Rapid and Complete Extraction of Free Fatty Acids from Oilseeds for Acid Value Determination

O.Yu. Berezin, Ya.I. Tur'yan, I. Kuselman*, and A. Shenhar

The National Physical Laboratory of Israel, Jerusalem 91904, Israel

ABSTRACT: The rapidity and uncertainty of the acid value determination in oilseeds are defined by the rate and completeness of the free fatty acid extraction. The strategy of reagent development for extraction has been discussed, and a set of reagents that provide rapid and complete extraction has been obtained. The set consists of two reagents. Reagent A contains triethanolamine in a mixture of water, isopropanol, and heptane. Reagent B contains a strong acid and inorganic salt in water. Reagent A allows the carrying out of rapid (1–2 min) solid–liquid extraction of the free fatty acids and some other acids from oilseeds. Reagent B provides the separation of the free fatty acids only into the heptane phase (~5 min), which can be used directly for the free fatty acid determination. Two techniques for this determination have been applied: pH-metric and titrimetric. The advantages of the proposed set of reagents are described.

JAOCS 73, 1707–1711 (1996).

KEY WORDS: Acid value, extraction, free fatty acids, heptane, isopropanol, oilseeds, set of reagents, triethanolamine.

The acid value (AV) is an important characteristic of an oilseed's quality. AV is expressed by the KOH necessary (in mg) for titration of the free fatty acids (FFA) contained in 1 g oil. Therefore, for AV calculation, the oil content (% mass) should be known.

Because a technique for AV determination in oilseeds without preliminary FFA extraction is unlikely, the time of FFA extraction is an important part of the overall analysis time. By the standard technique (1), the time for oil and FFA extraction into the hydrocarbon medium requires 4 h and more (2). After the extraction and oil weighing, the comparatively rapid step, titration of FFA, is carried out (1).

The advantage of the standards (1,2) is a consecutive determination of the oil content in oilseeds and AV. But today, more rapid techniques for determination of oil content are developed: for example, by supercritical fluid extraction with carbon dioxide (3,4) or by nuclear magnetic resonance (NMR) (5). Therefore, the development of a rapid and complete extraction of only FFA from oilseeds is an important task to accelerate AV determination. A solution of this problem is described in the present work.

*To whom correspondence should be addressed at The National Physical Laboratory of Israel, Danciger "A" Bldg., Givat Ram, Jerusalem 91904, Israel.

STRATEGY OF THE REAGENT DEVELOPMENT FOR FFA EXTRACTION

For acceleration of the FFA extraction from plasma (blood), Dole (6) proposed to substitute nonpolar hydrocarbon solvent by a mixture of nonpolar and polar solvents: heptane–isopropanol–water. After extraction, FFA were separated into the heptane phase by introducing an additional amount of heptane and water. We call the separation "reextraction" in the following.

The increase of solvent polarity for extraction accelerates the destruction of associations of lipids and FFA with cell membranes or with lipoproteins (7). Besides, it improves the wetting of the relatively polar seed material, which also accelerates the FFA extraction. Addition of a polar solvent, such as alcohol, is also important for FFA reextraction. The polar solvent decreases the difference between the surface tensions on the phase boundary and improves phase separation (8).

However, the solvent polarity should be optimal because its further increase could limit the solubility of lipids or FFA and lead to hydrolysis (solvolysis) of lipids (7). Another drawback of the polar solvent is caused by extraction of other acids (besides FFA), probably from proteins. To eliminate this drawback, Dole (6) has proposed to improve the FFA reextraction by addition of water and H_2SO_4 to the system. Addition of H_2SO_4 (6) or a base (9) upon FFA extraction is important also to suppress lipid hydrolysis caused by lipase catalysis. It is known (10) that the pH range for lipase catalysis is 4.0–5.0.

We call solvents for extraction and reextraction with added substances "reagents" in the text below.

The principle of using a polar reagent for FFA extraction was applied for oilseeds by Lapshina *et al.* (9). Their reagent (0.15–0.20M triethanolamine (TEA) dissolved in the mixture of 80% diethyl ether + 19% ethanol + 1% water, % vol) accelerates the FFA extraction not only because of polarity but also because of amine presence. Instead of reextraction, Lapshina *et al.* (9) carried out the analysis by limitation of the extraction time to 1 min to obtain an AV close to that obtained by the standard method (it was supposed that extraction rate for FFA is higher than for other acids). But even within 1 min, the extraction of other acids was not eliminated completely. This fact can be a source of positive errors and/or insufficient reproducibility.

