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A RAPID SILICA GEL METHOD FOR MEASURING TOTAL FREE FATTY ACIDS IN MILK[1,2]

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Analyses for free fatty acids resulting from lipolytic activity in milk have been, in general, either too time consuming or inaccurate, or both. In the past, considerable attention has been given to methods involving solvent extraction of the fat and free fatty acids followed by titration of the extract. The main disadvantage of these methods is that the water-soluble fatty acids are only partially recovered *(1, 3, 5)*. More recently, chromatographic analyses have been applied to measure the total free fatty acids in saponified milk fat *(2, 4)*. These techniques have shown promise of meeting the requisites of the routine analyst, i.e., they are quantitative, sensitive, and relatively rapid. However, little attention has been given to the possibility of utilizing an inert support, such as silica gel, for the rapid separation of relatively small quantities of free fatty acids from milk, such as may be produced by lipolysis.

This report describes a method for the rapid, quantitative recovery of small amounts of free fatty acids from milk and cream by directly separating the acids from the product without prior fractionation.

PROCEDURE

The procedure of Harper and Armstrong *(2)* for the determination of butyric acid and higher fatty acids was modified so that the total quantity of free fatty acids could be recovered in one fraction and with one solvent in approximately 15 minutes, instead of 60 minutes as required with the original method. This modified method is outlined briefly below. The reader is referred to the original paper for a more detailed description of equipment and reagents.

A. *Equipment.* A chromatographic column approximately 35 mm. I.D. by 250 mm. in length and with a perforated glass disc sealed into a 34/23 standard-taper joint is utilized; the column is attached to a suction flask.

B. The silica gel column is prepared in two sections; the bottom section contains a mixture of silicic acid and phosphate buffer, and the top section contains the acidified milk and silicic acid.

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1. *Bottom section.* Five g. of dry silicic acid (Mallinckrodt No. 2847) are mixed thoroughly with 3 ml. of 2 *M* KH_2PO_4-K_2HPO_4 buffer (pH 6.5) and slurried with 20 ml. U.S.P. chloroform. A stock suspension of the above ingredients may be prepared at one time and stored in a tightly stoppered brown bottle. Twenty-five ml. of the well shaken suspension is used for each analysis.

2. *Top Section.* A sample of milk is pipetted (or weighed) into a mortar and is acidified to pH 1.8-2.0 (predetermined) with 20% H_2SO_4 (V:V). Five g. of silicic acid is then added for every 3 ml. of milk. The size of the sample will depend on its content of fat, total solids, and free fatty acids. Up to 20 ml. of milk may be used. After the addition of silicic acid, the mixture is ground thoroughly, slurried with 5% *n*-butanol in chloroform, and transferred quantitatively on top of the bottom section. A small wad of cotton is stuffed tightly into the exit end of the column just prior to addition of the top section. The cotton serves to retain any silicic acid particles which may pass through with the eluant.

C. *Sample size.* The sample size may be varied from 5 to 20 g., depending on the fat content and nature of the sample. With fresh milk it is necessary to use at least 10 g. in order to minimize errors in analysis.

D. *Extraction procedure.* The column is attached to a suction flask and a vacuum is applied so that the solvent will flow through the column at the rate of about 50 ml. per 2 minutes. The extraction is completed when 150 ml. of the 5% butanol in chloroform solvent have been collected.

E. *Estimation of acids.* The acids in the eluate are titrated as previously described *(2)*, except that 15 ml. of neutral absolute alcohol and 0.3 ml. of the phenol red indicator are used. A 50/50 (V:V) mixture of either ethyl ether and absolute ethanol or ethyl ether and isopropyl alcohol may be used to replace the absolute ethanol *(7)*. A photometer may be used to standardize the end-point. The results are expressed in terms of acid degree, i.e., ml. N base necessary to neutralize 100 g. fat.

EXPERIMENTAL RESULTS AND DISCUSSION

The reliability of the method was established by determining the recovery of known acids (butyric, caproic, capric, lauric, palmitic, and oleic) added individually to milk prior to acidification. Four trials were made in which the concentration of added acids was 10, 20, 40, and 60 microequivalents. The concentration of acid had no effect on the recovery. The results are summarized in Table 1. All the acids were quantitatively recovered; the poorest recovery occurred for palmitic acid, 90% of which was recovered.

The reproducibility of the method is illustrated in Table 2 for five replicate analyses of six different samples. The maximum deviation from the mean for any given sample was from 4.3 to 10.1%; and the average deviation from the mean was from 1.5 to 5.9%. The average maximum deviation was 7.6% and the average of the mean deviation was ±4.05%.

