Hydrocarbons, Fatty Alcohols and Sterols from three Varieties of Mango Kernels Fat

[1]ZEESHAN ALI*, [2]RANA ALTAF HUSSAIN AND [2]HAMID LATIF SIDDIQUI
[1]*Applied Chemistry Research Center,*
Pakistan Council of Scientific and Industrial Research Laboratories, Complex,
Shahrah-E- Jalal-ud-Din Roomi Road, Lahore-54600, Pakistan.
[2]*Institute of Chemistry,*
University of the Punjab, Lahore, Pakistan

(Received 19th July 2007, revised 20th September 2007)

Summary: Three varieties of mangoes (*Mangifera indica*) *Chaunsa, Sindhri,* and *Desi in Pakistan* have been selected to find out unsaponifiable matter 1.5-2.31 %. It consists of hydrocarbons 8.6-9.8 % of carbon chain length C_{12}-C_{36}, whereas, squalene 45.26-54.19 % is determined in these varieties. The primary fatty alcohols 11.5-12.3 % of carbon chain C_{12}-C_{30} are also separated and identified. Octacosanol is the predominant alcohol in each of the variety. The sterols are separated by preparative TLC and identified as 4,4 dimethyl sterols 15.1-16.0 %, 4-methyl sterols 6.4-7.0 % and 4-desmethyl sterols 53.5-54.0 % and unidentified matter 2.5-3.0 %. Thin layer chromatography was used for separation and purification, whereas gas chromatography and spectroscopic techniques i.e., IR and MS were applied for identification and characterization of various classes of compounds.

Introduction

The agro-industrial waste materials such as rice bran, tobacco seeds and mango kernels are utilized in the world for the production of edible oil [1]. The edible oil is also produced from tobacco seeds in countries like Bulgaria, Greece, Romania and Italy [2]. The mango seeds as a byproduct of food industry are being used in India for the production of fat which can be utilized for edible purposes / industrial products [3].

Pakistan being an agricultural country is facing an acute shortage of edible oils therefore, a huge foreign exchange is spent on the import of this commodity. The import expenditure of oil is from 28284 million rupees [4] was increased to 44975 million rupees [5] to fulfill the demand of the country. Pakistan should have utilized the potential resources of agro industrial waste materials as pointed out for the production of oils in order to reduce the foreign exchange spent on the import of oils and fats. Three varieties of mangoes i.e., *Chaunsa, Sindhri and Desi in Pakistan* have been selected to have through investigation on their kernel fat for the first time in Pakistan. The juice producing factories is a source for the collection of waste mango seeds. The mango belongs to genera *Mangifera indica* of Anacardiciae family. There are 73 genera and 600 species of trees and shrubs mostly found in tropical countries in addition to America, Southern Europe and temperate Asia [6]. The fat 4550 tones can be produced from 1,037,200 tones of mangoes in the country [7].

Studies on mango kernel have been divided into two parts. One part is concerned with the components of mangoes and composition of fatty acids reported separately, whereas detailed investigations on unsaponifiable material (hydrocarbons, fatty alcohols and sterols) have been carried under the second part. The presence of hydrocarbons and fatty alcohols have been found out first time in unsaponifiable portion of the kernel lipids in addition to classification and quantification of sterols which have not been claimed by any previous worker in Pakistan [8-9]. The composition of mango fat is such that it can be utilized in cosmetic industry due to the presence of the sterols and also as a substitute for cocoa butter in food industry. The palm oil as regards to its fatty acids composition can be correlated to mango kernel fat. Therefore, it can be claimed as substitute for palm oil which has enormous industrial applications. Explanation for the utilization of mango kernel fat on industrial scale would have a great impact on the socioeconomic development of the country.

*To whom all correspondence should be addressed.

Results and Discussion

Although there is a low percentage of total sterol in kernel lipids, even then it plays an important role as precursor of other steroids, called hormones and as membrane components, the kernel fat can be used for the preparation of cosmetic preparations being an emollient. Mango kernel fat is semi-solid fat at room temperature. Its melting point is 35° C is nearer to body temperature [10] which indicates the easy digestion of the fat without hydrogenation process. The fat can also be used for shortenings, margarine and as a cocoa butter substitute [11-13] which consists of saturated fatty acids 59.5 % and unsaturated fatty acids 38.5 %. It can be compared with three varieties f mango kernel fat as regards to saturation 52.79- 60.51 % and unsaturation 39.49-47.21 % [14]. The cocoa butter is used for chocolates, candies coatings and also consumed in the production of solid chocolate confections [15].