May and Hume (11) used a reagent that consisted of 0.01M H_2SO_4 in a mixture of 78% isopropanol + 20% heptane + 2% water (% vol). The time for FFA extraction from oilseeds was 15 min. The removal of other acids extracted into this reagent was carried out by FFA reextraction into the upper heptane layer after addition of heptane and water, by analogy to Dole *et al.* (6). The H_2SO_4 concentration was probably insufficient for complete FFA reextraction because of its partial neutralization by the oilseed material. These reagents for extraction and reextraction obtained only 64–96% of the FFA compared to the standard method. Furthermore, the phase separation after FFA reextraction in the two-phase system (upper heptane-rich phase and lower isopropanol–water–oilseed material phase) is relatively slow; according to our experience with some oilseeds, it takes 10–15 min.

In the present work, we used an amine in a polar reagent (9) and of the FFA reextraction (11). However, we increased the H_2SO_4 concentration for (amine) neutralization, suppression of FFA dissociation, and their complete reextraction into the heptane layer. Besides, we decided to add an inorganic salt during reextraction to improve the phase separation. This decision was based on the known fact that addition of an inorganic salt to "oil–surfactant–water" systems can lead to formation of three phases (12). We expected the same phase behavior in the system "heptane–isopropanol–water." In this case, the heptane-rich phase should be the upper layer, isopropanol–water phase the middle one, and water–salt the lower one. Because the density of the oilseed material is between the density of middle and lower phases, the material should be localized on the boundary of these two phases, and it will provide the acceleration for the upper phase (with FFA) separation.

EXPERIMENTAL PROCEDURES

Chemicals. TEA was supplied by Fluka (Buchs, Switzerland); heptane, diethyl ether, ethanol, isopropanol and potassium hydroxide were from Frutarom (Haifa, Israel); sulfuric acid from Palacid (Haifa, Israel); sodium sulfate from Merck (Darmstadt, Germany); hydrochloric acid, boron trifluoride methanol complex, and buffers at pH = 7.00 and pH = 9.22 from BDH (Poole, England); potassium chloride and nitrate from Baker (Phillipsburg, NY). Heptadecanoic acid and oil reference standards AOCS Nos. 1, 2, 4 were from Sigma (St. Louis, MO). The oilseeds (sunflower, soybean and canola) were purchased from local suppliers.

Apparatus. SEB (Rumilly, France) coffee grinder; Tuttenauer (Jerusalem, Israel) drying oven; Radiometer pH/ion meter PHM95 (Copenhagen, Denmark) with glass indicator electrode, Ag/AgCl aqueous reference electrode and saturated KNO_3 electrolytic bridge; HP 5880A (Avondale, PA) gas chromatograph with flame-ionization detector (FID) and fused-silica capillary column Vitocap-Al-0.2, L = 25 m, d = 0.22 mm, d_f = 0.45 μkm (analogous to immobilized Carbowax 20M) from Vitochrom Ltd. (Moscow, Russia); Nessler cylinders (Duran, Mainz, Germany) were used.

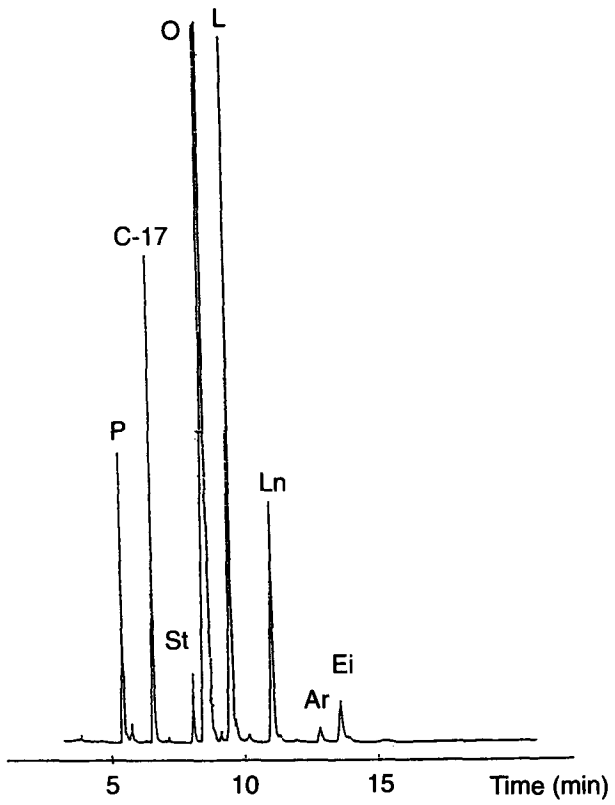

FIG. 1. Gas chromatogram of free fatty acids extracted from the canola sample by the new technique. The peaks in the chromatogram are: P—palmitic, C-17—heptadecanoic, St—stearic, O—oleic, L—linoleic, Ar—arachidic, Ei—11-eicosenoic acids.