TABLE 1

Reliability of the silica gel extraction method for the recovery of various fatty acids added to milk[a]

Acid	Per cent recovery		Average deviation from mean
	Range	Average	
Butyric	95-106	99.0	± 3.5 per cent
Caproic	96-104	100.0	± 4.0 per cent
Capric	92-94	93.0	± 1.0 per cent
Lauric	92-101	94.0	± 4.6 per cent
Palmitic	88-93	90.0	± 2.0 per cent
Oleic	92-98	96.0	± 3.0 per cent

[a] The percentage recovery of each acid is based on four replicate trials.

The silica gel extraction method was compared with the chromatographic method of Harper and Armstrong *(2)*. In order to obtain sufficient concentration of free acids in fresh milk without using large volumes for analysis with the silica gel method, 20 ml. of milk was neutralized and lyophilized prior to analysis. The freeze drying did not influence the results of the analyses. During

TABLE 2

Reproducibility of silica gel extraction method with milks of different acid degrees

Sample No.	Acid degree range	Average acid degree[a]	Per cent deviation from average acid degree		
			Minimum	Maximum	Average
1	0.63- 0.75	0.67	1.1	10.1	±5.9
2	1.22- 1.34	1.26	0.0	6.3	±1.5
3	1.85- 2.20	2.00	2.5	10.0	±4.5
4	2.45- 2.80	2.60	1.6	7.5	±5.5
5	4.50- 4.90	4.71	0.3	4.3	±3.6
6	13.70-15.00	14.80	0.9	7.4	±3.3
Average				7.6	±4.05

[a] Average of five trials.

the study, 40 samples varying in acid degree values from 0.51 to 6.0 were analyzed. The results are presented in Table 3. The data are grouped in four different acid degree ranges in order to determine the applicability of the method for samples of widely varying free fatty acid content. The results show that the silica gel extraction method gave results comparable to the chromatographic

TABLE 3

Comparison of the silica gel extraction method and the chromatographic method

Acid degree range	No. of samples	Per cent variation for mean acid degree value[a]		
		Minimum	Maximum	Mean
0.5-1.0	9	8.1	7.6	±3.7
1.1-2.0	12	9.3	10.9	±4.6
2.1-3.0	8	5.3	8.6	±5.1
3.0-6.0	10	7.3	9.2	±4.8
Average		9.3	10.9	±4.6

[a] Acid degree values by the silica gel extraction method compared to average acid degree values obtained by chromatographic method of Harper and Armstrong *(2)*.

method. The average deviation from the mean was within the experimental error of the longer method *(2)*.

The silica gel extraction method was also compared with the solvent extraction method of Johnson and Gould *(3)* as modified by Thomas *et al.* *(6)*. Twenty-six samples, which varied in acid degree values from 0.37 to 7.0, were analyzed in duplicate by each method. The average acid degree values are compared in Table 4. The data are divided into two groups, the samples with

TABLE 4

Comparison of the silica gel extraction method with the solvent extraction method

Acid degree values			
Solvent extraction	Silica gel extraction	Solvent extraction	Silica gel extraction
0.37	0.39	1.50	1.55
0.70	0.68	1.87	1.88
0.92	0.98	1.82	2.10
0.94	0.94	1.92	2.15
0.94	0.96	2.12	2.30
1.02	0.91	2.20	2.22
1.05	0.95	2.20	2.35
1.07	1.01	2.35	2.50
1.19	0.94	2.98	3.05
1.13	1.18	3.64	3.87
1.22	1.28	3.80	4.10
1.25	1.20	4.40	4.61
1.34	1.40	6.70	7.05

acid degrees of less than 1.5 and those with 1.5 or more. For samples with acid degrees of less than 1.5, the values obtained by the two methods were in very good agreement. However, for samples with acid degree values greater than 1.5, the silica gel extraction method gave slightly higher values. This is especially true for all samples with an acid degree value of over 2.3, for which the silica gel method gave results that are consistently about 5% higher than those obtained by solvent extraction. The difference in the two methods is thought to be related to the better recovery of the lower fatty acids by the silica gel extraction method.

SUMMARY

A silica gel extraction procedure is described for determining the total free fatty acids in milk without prior extraction of the fat. The method is rapid and provides quantitative recovery of the free fatty acids in milk. It requires only 15 minutes instead of about 60 minutes as required in previous methods. The method is more quantitative in respect to the recovery of free fatty acids than any of the previous methods used for the measurement of free fatty acids in milk. However, in samples of acid degrees of less than 1.5 the results obtained by the silica gel extraction method are comparable to those obtained by the solvent extraction method and the chromatographic method. With acid degrees higher than 1.5 the silica gel extraction method generally reveals a higher free fatty acid content.

Preliminary results also indicate that the solvent extraction method is applicable to the analysis of other dairy products, such as dry milk, condensed milk, ice cream mix, and butter.

To minimize the expense of reagents, freeze-dried samples may be used if desired.

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