There is no remarkable difference between the fatty acids composition of palm oil and mango kernel fat. However, the major difference between palm oil and mango kernel fat is of palmitic acid and stearic acid which does not matter significantly for the preparation of industrial products. The stearic acid can be produced from mango kernel fat which has vast industrial applications for the production of toilet soaps, cosmetics [16] and metallic soaps.

The lipids of three varieties of mango kernels are made free from unwanted materials such as glucose, salts, urea, sucrose etc., as described under materials and methods to avoid any interference. The percentages of purified fat extracted with chloroform/ methanol on the weight basis of mangoes and dried kernels are given in Table-1. It shows that *Desi* variety dried kernels contains highest percentage of fat 11.8 % as compared to *Sindhri* 10.9 % and *Chaunsa* 9.6 %.

The fat 60 g of each variety gives unsaponifiable matter in *Chaunsa, Sindhri and Desi* 1.50 %, 1.68 % and 2.31 % respectively (Table 1). So, the unsaponifiable matter of *Desi* is highest as compared to other two varieties. The literature reveals that mango seed fat consists of unsaponifiable matter 1.0-5.3 % [17] in comparison to that of the Malagasy mango seed (African variety) 0.9- 2.8 % [8]. However, unsaponifiable 5.3 % of previous work looks higher which may be due to different agronomical conditions and eventually different varieties of mangoes.

Table-1: The weights and percentages of mangoes, kernels, fats and unsaponifiables.

Products	Chaunsa (g)	Sindhri (g)	Desi (g)
Mangoes (g)	4786.2	4045.0	3434.3
Dried kernels (g)	259.5	125.9	127.7
Fat (g)	24.9	13.7	15.1
Fat* (%)	9.6	10.9	11.8
Fat** (%)	0.5	0.3	0.4
Unsaponifiable/ 60 g of oil	0.906	1.008	1.387
Unsaponifiable (%)	1.50	1.68	2.31

*Fat percentage on the weight basis of dried kernels. **Fat percentage on the weight basis of mangoes.

The thin layer chromatograms of thickness of 0.25 mm are used for fine separations of unsaponifiable matter into five fractions for further work. The pattern of separation for unsaponifiable compounds of three varieties is similar. The R_f value of each fraction is shown in Fig. 1. It shows R_f value of hydrocarbons 0.83, primary fatty alcohols 0.70, 4,4-dimethyl sterols 0.60, 4-methyl sterols 0.42 and 4-desmethyl sterols 0.36 and unidentified 0.01. It reflects that by using polar solvent system; hexane-diethyl ether-acetic acid (80: 20: 2) the separation is according to polarity of compounds [18]. The hydrocarbons are closest to solvent front, the primary fatty alcohols are located after hydrocarbons, next 4,4-dimethyl sterols (triterpene alcohols) and the resolution of 4-methyl sterol is between 4,4-dimethyl sterols and 4-desmethyl sterols.

The unsaponifiable matter (300 mg) of each variety is fractionated by the application of thin layer chromatography into hydrocarbons 8.6-9.8 %, primary fatty alcohols 11.5-12.3 %, 4,4-dimethylsterols 15.1-16.0 %, 4-methylsterols 6.4-7.0 %, 4-desmethylsterols 53.5-54.0 % and unidentified 2.5-3.0 % (Table-2).The highest percentage of 4-desmethylsterols has been found out and hydrocarbons are of lowest percentage.

The literature reveals that unsaponifiable matter of *Vernonia anthelmintica* seed oil contains hydrocarbons C_{25}-C_{37} [19] and ten Indian seed oils

Table-2: Fractionation of unsaponifiable matter (300 mg) of each variety of mango kernel fat.