Procedure. The oil contents in the oilseed samples were determined by standard extraction technique (2), and the AV values by standard titration technique (1). They were assumed to be the correct AV values. The same samples of oilseeds were used for the FFA extraction with our new reagents. The analysis of the extract was carried out by pH-metric technique (13) or by standard titration (1). Other methods (14) also may be used. The identification of FFA was performed by gas chromatography after the FFA derivatization to methyl esters with BF_3/CH_3OH (15). The chromatograms (Figs. 1–3) were recorded at an oven temperature from 180°C (hold 4 min) to 250°C with a gradient of 3°C/min (hold 10 min); carrier gas was helium at a flow rate of 0.8 mL/min; injector and detector temperature was 250°C.

RESULTS AND DISCUSSION

Development of the reagent set. The set of reagents for rapid and complete extraction of FFA from oilseeds developed by us (16) consists of two reagents: reagent A and reagent B.

Reagent A includes 0.05–0.15 M TEA in the solvent mixture of 50–65% isopropanol + 28–48% heptane + 1–7% water (% vol). This solvent is a one-phase system in accordance with the phase diagram (17). Introduction of TEA in the given

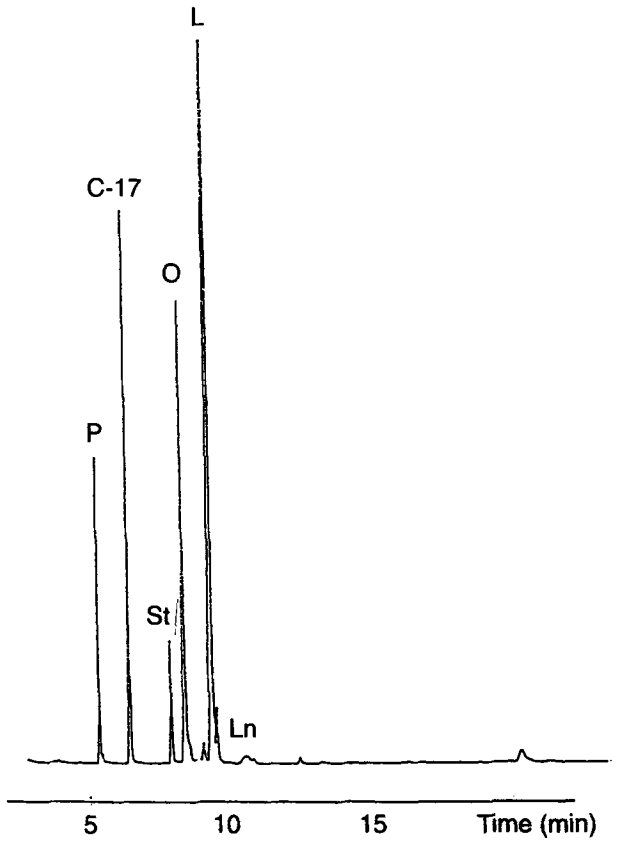

FIG. 2. Gas chromatogram of free fatty acids extracted from the sunflower sample by the new technique. The symbols of the acids are the same as in Figure 1.

