (Fe(III) SCN) and the TBARS analytical methods. Fig. 2 shows the absorbance-time graphs obtained for peanut oil, hazelnut oil, coconut oil and palm oil. According to these graphs, all of the fatty acids approached an absorbance of 1.0 based on the Fe(III) SCN method. Peanut oil oxidation was observed for about 30 h while hazelnut oil was observed for 60 h. Coconut oil and palm oil were also examined at certain intervals for about 100 h. Absorbance-time curves for all oils gave a sigmoidal curve for both methods.

The secondary oxidation products were slower to form than the primary oxidation products, and also showed a stable state after the oxidation progression step. In previous studies, it was reported that free fatty acids were oxidized faster than esters, and this effect was due to the participation of carboxyl groups in the decomposition of peroxides[20]. The amount of oleic and linoleic acid has also been seen to affect oxidative stability. The presence of both oleic and linoleic acid are a significant quality feature of oils. In this study, oleic acid (C18:1) values of unsaturated fatty acids in hazelnut and peanut oil were found to be 83.24±4.16 and 58.77±2.93 g/100 g, respectively. Previously, the addition of stearic acid has been reported to accelerate the peroxidation of methyl linoleate[21]. In contrast, in our study, it is seen that coconut

Table 1. Fatty-acid composition and *trans* fatty-acid content of essential oils prepared by fatty acid methyl ester analysis (FAME) of peanut, hazelnut, coconut and palm oils

Fatty acid	Fatty acid content ± SD (g per 100 g)			
	Peanut oil	Hazelnut oil	Coconut oil	Palm oil
C 8:0*	–	–	10.01±0.50	5.76±0.28
C 10:0	–	–	7.58±0.37	4.75±0.23
C 11:0	–	–	–	0.04±0.00
C 13:0	–	–	0.03±0.00	0.04±0.00
C 14:0	–	–	20.47±1.02	13.51±0.67
C 16:0	7.63±0.38	6.21±0.31	9.03±0.45	10.05±0.50
C 17:0	0.05±0.00	0.04±0.00	–	0.03±0.00
C 18:0	2.68±0.13	3.74±0.18	45.89±2.29	64.99±3.24
C18:0 c9,c10 (ep.-9)	0.14±0.00	0.06±0.00	–	–
C18:0 (2,3- epoxypropil)	–	–	–	0.04±0.00
C 20:0	0.55±0.02	0.16±0.00	0.08±0.00	0.36±0.02
C22:0	–	–	–	0.05±0.00
C23:0 (iso-20)	0.88±0.04	–	–	–
Σ SFA	**11.93**	**10.21**	**93.09**	**99.62**
C16:1w7	0.36±0.01	0.12±0.00	–	–
C17:1	0.07±0.00	0.06±0.00	–	–
C18:1w9	58.77±2.93	83.24±4.16	5.85±0.29	0.32±0.01
C20:1w9	0.42±0.02	0.19±0.01	–	–
Σ MUFA	**59.62**	**83.61**	**5.85**	**0.32**
C18:2w6	–	6.07±0.30	1.02±0.05	0.06±0.00
Σ PUFA	–	**6.07**	**1.02**	**0.06**
C18:1 t9 (1,3-dielaidin)	–	0.10±0.00	–	–
C18:2 t10,c12	27.88±1.39	–	–	–
C18:1 t13	0.12±0.00	–	–	–
Σ *Trans*	**28**	**0.1**	–	–
Lauron	–	–	0.04±0.00	–
C18:0 (9,12,15-cyclopropil)	0.45±0.02	–	–	–
Σ Others	**0.45**	–	**0.04**	–

*C8:0, Caprylic acid; C10:0, Capric acid; C11:0, Undecanoic acid; C13:0, Tridecilic acid; C14:0, Myristic acid; C16:0, Palmitic acid; C17:0, Margaric acid; C18:0, Stearic acid; C18:0 c9,c10 (ep.-9), Epoxyoleic acid; C18:0 (2,3-epoxypropil), Glycidol stearate; C20:0, Arachidic acid; C22:0, Behenic acid; C23:0 (iso-20), Henicosanoic acid; C16:1 w7, Palmitoleic acid; C17:1, *cis*-10-Heptadecenoic acid; C18:1 w9, Oleic acid; C20:1 w9, Gadoleik asit; C18:2 w6, Linoleic acid; C18:1 t9, 1,3-Dielaidin; C18:2 t10,c12, 10-*trans*-12-*cis*-octadecadienoic acid; C18:1 t13, *trans*-13-Octadecenoic acid; Lauron, 12-Tricosanone; C18:0 (9,12,15-cyclopropil), Cyclopropaneoctanoic acid 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl].

oil and palm oil are stearic acid rich (C18:0), which is a saturated fatty acid. In Fig. 2, it can be seen that the oxidation rates of coconut and palm oils were slower than those of other oils.