No	Components	R_f values	Chaunsa		Sindhri		Desi	
			(mg)	(%)	(mg)	(%)	(mg)	(%)
1	Hydrocarbons	0.83	25.8	8.6	27.0	9.0	29.5	9.8
2	Fatty alcohols	0.70	36.9	12.3	36.0	12.0	34.5	11.5
3	4,4-Dimethylsterols	0.60	45.3	15.1	47.7	15.9	48.0	16.0
4	4-Methylsterols	0.42	21.0	7.0	19.2	6.4	20.0	6.7
5	4-Desmethylsterols	0.36	162.0	54.0	161.4	53.8	160.5	53.5
6	Unidentified	0.01	9.0	3.0	8.7	2.9	7.5	2.5

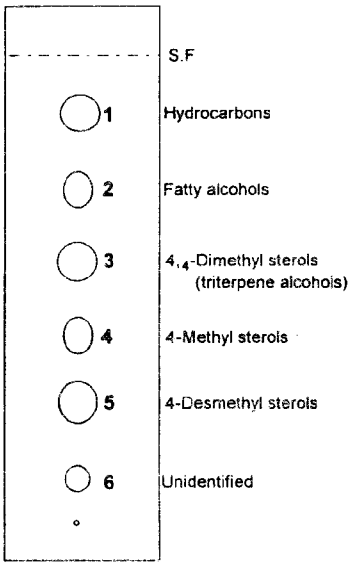

Figure-1: Separation of unsaponifiable lipid compounds.

consist of hydrocarbons C_{16}-C_{37} [20]. The virgin olive oil shows the range of hydrocarbons C_{14}-C_{35} [21]. The work under investigation shows the hydrocarbon chain length C_{14}-C_{35} (Table-3). The squalene was separated and purified by $AgNO_3$ impregnated TLC. Mass spectra showed *m/z* 410 $[M]^+$ is in agreement with its molecular formulae of ($C_{30}H_{50}$). Squalene was found out by previous workers in sunflowers, olive, soybean, rapeseed and pumpkin seed oils [22-23].

The previous workers have seperated primary alcohols from vegetables as well as animals. The Japonica fat contains alcohols C_{12}-C_{26} [24], *Jojoba* oil C_{18}-C_{24} [25], *Calotropis procera* C_{12}-C_{18} [26], *Anthelmintica* seed oil C_{18}-C_{28} [17], *Plant wax* C_{18}-C_{29} [27], *Sperm whale* oil C_{12}-C_{20} [28] in addition to hexacosanol, octacosanol and triacontanol from kernels, nuts, seeds fruits and cereals [29].

Table-3: Hydrocarbons C_{12}-C_{36} analyzed by Gas Chromatograph.

Chain Length	RRT	Chaunsa	Sindhri	Desi
C_{12}	12.861	0.64	0.92	0.13
C_{13}	13.145	-	-	0.14
C_{14}	14.142	2.12	1.31	0.30
C_{15}	15.028	1.30	0.65	0.41
C_{16}	16.048	1.81	1.74	1.18
C_{17}	17.277	3.14	3.33	0.97
C_{18}	18.193	4.80	1.85	2.13
C_{19}	19.369	8.42	5.31	2.34
C_{20}	20.392	8.82	7.84	4.75
C_{21}	21.669	1.31	1.65	1.02
C_{22}	22.158	5.42	6.75	1.26
C_{23}	23.703	1.24	3.56	3.48
C_{24}	24.158	1.18	1.84	2.67
C_{25}	25.681	1.12	3.54	1.80
C_{26}	26.881	2.84	1.21	4.73
C_{27}	27.077	0.38	0.18	0.14
C_{28}	28.752	0.16	0.12	0.29
C_{29}	29.693	1.38	1.37	2.20
C_{30}	30.677	45.26	49.51	54.19
C_{31}	31.282	0.58	0.82	2.78
C_{32}	32.770	0.09	0.13	2.35
C_{33}	33.432	0.84	0.72	1.98
C_{34}	34.897	1.68	1.80	2.79
C_{35}	35.149	2.28	1.71	1.76
C_{36}	36.221	3.19	2.14	4.21

The work on primary fatty alcohols C_{12}-C_{30} of mango kernel fat is reported in Table-4. The highest percentage of octacosanol has been found out in each variety. The fatty acid compositions C_{14}-C_{22} of three varieties of mangoes, reported separately are different in chain length from fatty alcohols. The distribution of fatty acids and fatty alcohols reflects that the synthesis of fatty alcohols and fatty acids leads to different roots in nature rather to believe the theory that fatty alcohols and fatty acids are in reversible equilibrium and are directly interconvertible.