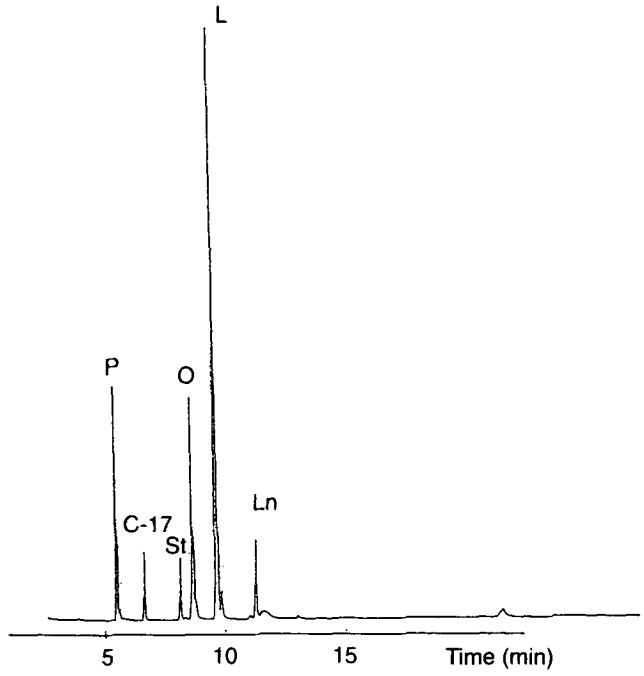

FIG. 3. Gas chromatogram of free fatty acids extracted from the soybean sample by the new technique. The symbols of the acids are the same as in Figure 1.

concentration range in the solvent did not change its number of phases (one).

The complete solid–liquid extraction of FFA (with partial extraction of other acids) into reagent A is carried out during 1–2 min. After the extraction, reagent B should be added to the system for a 5-min reextraction (liquid–liquid) of FFA only.

Reagent B is an aqueous solution of H_2SO_4 and Na_2SO_4. Introduction of this solution into reagent A in volume ratio A:B = 35:15 leads to the phase separation. When the concentration of Na_2SO_4 is lower than 0.5 M, one can observe only two phases (heptane-rich phase and isopropanol–water–salt phase). Increasing the salt concentration leads to formation of the three phases named above in the paragraph "Strategy" (the upper—heptane, the middle—isopropanol, and lower—aqueous phase). The volume of the upper phase depends on the salt concentration due to salt-out effect. At a Na_2SO_4 concentration of 1.3–1.5 M in reagent B, the upper phase volume equals the volume of heptane introduced into reagent A.

The range of H_2SO_4 concentrations (0.5–2.5 M) was chosen because if the acid concentration is less than 0.5 M, the reextraction of FFA is incomplete, and if it is more than 2.5 M, the obtained AV values are larger than those determined by the standard method (probably as a consequence of the hydrolysis of triglycerides).

Sulfuric acid is absent in the heptane layer (based on our experiments without oilseeds). Substitution of H_2SO_4 and Na_2SO_4 with hydrochloric acid and its sodium salt leads to the introduction of acid into the heptane layer and to corresponding interference during the FFA determination.

Technique of reagent set use. An exact amount (2–5 g) of dried and ground oilseed is introduced into a Nessler cylinder, 35 mL of reagent A containing 15 mL heptane is added, the cylinder is closed and shaken for 1–2 min. Then, 15 mL of reagent B is added and shaken for 1 min. The cylinder is left for ~5 min and is periodically hand-turned vertically (and not upside down) for better phase separation (Fig. 4). The upper phase, volume V_u = 15 mL, is removed from the cylinder and treated with dry sodium or magnesium sulfate for rapid elimination of opalescence. An aliquot, V_a = 8–10 mL, is used for pH-metric FFA detection or standard titration, i.e., the determination of the acid value in the extract, AV_e (related to oil weight $W = mp/100$, where m is weight of the oilseed sample in g and p is the oil content in the oilseed in percentage). The acid value in an oilseed sample is $AV_{os} = AV_e (V_u/V_a)$.

Identity of the extracted acids. The identity of the acids extracted by our technique and by the standard procedure (2) was estimated by comparison of the corresponding gas chromatograms. The chromatograms looked similar; therefore, they are shown in Figures 1–3 only for the new technique.

Qualitative composition of FFA (Figs. 1–3 and Table 1) was established by comparison of the relative retention times for the FFA in our samples and those in the oil reference stan-

