

THE ESTIMATION OF FAT IN MILK, USING PETROLEUM ETHER AS A SOLVENT.

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THE almost universally used solvent for fat in milk analysis is ether, and the form usually employed in this country is methylated ether. This solvent has certain undesirable qualities, as it is composed largely of methyl-ethoxide, which, being very volatile, causes a considerable loss of solvent.

Petroleum ether is free from these drawbacks, and has the further advantage of being an excellent solvent for fats, and not dissolving many other substances. Another property in which petroleum ether differs from ether is its insolubility in water, a property which has been considered as an advantage, but which is the one property to which many failures to successfully employ this solvent is due.

In order to see plainly the conditions under which petroleum ether can be used to extract fat from milk, it is necessary to consider slightly the theoretical bearings of the subject.

Milk may be considered for this purpose as an emulsion of small fat globules in a somewhat viscous liquid; the relation between the surface energy of the fat and the liquid is such that a layer is condensed around the fat-globules, which prevents its being touched by anything except the liquid, unless special forces are brought into play. It would therefore be quite hopeless to expect to extract fat from milk by petroleum ether or any solvent which does not dissolve in the liquid portion.

By so modifying the relation between the surface energy of the fat and the liquid that the layer becomes a semi-permeable membrane, a solvent such as petroleum ether would extract fat from this liquid, provided the solvent could be brought into contact with each globule. A solvent like ether soluble in the liquid would be very much more efficient in this respect, as it would dissolve in the liquid, and distribute itself between liquid and fat in proportion to its solubility in each; as ether is very soluble in (liquid) fat, and fairly so in aqueous liquids, the fat globules would soon take up large amounts of ether, and become practically solutions of fat in ether; from their large size and low density they would rapidly rise to the surface, and coalesce to form a liquid layer, and the fat would without difficulty become evenly distributed throughout the ether on shaking.

To attain the same end with petroleum ether, the mixture must be so perfect that every fat globule is brought into contact with petroleum ether. This state of things can only be attained in practice when a sufficient length of time is allowed for the fat globules to rise to the surface, which would prolong the time necessary for an estimation of fat to many hours. Under these circumstances the use of petroleum ether would be a disadvantage.

The employment of amyl-alcohol to expedite the time taken for the fat to rise to the surface, devised by Leffmann and Beam, and elaborated by Gerber, appeared to be a condition likely to insure the extraction of fat by petroleum ether in a short time. We had no fear that amyl-alcohol would be extracted with the fat, as we were

convinced by previous experiment that it combined with the sulphuric acid used to make the milk suitable for extraction, and formed a compound very soluble in water.

The mode of procedure described below was devised entirely from theoretical considerations, though we hoped at first to be able to omit the washing with water. The fact that the process founded on theory worked well in practice incidentally affords evidence of the correctness of the views set forth above.

The following experiments illustrate the slowness with which petroleum ether extracts fat from milk boiled with hydrochloric acid, when nothing is present to hasten the separation of the fat. Three samples of milk were treated by the Werner-Schmid process, petroleum ether being substituted for ether.

	I.	II.	III.
Percentage of fat (Storch)	3.95	3.74	3.84
Fat deduced from the evaporation of an aliquot portion of petroleum ether	—	2.28	—
Fat in three extractions with petroleum ether	2.59	3.20	2.83
Fat in a second three extractions with petroleum ether	2.84	3.31	2.94

We next substituted sulphuric acid for the hydrochloric acid, and added amyl-alcohol—in fact, we used the Gerber method as far as mixing was concerned, but instead of placing in a centrifugal machine we cooled and added petroleum ether. This was allowed to separate, re-mixed, allowed to separate again, re-mixed a second time, and finally allowed to separate; an aliquot portion was evaporated. It was observed that the petroleum ether, after the first separation, was not homogeneous, a more highly-refracting layer being easily seen at the bottom. This was expected, and the re-mixing was adopted to guard against this. We found that the layer of petroleum ether containing fat always occupied a volume greater than the petroleum ether added, and the excess was practically equal to the volume of the fat. It was found also that petroleum ether extracted from a mixture of sulphuric acid, water, and amyl-alcohol, a small amount of substance having an ethereal smell.