The percentage of 4-desmethyl sterols and 4-methyl sterols is highest and lowest respectively among sterols of three varieties as determined by thin layer chromatography. The polar solvent system hexane-diethyl ether-acetic acid (80: 20: 2)

Table-4: Primary fatty alcohols acetates C_{12}-C_{30} analyzed by Gas Chromatograph.

Chain Length	RRT	Chaunsa (%)	Sindhri (%)	Desi (%)
$C_{12:1}$	9.054	0.2	—	0.1
$C_{12:0}$	9.567	0.5	0.7	0.6
$C_{14:1}$	10.040	0.1	—	—
$C_{14:0}$	10.464	0.7	0.8	0.6
$C_{16:1}$	11.304	2.3	2.1	2.4
$C_{16:0}$	12.004	8.2	8.4	8.3
$C_{18:1}$	13.454	11.3	11.0	10.9
$C_{18:0}$	14.074	51.0	51.3	51.4
$C_{20:1}$	14.452	2.3	1.2	1.0
$C_{20:0}$	14.908	9.0	10.0	10.2
$C_{22:1}$	16.857	0.4	0.3	0.1
$C_{22:0}$	17.413	4.4	4.5	4.7
$C_{24:1}$	18.136	0.5	0.3	0.1
$C_{24:0}$	18.924	2.0	2.3	2.5
$C_{26:0}$	23.508	3.8	3.5	3.2
$C_{28:0}$	26.181	2.5	2.9	3.0
$C_{30:0}$	27.917	0.8	0.7	0.9

is used for the separation of sterols. The literature reveals that previous workers analyzed sterols of different varieties of mangoes [8-9, 20, 30-31]. The percentage of sterols in unsaponifiable matter is highest as compared to hydrocarbons and primary fatty alcohols, but the percentage of sterols is very low comparatively to the total fat due to the low percentage of unsaponifiable matter.

Experimental

Materials

The waste seeds of known mango varieties i.e., *Chaunsa, Sindhri and Desi* were collected from juice factories (Shezan International Ltd., Lahore and Maahar Food Industries Pvt Ltd., Lahore). The solvents and reagents were purchased from Merck (Darmstadt, Germany) and Winlab (Leicestershire, UK). All of the solvents/reagents used were of analytical-grade. Silica gel 60 HF_{254}, Merck Ref. 7739 was used for TLC. The standards; hydrocarbons and fatty alcohols are product of BDH (Poole, England). The squalene (Sigma-Aldrich, Germany) and stigma sterol (Winlab, UK) were obtained on request from Loughborough University UK for research purpose. Lanosterol was extracted from wool wax and purified in the laboratory. The pyridine (Riedel-de-Haën, Germany) and acetic anhydride (Fluka, Germany) were used for the acetylation of fatty alcohols.

Extraction of Lipids

The kernels of the varieties i.e., *Chaunsa, Sindhri and Desi* were dried and 60.0 g powder of each was taken in separate flasks and 500 mL solvent mixture; chloroform/methanol (2: 1 v/ v) was added into each and stirred vigorously with a magnetic stirrer for half an hour at room temperature. It was filtered and the material was stirred again with half quantity of 250 mL for fifteen minutes to extract maximum lipids. The process was repeated and extracts were pooled together in a round bottom flask. The solvent containing lipids was dried over anhydrous sodium sulfate and filtered. The mixture was concentrated under vacuum by rotary film evaporator to get lipids followed by treatment with a mixture of chloroform/methanol/0.9 % sodium chloride solution (3:48:47 v/v) to remove impurities [32]. The dried kernel powder of *Chaunsa, Sindhri and Desi* produced 3.00 g, 3.23 g and 3.45 g fats respectively on the weight basis of 25 g of each variety. These fats were stored under nitrogen for further analysis.

Saponification of fats

The kernel fat 60 g of each variety of mangoes was refluxed with 0.5 N ethanolic potassium hydroxide 900 mL for three hours. The ethanol was distilled off, then water and diethyl ether was added [33]. The soap solution was separated and the upper organic layer was distilled off to get unsaponifiable matter of *Chaunsa* 0.906 g, *Sindhri* 1.008 g and *Desi* 1.387 g.