In a small number of studies the effect of monounsaturated fatty acids (MUFA), such as oleic acid (C18:1), which make up a significant portion of oils, have been examined. It has shown that monounsaturated oleic acid is more readily oxidised from saturated chain fatty acids (stearic acid C18:0) or PUFA (linoleic acid C18:2) by using stable isotope-labeled fatty acids[22]. This result shows that particular attention should be paid to the amount of free fatty acids in fats and oils, since they can affect the oxidative stability of fats.

In accordance with the pseudo-first order rate constant (k) is calculated from the slope of the ln [(1 – A)/A] curve according to t[14].

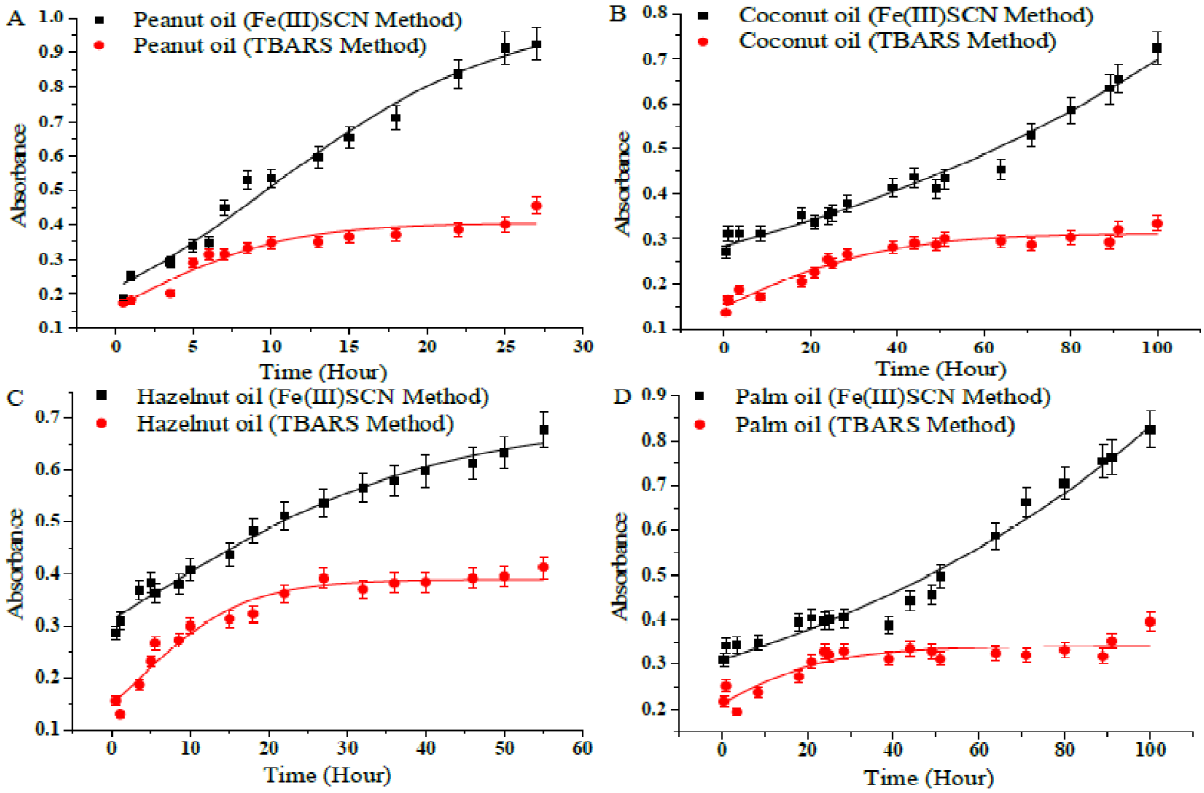

Fig. 2. The absorbance-time graph of the oxidation of (A) peanut oil, (B) coconut oil, (C) hazelnut oil, (D) palm oil (measurements made with Fe(III) SCN and TBARS method).

$$\ln (1 - A_t)/A_t = \ln (1 - A_0)/A_0 - k.t \quad (1)$$

A_0 is absorbance at baseline and A_t is absorbance at time t, which is proportional to the total concentration of hydroperoxides or aldehydes.

The formation rates of the primary and secondary oxidation products for the lipid oxidation system with Cu(II) initiator are given in Table 2. The rate of oxidation observed for peanut and hazelnut oil was higher than that of coconut and palm oil, and this is related to having fewer saturated fatty acids in the oils. In addition, the total *trans* fat content observed in peanut oil was thought to have been a significant contribution to the rate of oxidation.

The correlation between fatty acids in our study was evaluated statistically. A negative correlation ($r = -0.974$) was found between the saturated fatty acids (SFA) and the monounsaturated fatty acids (MUFA) of peanut, hazelnut, coconut and palm oils at $p = 0.026$ significance level. In the same way, a negative correlation ($r = -0.997$) was found between the saturated fatty acids (SFA) and the polyunsaturated fatty acids (PUFA) at $p = 0.053$ significance level. The correlation between monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) was found to be ($r = 0.996$) at $p = 0.057$ significance level.