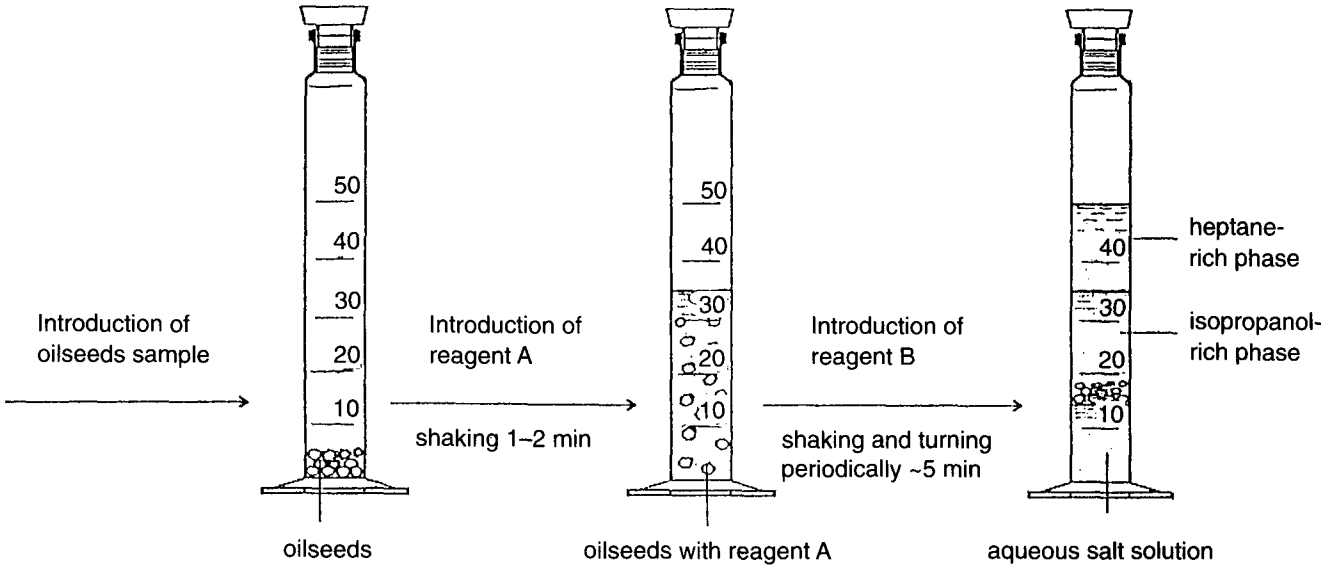

FIG. 4. Diagram for use of the reagent set.

dards AOCS Nos. 1, 2, and 4. As internal standard, heptadecanoic acid was used.

The ratio of the peak area of a FFA to the sum of the peak areas on the chromatogram (%) was used for the comparison of "profiles" of the chromatograms (Table 1). One can see that there are practically no differences between the chromatograms of FFA extracted by the new technique and by the standard method.

Precision and accuracy. Table 2 shows the average results obtained by standard AV_{st} and proposed AV_p techniques from $n = 5$ replicates for each oilseed; standard deviations for these replicates, S_{st} and S_p; Fisher's ratio, $F = S_p^2/S_{st}^2$; and Student's ratio, $t = |AV_{st} - AV_p|/[(S_{st}^2 + S_p^2)/5]^{0.5}$.

The critical value for Fisher's ratio is 6.39 at the 5% level of confidence and the number of degrees of freedom $n - 1 = 4$. For Student's ratio, the critical value is 2.31 at the 5% level of confidence and $2(n - 1) = 8$ degrees of freedom. From comparison of the F-data with the critical value, it follows that the difference between precision of results obtained by standard and proposed techniques is insignificant (all F values are less than the critical one). The accuracy for these techniques is approximately the same in terms of the deviations of the average results obtained by the proposed technique (AV_p) from the average results obtained by the standard technique (AV_{st}), which are insignificant in comparison with the random errors: all t values are less than the critical value (2.31).

Advantage of the novel reagents and their use. The main advantage of the suggested set of reagents for free fatty acid extraction from oilseeds for the purpose of AV determination is the short extraction time (up to ~10 min) while maintaining the uncertainty of the AV determination on the acceptable level.

ACKNOWLEDGMENT

The authors express their gratitude to Prof. E. Schoenberger for his valuable advice.

TABLE 1
Composition of Free Fatty Acid Extracted from Oilseeds[a]

	Sunflower oilseeds		Canola oilseeds		Soybean oilseeds	
Fatty acid	New technique (%)	Standard (%)	New technique (%)	Standard (%)	New technique (%)	Standard (%)
Palmitic	8.4	8.4	6.0	5.9	14.6	15.7
Stearic	4.8	4.3	2.0	1.4	4.8	4.6
Oleic	19.1	17.5	59.3	57.9	19.2	16.4
Linoleic	65.1	69.0	21.8	25.0	54.1	54.9
Linolenic	2.5	0.5	8.2	8.1	7.2	8.3
Arachidic	0.1	0.4	0.7	<0.1	<0.1	<0.1
11-Eicosenoic	n.d.	n.d.	1.9	1.7	n.d.	n.d.

[a]By the new technique and by the standard method (in % of total peak area on the chromatogram). n.d., Not detected.

