Note

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Toshiro Murata and Nagayuki Miyazaki : Determination of Free Fatty Acids in Fats and Oils by Non-aqueous Titration Method.

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One of the important chemical methods for examining oils and fats is estimation of their content of free fatty acids. The amount of free acids is expressed in the term of acid value, which indicates the number of milligrams of potassium hydroxide required to neutralize the free fatty acid in 1 g. of fat or oil. In order to determine their acid value, a sample is mixed with ethanol, methanol, amyl alcohol,[1] or a mixture of ethanol and ether,[2] and titrated with standardized aqueous or alcoholic alkali, with phenolphthalein as an indicator.

The acid value of natural fats and oils is variable, entirely depending on the purity of an oil, its age, extent of hydrolysis, and the degree of oxidation suffered. The importance of acid value lies in the fact that it indicates the quality of an oil or fat.

However, in the above-described common method, some problems often arise in obtaining clear end point of the color change, i.e., on titration, the precipitation of fat and oil causes an unpleasant emulsion or cloudiness in the solution. Thus, sometimes warming and addition of a solvent are required to overcome such a disadvantage. Especially, when solid fat is used as a sample, it is often difficult to estimate the acid value precisely.

Fritz and others[3] successfully determined some fatty acids in a solvent mixture of methanol and benzene, with titrant of sodium methoxide, using Thymol Blue as an indicator. Such a titric estimation in non-aqueous solvent was used for determination of the amount of free fatty acid in fats and oils in the present series of work.

Experimental and Results

1) Reagents—a) 0.1*N* Sodium Methoxide Solution : To 16.7 cc. of MeOH, 1.2 g. of metallic Na was dissolved with cooling in H_2O and exclusion of CO_2. After complete solution of Na, 30 cc. of MeOH and 250 cc. of benzene were added to the solution.

b) MeOH and benzene mixture (1:3)

c) Thymol Blue reagent : The indicator was prepared by dissolving 0.1 g. of Thymol Blue in 100 cc. of MeOH.

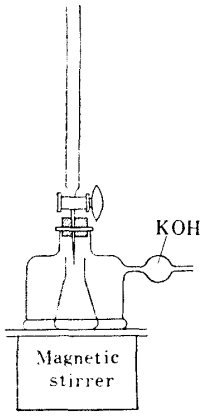

Fig. 1.

2) Standardization of 0.1*N* Sodium Methoxide Solution—About 0.05~0.1 g. of BzOH was accurately weighed, dissolved in 20 cc. of benzene-MeOH mixture, and Thymol Blue reagent was added to the solution. Titration was carried out in the apparatus shown in Fig. 1, excluding CO_2 from air and human respiration. A magnetic stirrer was used for agitation of the titrated mixture. The titration was completed when the color of the solution changed to deep blue. A blank test was carried out at the same time.

3) Estimation of Stearic Acid—Stearic acid (commercial sample, m.p. 70.1°) was accurately weighed and estimated by the titric method as described above. This analytical method is called "non-aqueous titration" in this paper. The results are shown in Table I.

4) Estimation of Palmitic Acid—Palmitic acid (m.p. 61.3°) was titrated with MeONa in non-aqueous solvent as described above. The results are shown in Table II.

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1) A. Halphen : Ann. chim. anal. appl., **6**, 133 (1901).
2) E. Boedtker : Chemiker Ztg., 548 (1911).
3) J. S. Fritz, N. M. Lisicki : Anal. Chem., **23**, 589 (1951).

Table I. Determination of Stearic Acid (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	Found (g.)	Found (%)	Error (%)
0.0218	0.810	0.0230	105.2	5.50
0.0212	0.798	0.0227	106.8	7.07
0.0163	0.635	0.0181	110.6	11.04
0.0316	1.192	0.0339	108.0	7.28
			(Av.) 107.65±0.35	

Table II. Determination of Palmitic Acid (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	Found (g.)	Found (%)	Error (%)
0.0478	1.859	0.0477	99.7	0.21
0.0543	2.143	0.0549	101.3	1.10
0.0448	1.801	0.0462	103.1	3.13
			(Av.) 101.37±0.66	

Palmitic acid was recrystallized to melt at 61.9° and the recrystallized sample was estimated by the same method as above. The recovery rate is shown in Table III.

Table III. Determination of Recrystallized Palmitic Acid (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	Found (g.)	Found (%)	Error (%)
0.0351	1.378	0.0353	100.7	0.57
0.0386	1.514	0.0388	100.6	0.52
0.0805	3.166	0.0812	100.9	0.87
			(Av.) 100.73±0.05	

The recrystallized palmitic acid was estimated also by the usual acid-value determination method. The acid was dissolved in EtOH-Et_2O mixture (1:1) and titrated with 0.1*N* KOH with phenolphthalein as an indicator. This method is called "aqueous titration" in this paper. The results are shown in Table IV.

Table IV. Determination of Recrystallized Palmitic Acid (Aqueous titration)

Sample (g.)	0.1*N* KOH (cc.)	Found (g.)	Found (%)	Error (%)
0.0300	1.135	0.0291	97.04	3.00
0.0278	1.078	0.0286	99.43	2.91
0.0331	1.253	0.0321	97.08	3.02
			(Av.) 97.85±0.61	

5) **Estimation of Free Fatty Acids in Butter**—a) By Aqueous Titration : About 0.5 g. of butter was dissolved in 40 cc. of EtOH-Et_2O mixture (1:1), to obtain a yellow emulsified solution. Further 20 cc. of the solvent mixture was added to the emulsified solution, but the solution was not entirely clarified. The solution was titrated with 0.1*N* KOH solution, using phenolphthalein as the indicator, up to the red coloration of the solution. Sometimes, vigorous shaking was required to prevent cloudiness in the solution by the precipitation of fatty acid salt from Et_2O layer. The estimated acid values are shown in Table V.