Thin Layer Chromatography (TLC)

Fifteen thin layer chromatograms (20 cm × 20cm) of 0.25 mm thickness were prepared by Quickfit TLC applicator, the silica gel 30 g/ distilled water 60 mL (1: 2) was used for the preparation of five plates. The chromatograms were air dried and activated by heating at 105° C for an hour. Ten more chromatograms of same thickness were prepared under the same conditions. The unsaponifiable matter 300 mg of each variety was fractionated by using solvent system; hexane-diethyl ether-acetic acid (80: 20: 2) [32]. The non destructive locating reagent 2,7 dichlorofluorescein was used to have purple yellow colored bands under ultra violet light at 366 nm. The unsaponifiable matter was fractionated into hydrocarbons 25.8-29.5 mg, primary fatty alcohols 34.5-36.9 mg, 4,4-dimethylsterols 45.3-48.0 mg, 4-methyl sterols 19.2-21.0 mg, 4-desmethylsterols 160.5-162.0 mg and unidentified 7.5-9.0 mg. The bands of these compounds were scratched and the

materials were eluted by n-hexane and diethylether, dried by anhydrous sodium sulfate, filtered and the solvent was removed by rotary thin film evaporator under vacuum to get above mentioned compounds. The separated compounds were identified by using standards of hydrocarbons, primary fatty alcohols and sterols. The eluted chromatograms were sprayed by saturated solution of antimony trichloride in chloroform and on heating at 100 °C for 3 minutes; the red violet spots identified the presence of sterols [32]. The squalene was separated and purified by $AgNO_3$ (12.5 %) impregnated TLC by using hexane h-diethylether (95: 5) as eluting solvent.

Acetylation of Fatty Alcohols

The primary fatty alcohols of three varieties, each weighing 100 mg were stirred and refluxed with acetic anhydride 8 mL / pyridine 8 mL (1: 1) at room temperature for eighteen hours. The acetates of alcohols and sterols were extracted with diethyl ether (3×75 mL) after the addition of water 60 mL. The acetates were thoroughly washed to remove pyridine and acetic anhydride in the separating funnel then dried over anhydrous sodium sulfate prior to their recovery after the distillation of diethyl ether under vacuum. The fatty alcohols acetates were purified by preparative TLC [34].

Infrared Spectroscopy and Mass Spectrometry

The infrared spectrophotometer (Thermo-Nicolet IR-200) was used for the identification of hydrocarbons and purified alcohol acetates. The hydrocarbons showed infrared absorption at 2860 cm^{-1} (CH_3-stretch), 2940 cm^{-1}(CH_2-stretch), 1380 cm^{-1}(CH_2-bend), 1460 cm^{-1}(CH_3-bend), whereas the purified acetates of primary fatty alcohols showed infrared absorption at 1240 cm^{-1}(C=O-stretch), 1370 cm^{-1} (CH_3-bend), 1460 cm^{-1} (CH_2-bend), 1730 cm^{-1} (C=O-stretch), 2840 cm^{-1}(CH_3-stretch) and 2930 cm^{-1}(CH_2-stretch). The mass spectrum was recorded on JEOL MS Route instrument using ionization mode EI for the confirmation of the structure of squalene. Mass spectra showed *m/z* 410 $[M]^+$ in agreement with its molecular formulae i.e., $C_{30}H_{50}$.

Gas Chromatography

The gas chromatograph, Shimadzu GC-14A and data processor C-R-4A with SE-30 column of dimension (2.5m × 3mm ID) was used for the analysis of hydrocarbons and primary fatty alcohols under temperature programming 100-250 °C at the rate of 10 °C/ minute. The injector and detector temperatures were 250 °C and 280 °C respectively. The flow rate of nitrogen gas was 30 ml/ minute. The standards were used for the characterization of hydrocarbons and primary fatty alcohols.

Acknowledgement

We pay our gratitude to HEJ, Research Institute of Chemistry, University of Karachi for the measurement of MS and GC-MS.