The table below gives the results of our preliminary experiments :

	I.	II.	III.
Percentage of fat (Storch)... ..	3.34	3.88	3.88
Percentage of fat deduced from an aliquot portion, neglecting volume of fat... ..	3.38	3.91 3.88 3.87	3.91 3.88
Percentage of fat deduced from an aliquot portion, correcting for volume of fat	3.43	3.97 3.96 3.95	3.97 3.96
Percentage of fat deduced from an aliquot portion, correcting for volume of fat, and for blank	3.33	3.87 3.87 3.86	3.87 3.87
Percentage of fat deduced from complete extraction	3.52	—	—
Ditto corrected for blank	3.32	—	—
Percentage of fat deduced from complete extraction and washing the petroleum ether once with water...	—	3.90	3.86

In all experiments where the petroleum ether was not washed the fat darkened on drying, and an odour resembling that of amyl butyrate was developed; this we attribute to the extraction of a little amyl-hydrogen sulphate.

We found that when the petroleum ether was washed the fat was nearly white and odourless.

The following mode of procedure is now adopted: Nine c.c. of sulphuric acid (90-91 per cent. H_2SO_4) are measured into a tube holding about 50 c.c., and constricted just above the point where 20 c.c. reach; 10 grammes of milk are weighed into this tube, care being taken to prevent the milk and acid mixing; 0.9 c.c. of amyl alcohol is added, the tube corked, and well shaken; after cooling to about 25°C ., 20 c.c. of petroleum ether are added, and the tube well shaken. When separation is complete, the contents of the tube are again well mixed, and allowed to separate; a second re-mixture and separation is given, and the petroleum ether blown off into a tube containing 20 c.c. of water, with which it is shaken and allowed to separate. After separation from the water, the petroleum ether is blown off into a tared flask. Further portions of petroleum ether are added to the tube containing the acid liquid, blown off into the tube containing the water, and transferred to the flask.

We are in the habit of whirling the tubes in a Leffmann-Beam centrifugal machine to reduce the time necessary for separation.

The following additional results will show that this method compares well with others.

Petroleum Ether.	Other Methods.	Methods Used.
3.76	3.75	Storch
3.62	3.62	"
3.04	3.00	Ritthausen
3.63	3.59	Storch
3.64	3.60	"
3.67	3.66	"
4.00	4.00	"
64.72	64.70	Amyl alcohol extraction

We use this method chiefly because the employment of ether is avoided.

DISCUSSION.

Dr DYER thought that it was rather to the credit of petroleum ether that it did not give, in the Werner-Schmid process, the same good results which were obtained with ordinary ether. The Werner-Schmid method, although it gave excellent results, only did so in virtue of its errors being balanced in different directions. Scientifically speaking, in spite of its good results, it was a slipshod process. It was assumed that all the fat was obtained in the supernatant ether, but as a matter of fact a tangible proportion of fat remained in association with the ether that still remained in the acid fluid. However, the residue of the supernatant ether, which was called fat, contained, besides fat, nondescript substances, formed probably by the action of the acid on the milk sugar and curd. These impurities, by some happy natural provision, balanced the fat that was not dissolved in the supernatant ether, so that fairly accurate results were obtained. Petroleum ether apparently did not dissolve these substances, though after prolonged shaking higher results were obtained. The

Werner-Schmid process gave admirable results if it was carried out in a separator, the acid liquor being run off and washed with ether a second time, the total ether washings being then washed with a small quantity of water. In the ordinary way, if the ethereal residue were re-dissolved in ether after it was dry, there was a considerable quantity of material which did not dissolve. The errors, perhaps, were not very great, and balanced one another pretty closely in the case of ordinary milk, but in the case of separated milk the percentage error, having regard to the small proportion of fat, was often very large in the Werner-Schmid process as generally used.

Mr. CHAPMAN remarked that petroleum ether was somewhat variable in character, and inquired whether the particular kind of petroleum ether employed affected the results.

Mr. RICHMOND said that Mr. Rosier and he had only employed one kind of petroleum spirit, which was obtained by careful fractionation, and boiled below 80° C. As low a boiling-point as possible was chosen in order that the petroleum spirit might be readily driven off from the fat by drying in a water-bath. He might mention that the fat extracted by petroleum spirit was obtained perfectly white and free from odour if the spirit containing the fat were washed with water. If this were not done, a small quantity of acid was dissolved out, probably in the form of acid ethereal sulphate.