Table V. Estimation of Free Fatty Acids in Butter (Aqueous titration)

Sample (g.)	0.1*N* KOH (cc.)	Acid Value
2.354	0.531	1.266
2.180	0.493	1.268
2.355	0.520	1.240
		(Av.) 1.255±0.008

b) By Non-aqueous Titration : About 2～4 g. of the sample butter was titrated with 0.1*N* MeONa solution in MeOH-benzene mixture and consumed mg. of MeONa was calculated for corresponding mg. of KOH. The results are shown in Table VI.

Table VI. Estimation of Free Fatty Acids in Butter (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	MeONa (mg./g. of butter)	Calcd. KOH (mg./g. of butter)
2.452	0.546	1.204	1.250
2.689	0.601	1.207	1.252
3.773	0.844	1.208	1.252
		(Av.) 1.206±0.001	1.252±0.001

6) Estimation of Free Fatty Acids in Vegetable Oils—Free fatty acids in peanut oil, rape oil, and soy-bean oil were determined by both aqueous and non-aqueous titrations, and the results obtained are shown in Thbles VII～XII.

Table VII. Free Fatty Acids in Peanut Oil (Aqueous titration)

Sample (g.)	0.1*N* KOH (cc.)	Acid value
2.8196	1.041	2.071
2.8810	1.069	2.082
2.6118	0.965	2.068
2.5814	0.961	2.089
2.5632	0.940	2.058
2.7900	1.016	2.044
		(Av.) 2.069±0.005

Table VIII. Free Fatty Acids in Peanut Oil (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	MeONa (mg./g. of oil)	Calcd. acid value
2.7015	0.998	1.995	2.702
2.8397	1.057	2.011	2.088
2.7973	1.048	2.024	2.102
3.0508	1.132	2.005	2.082
2.9978	1.099	1.980	2.056
2.8387	1.066	2.029	2.107
		(Av.) 2.007±0.006	2.085±0.006

Table IX. Free Fatty Acids in Rape Oil (Aqueous titration)

Sample (g.)	0.1*N* KOH (cc.)	Acid value
1.9056	2.196	6.465
1.6611	1.910	6.453
1.6721	1.912	6.417
1.7415	1.986	6.398
1.8239	2.803	6.409
		(Av.) 6.428±0.01

Table X. Free Fatty Acids in Rape Oil (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	MeONa (mg./g. of oil)	Calcd. acid value
1.7422	1.960	6.080	6.314
1.8182	2.061	6.125	6.361
1.9734	2.240	6.133	6.369
1.9380	2.197	6.126	6.362
2.0611	2.327	6.099	6.334
		(Av.) 6.113±0.008	6.348±0.009

Table XI. Free Fatty Acids in Soy-bean Oil (Aqueous titration)

Sample (g.)	0.1*N* KOH (cc.)	Acid value
2.4310	2.040	4.709
2.3096	1.929	4.687
2.4306	2.042	4.715
2.1600	1.816	4.717
2.3187	1.939	4.691
	(Av.)	4.704±0.0052

Table XII. Free Fatty Acids in Soy-bean Oil (Non-aqueous titration)

Sample (g.)	0.1*N* MeONa (cc.)	MeONa (mg./g. of Oil)	Calcd. acid value
2.944	2.409	4.422	4.592
2.718	2.245	4.463	4.635
2.548	2.100	4.453	4.624
2.636	2.152	4.411	4.581
2.408	1.964	4.407	4.577
2.712	2.226	4.434	4.605
		(Av.) 4.432±0.007	4.602±0.008

Discussion

In the present series of experiments, recovery of stearic acid was greater than the value obtained by Fritz.[3] Recrystallization of the sample increased the recovery rate in the case of palmitic acid. These findings suggested that stearic acid used might have contained some impurities.

Butter contains some acidic substances other than free fatty acid. A principal component of the acidic substance is supposed to be lactic acid, which should be titratable in non-aqueous solvent. When using sodium methoxide as the titrant, the titration was more easy and clear than with aqueous potassium oxide solution as a titrant.

Such improvements by the use of sodium methoxide as titrant in non-aqueous solvent were also observed in determination of the amount of free fatty acid contained in three kinds of vegetable oils. Furthermore, in such a colored oil as the peanut, rape, and soy-bean oils, the color change of phenolphthalein is considerably difficult to identify, compared with the clear change of Thymol Blue.

Since fats are more soluble in benzene–methanol mixture than in ether–ethanol mixture, the amount of the former solvent to be used is smaller and procedure is carried out rapidly.

Except in the case of peanut oil, all acid values obtained by non-aqueous titration method were smaller than those obtained by aqueous titration method. Only peanut oil dissolved well in ether–ethanol mixture and was titrated with potassium hydroxide solution without producing any precipitation during the procedure. Therefore, the greater acid values obtained by aqueous titration method for other fats and oils might be due to difficulty of identifying the end point of titration.

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Summary

Free fatty acids in butter and in peanut, rape, and soy-bean oils were estimated in benzene–methanol mixture by titration with 0.1*N* sodium methoxide, using Thymol Blue as an indicator, and accurate estimations were able to be made rapidly and with ease.

The values obtained by this method were compared with those obtained by the usual acid value estimation method.

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