TABLE 2
Comparison of Acid Value (AV) Determined by the New Technique and by the Standard Method[a]

Oilseed	AV_p	S_p	AV_{st}	S_{st}	F	t
Sunflower	1.66	0.21	1.69	0.19	1.21	0.30
Soybean	1.97	0.07	2.06	0.15	2.00	1.16
Canola	2.67	0.07	2.60	0.13	1.87	1.13

[a]AV_p, average acid value obtained by proposed technique; S_p, standard deviation of the replicates by proposed technique; AV_{st}, average acid value obtained by standard technique; S_{st}, standard deviation of the replicates by standard technique; F, Fisher's ratio; t, Student's ratio.

REFERENCES

1. Oilseeds—Determination of Acidity of Oils, Standards of the International Organization for Standardization, Switzerland, 1988, ISO 729: 1988-11-01.
2. Oilseeds—Determination of Hexane Extract (or Light Petroleum Extract), Colled "Oil Content," *Ibid.*, Switzerland, 1988, ISO 659: 1988-02-15.
3. Bulley, N.R., M. Fattori, A. Meisen, and L. Moyls, Supercritical Fluid Extraction of Vegetable Oil Seeds, *J. Am. Oil Chem. Soc. 61*:1362–1365 (1984).
4. Taylor, S.L., J.W. King, and G.R. List, Determination of Oil Content in Oilseeds by Analytical Supercritical Fluid Extraction, *Ibid. 70*:437–439 (1993).
5. Tiwari, P.N., and R.N. Gambhir, Seed Oil Determination Without Weighing and Drying the Seeds by Combined Free Induction Decay and Spin-Echo Nuclear Magnetic Resonance Signals, *Ibid. 72*:1017–1020 (1995).
6. Dole, V.P., A Relation Between Nonesterified Fatty Acids in Plasma and the Metabolism of Glucose, *J. Clin. Invest. 35*:150–154 (1956).
7. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, Oxford, 1982, pp. 17–19.
8. Dole, V.P., and H. Meinertz, Microdetermination of Long-Chain Fatty Acids in Plasma and Tissues, *J. Biol. Chem. 235*:2595–2599 (1960).
9. Lapshina, T.M., Ya.I. Tur'yan, and S.I. Danilchuk, pH-metric Method for the Determination of Acid Numbers of Oils, *J. Anal. Chem. of USSR 46*:833–840 (1991).
10. Pritchard, J.L.R., Analysis and Properties of Oilseeds, in *Analysis of Oilseeds, Fats and Fatty Foods*, edited by J.B. Rossell, and J.L.R. Pritchard, Elsevier Applied Science, London and New York, 1991, pp. 39–101.
11. May, W.E., and D.J. Hume, An Automated Gas–Liquid Chromatographic Method of Measuring Free Fatty Acids in Canola, *J. Am. Oil Chem. Soc. 70*:229–233 (1993).
12. Bourrel, M., and R.S. Schechter, Microemulsions and Related Systems. Formulation, Solvency and Physical Properties, New York and Basel, Marcel Dekker, 1988, pp. 127–140.
13. Tur'yan, Ya.I., O.Yu. Berezin, I. Kuselman, and A. Shenhar, pH-metric Determination of Acid Values in Vegetable Oils Without Titration, *J. Am. Oil Chem. Soc. 73*:295–301 (1996).
14. Berezin, O.Yu., Ya.I. Tur'yan, I. Kuselman, and A. Shenhar, Alternative Methods for Titratable Acidity Determination, *Talanta 42*:507–517 (1995).
15. Ke-Shun, Liu, Preparation of Fatty Acid Methyl Esters for Gaschromatographic Analysis of Lipids in Biological Materials, *J. Am. Oil Chem. Soc. 71*:1179–1187 (1994).
16. Tur'yan, Ya.I., O.Yu. Berezin, I. Kuselman, and A. Shenhar, Set of Reagents for Rapid Extraction of Free Fatty Acids from Oilseeds, Israel, Patent Appl. 115677, 19.10.95.
17. Vorob'eva, A.L., and M.Kh. Karapet'yants, Miscibility in Systems Water-aliphatic Alcohol-*n*-alkane. III. The Systems Water-Isopropanol-*n*-C_nH_{2n+2} (n = 6,7,8,9), *Russian J. Phys. Chem. 41*:1061–1063 (1967).

[Received March 15, 1996; accepted August 27, 1996]