Table 2. The rate constants of primary- and secondary-oxidation products formation from oil-Cu(II) system

	Fe(III) SCN method		TBARS method	
Oil species	$k_1 \pm S_k$ (h^{-1})x10^{-1}	r^2	$k_2 \pm S_k$ (h^{-1})x10^{-1}	r^2
Peanut oil	1.41±0.05	0.983	0.42±0.07	0.749
Hazelnut oil	0.27±0.01	0.955	0.22±0.03	0.693
Coconut oil	0.16±0.00	0.945	0.09±0.01	0.735
Palm oil	0.21±0.01	0.933	0.06±0.01	0.576

k_1: pseudo-first order rate constant with respect to hydroperoxides formation (measured by Fe(III) SCN method).

k_2: pseudo-first order rate constant with respect to malondialdehyde formation (measured by TBARS method).

The relationship between the pseudo-first order rate constant and fatty acids for all oils was statistically evaluated. According to the evaluation, the pseudo-first order rate con-

stant showed a negative correlation (r = –0.616) with saturated fatty acids, and also positively correlated with monounsaturated fatty acids and polyunsaturated fatty acids (r = 0.423) and (r = 0.814), respectively. These results showed that the structures of the oxidation products were closely related to the presence of saturated and unsaturated fatty acids.

Conclusions

Oxidation studies show that fatty acids are primarily responsible for the diversity of oxidation products. When the contribution of saturated, *cis* unsaturated and *trans* unsaturated fatty acids to the rate of oxidation of oils is evaluated together, it will facilitate the emergence of new studies and predictions in the food and cosmetic industry. The results of this study aimed to demonstrate the clarification of lipid oxidation mechanism by correlating with the physical properties of fat. Thus, it will help to develop new technologies for preventing cellular damage and inhibition studies that may arise from lipid oxidation.

Acknowledgements

The author gratefully acknowledge to thank Doğavita Pharmaceutical Food Industry and Trade Co. (Çiftcizade, Turkey) for the oils used.

References

1. L. T. Rael, G. W. Thomas, M. L. Craun, C. G. Curtis, R. Bar-Or and D. Bar-Or, *J. Biochem. Molecular Bio.*, 2004, **37**, 749.
2. S. Duru and D. B. Konuşkan, *Gıda*, 2014, **39**,1.
3. E. Karaca and S. Aytaç, *J. Faculty Agr. (OMU)*, 2007, **22**, 123.
4. Q. Wu, T. Liu, H. Liu and G. Zheng, *African J. Biotech.*, 2009, **8**, 1782.
5. V. D. Zheljazkov, B. A. Vick, B. S. Baldwin, N. Buehring, C. Coker, T. Astatkie and B. Johnson, *Indian Crops Product*, 2011, **33**, 537.
6. G. Wolfram, *Eur. J. Med. Res.*, 2003, **8**, 321.
7. A. S. Czerniak and D. Rabiej, *Eur. Food Res. Tech.*, 2018, **244**, 1397.
8. E. Choe and D. B. Min, *Comprehensive Rev. Food Sci. Food Safety*, 2006, **5**, 169.
9. D. Duxbury, *Food Tech.*, 2005, **59**, 34.
10. W. Chaiyasit, R. J. Elias, D. J. McClements and E. A. Decker, *Critical Rev. Food Sci. Nutrition*, 2007, **47**, 299.
11. American Oil Chemists' Society, Method Cd 1-25: Iodine Value of Fats and Oils. Wijs Method., AOCS Press, Firestone, 1998, 5th ed.
12. A. K. Hassan, *Indian J. Pharm. Sci.*, 2015, **77**, 461.
13. International Union of Pure and Applied Chemistry (IUPAC), Standard Methods for the Analysis of Oils, Fats and Derivatives, Pergamon Press, Oxford (UK), 1992, 7th ed.
14. B. Yildoğan-Beker, T. Bakir, İ. Sonmezoğlu, F. İmer and R. Apak, *Chem. Phys. Lipids*, 2011, **164**, 732.
15. H. Ohkawa, N. Ohishi and K. Tagi, *Anal. Biochem.*, 1979, **95**, 351.
16. J. C. Miller and J. N. Miller, "Statistics for Analytical Chemistry", Ellis Horwood Limited, Prentice, 1993, 3rd ed.
17. R. Kowalski, *Acta Chromatographica*, 2007, **18**, 15.
18. T. D. Parker, D. A. Adams, K. Zhou, M. Harris and L. Yu, *J. Food Sci.*, 2003, **68**, 1240.
19. D. B. Min and G. D. Bradley, "Fats and Oils Flavors, Wiley Encyclopedia of Food Science and Technologhy", John Wiley and Sons, Inc, New York, 1992, pp. 828-832.
20. R. T. Holman and O. C. Elmer, *J. American Oil Chemists' Soc.*, 1947, **24**, 127.
21. K. Miyashita and T. Takagi, *J. American Oil Chemists' Soc.*, 1986, **63**, 1380.
22. P. J. Jones, P. B. Pencharz and M. T. Clandinin, *The American J. Clinic. Nutr.*, 1985, **42**, 769.