References

1. Y. Takeshita, H. Yoshida, and M. Y. Raie, Global trend of fat resource and technology, *Transaction of the Kakushikan University Japan, Deptt. of Eng.*, **14**, 86 (1981).
2. T. Yazicioglu, Regional problems of edible oils and fats, *Cento Scientific Program Panel*, Lahore, Pakistan, **7**, (1975).
3. B. L Narasimha, B. R. Reddy and S. D. Thirumala Rao, *J. Am. Oil Chem. Soc.*, **54**, 494 (1977).
4. Government of Pakistan, *Annual Report, State Bank of Pakistan*, **100** (2000).
5. Government of Pakistan, *Agricultural Statistics Economic Wing*, 211, 256 (2005).
6. G.L. Chopra, *Angiosperm*, Unique Publishers, Lahore, Pakistan, **9** (1970).
7. Government of Pakistan, *Pakistan Statistical Year Book, Federal Bureau of Statistics*, **141** (2003).
8. E. M. Gaydou and P. Bouchet, *J. Am. Oil Chem. Soc.*, **61**, 1589-93 (1984).
9. M. A. Ali, M. A Gafur, M. S. Rahman and G. M. Ahmed, *J. Am. Oil Chem. Soc.*, **62**, 520-23 (1985).
10. J. Hemavathy, J. V. Prabhakar and Sen, D. P., *J. Food Sci.*, **52**, 833 (1987).
11. Fincke, *Deutsche Lebensm*, Rundsch, **76**, 187 (1980).
12. W. Hemker, *J. Am. Oil Chem. Soc.*, **58**, 110 (1981).
13. C. Bandyopadhyay and A. S. Gholap, *Cusr. Sci*, **48**:935 (1979).
14. Z. Ali, H. L. Siddiqui and S. Hamid, *Sci. Int*, **19**, 51 (2007).
15. D. Swern, *Bailey's Industrial Oil and Fat Products*, 4th edition, A Wiley Interscience Publication, John Wiley & Sons, New York, 1

(1979).
16. Bhattacharya, Kaustuv, Shukla, Vijai K.S., *Cosmetics & Toiletries,* **117**, 65 (2002).
17. G. Lakshminararayana, T. Chandrasekhara Rao and P. A. Ramalingaswamy, *J. Am. Oil Chem. Soc.,* **60**, 67 (1983).
18. M. Waheed, N. Kausar, M. N. Nawazish and Z. Hussain, *Pak. J. Biol.,* **XVI**, 71 (1981).
19. J. A. Fioriti, Margaret, J. Kanuk and R. J. Sims, *J. Am. Oil Chem. Soc.,* **48**, 240 (1971).
20. M. T. Saeed, R. Agrawal, M. W. Y. Khan, F. Ahmad, S. M. Osman, T. Akihisa, K. Suzuki and T. Matsumoto, *J. Am. Oil Chem. Soc.,* **68**, 193, (1991).
21. A. Lanzon, T. Albi, A. Cert and J. Gracian, *J. Am. Oil Chem. Soc.,* **71**, 285 (1994).
22. M. Bastic, L. Bastic, J. A. Jovanovic and G. Spiteller, *J. Am. Oil Chem Soc.,* **55**, 886 (1978).
23. P. Bondioli, C. Mariani, A. Lanzani, E. Fedeli and A. Muller, *J. Am. Oil Chem. Soc.,* **70**, 763 (1993).
24. M. Y. Raie, S. Zaka and M. Saleem, *Fette. Seifen. Anstrichmittel,* 85 Jahrgang, Nr **8**, 325-26 (1983).
25. R. J. Hamilton and M. Y. Raie, *Chemistry and Physics of Lipids,* **14**, 92 (1975).
26. M. Y. Raie, M. A. Javeed and I. Ahmad, *Fat. Sci. Technol,* 97 Jahrgang, 553-54, Dezember (1995).
27. S. Satgur Khama and G. Edward Perkins, *J. Agri. Food Chem.,* Illinois Urbania II, **18** (2), 253-55 (1976).
28. R. J. Hamilton, M. Long and M. Y. Raie, *J. Am. Oil Chem. Soc.,* **49**, 307 (1972).
29. K. Kawanishi, K. Aoki, Y. Hashimoto and A. Maisunobu, *J. Am. Oil Chem. Soc.,* **68**, 869 (1991).
30. T. Itoh, T. Tamura and T. Matsumoto, *J. Am. Oil Chem. Soc.*, **50**, 122 (1973).
31. T. Itoh, T. Tamura and T. Matsumoto. *J. Am. Oil Chem. Soc.,* **50**, 300 (1973).
32. M. Y. Raie, Ijaz Ahmed, M.A. Javed and I. Waheed, *Proc. Pakistan Acad. Sci.*, **26**, 199 (1989)
33. AOAC, *Official Methods of Analysis*, **41**, 1.39 International (2005).
34. R. J. Hamilton and M. Y. Raie, *J. Am. Oil. Chem. Soc.,* **53**, 748 (1976).