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MODIFICATION OF THE MOJONNIER
METHOD OF FAT DETERMINATION
BY SUBSTITUTION OF SOLVENTS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Richard Emerson Marland
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**Modification of the Mojonnier Method
of Fat Determination by Substitution
of Solvents**

presented by

Richard E. Marland

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of the requirements for

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by

Richard Emerson Marland

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MODIFICATION OF THE MOJONNIER METHOD OF FAT DETERMINATION
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INTRODUCTION

For many years the Mojonnier method of determining the fat content of dairy products has been the standard laboratory control procedure for the dairy industry. This method, which is a modification of the Rose-Gottlieb technique only in regard to apparatus, utilizes ethyl and petroleum ethers as solvents.

The relatively high costs of these two ethereal agents, along with recent war-time shortages of ethyl ether, have created a need for an investigation into the possibility of replacing these reagents with less expensive and more easily obtainable materials. Although substitutes for these solvents have been suggested before, available data are limited and inconclusive.

Furthermore, despite the fact that the Rose-Gottlieb (Mojonnier) method has been a standard for several years, little information is available on the efficiency of extraction when variable quantities of solvents or other reagents are used. Similarly, a scarcity of specific data exists concerning certain of the fundamental concepts of the functions of the various reagents, especially in regard to the interactions resulting from the addition of the solvents.

In view of the present shortage and economic considerations involving the solvents, and because of the need for additional information pertaining to the principles of the Mojonnier method, investigations along these lines appear desirable.

REVIEW OF LITERATURE

The existing procedures for the gravimetric determinations of fat in dairy products may be classified into two groups: (a) dry extraction or (b) wet extraction. Dry extraction procedures involve a preliminary drying of the milk, or other product, to an almost anhydrous condition before proceeding with the extraction. In wet extraction procedures, the milk, or other product, is extracted without any preliminary dehydration process.

Dry Extraction Procedures Involving Ether

The Adams Method: The Adams method as described by Richmond (59) and Croll (12) is purportedly the first gravimetric method developed for the analysis of milk for fat and was adopted by the Society of Public Analysts as a quasi-official method (59). The procedure is as follows: A weighed quantity of milk is absorbed by blotting paper and the milk and paper dried to constant weight at 100° C. The dried paper coil is placed in a Soxhlet extractor and extracted with ether. The ether is then evaporated and the fat dried to constant weight.

Richmond (59) describes the work done by numerous investigators concerning the accuracy of this method. These workers agreed that the blotting papers contained ether soluble substances causing too high a value to be obtained. Richmond (59) overcame this difficulty by extracting a piece of blotting paper as a blank along with the milk samples.

Later studies by Hals and Klykksen (24) showed the Adams test to give lower results than the Gottlieb, Werner-Schmid or Gerber tests. Eichloff (14) objected to the use of the Adams method when milk was abnormally acid as the results obtained were too high. Thomsen (77) modified the Adams method by treating the milk sample with pepsin and hydrochloric acid before drying. He

found this treatment improved the results obtained when skim milk and butter milk were analyzed. Timpe (78) recommended the addition of 25 per cent of sulphuric acid to the ether used to dehydrate it, thus eliminating the water soluble residue from the fat. Rijnfeld (62) compared the Soxhlet extraction method with the Bonnema. The results indicated that both methods were equally good. The Bonnema method, as described by Croll (12) was based on principles similar to the Rose Gottlieb method, but included the addition of gum tragacanth (originally suggested by Rusting (67)) to gelatinize the aqueous layer, making separation easier. Other workers (3, 6, 16, 24) have found the Adams method to be inaccurate in the analysis of certain dairy products, chiefly concentrated products, and have recommended other methods.

Storch Method: The Storch method of analysis as described by Richmond (59) was another early development. This method calls for the drying of milk on pumice at 100° C, grinding to a very fine powder, and extracting the fat by percolation using ether as an extraction medium. The grinding and extraction were repeated, and all ether washings were collected in a tared flask, distilled, and the fat dried and weighed. Richmond (59) commends the accuracy of the Storch method. However, due to its length and the difficulty of grinding with pumice, he proposes the use of Kieselguhr for pumice and the use of the Soxhlet extractor. This worker states that plaster of Paris and kaolin also could be used satisfactorily as grinding substances.

Other Methods: The Somerset House Method of Fat Extraction, which was developed by Bell of England (59) differs from that of Storch in that the milk is neutralized with N/11 strontia solution using phenolphthalein as an indicator. The neutralized product is dried on a hot plate with continual mixing. When the texture is such that it can be broken up, 20 ml. of methylated ether are added and rubbed up with the dried powder. The ether is decanted through

a weighed filter. This extraction and maceration process is continued for eight extractions. When the ether extractions are finished, the same procedure is followed using alcohol in place of the ether for two extractions. These are followed by eight more ether extractions. The filter is washed with ether, the ether distilled off and the fat dried and weighed. The author claims that the slight error due to phenolphthalein and strontia in the residue can be ignored.

A somewhat similar method has been proposed by Mitchell and Alfend (47) for the analysis of butter. This method is commonly called the Sand-Gooch method and was found to be inferior to the Kohman technique (38).

Richmond (59) describes a method devised by Babcock in which the milk is dried in a perforated metal cylinder, using specially prepared asbestos as the drying material. The extraction is carried out in the same container using anhydrous ether. The results using this method agreed closely with those secured by using the Adams procedure.

The refractometer has been used in estimating the fat in ethereal extracts of milk by some workers. Such a method is the Woolney technique (12, 23). In this method, an aliquot portion is taken, and the fat calculated by use of the refractometer. The value obtained is used for calculation of the fat in the sample. Hals and Gregg (23) found the method to lack rapidity and accuracy.

Several miscellaneous dry extraction methods have been reviewed by Richmond (59). All of these methods were proposed by some of the earlier workers (Wanklyn, Moore, Piggott, Lieberman, Baynes, Marpmann, Ganutter, Duclaux, Wiley, Johnson, Fernandez-King, Hampe and Froidevaux).

Wanklyn extracted the fat from dried milk solids without any other medium being present except the ether. Morre, Piggott and, later, Liebermann

dehydrated the milk by adding to it anhydrous copper sulphate. The dry residue was extracted with petroleum ether. Baynes employed powdered glass as a grinding medium in his modification of the Storch method. Marpmann used cotton wool, Ganutter used wood-fiber, Duclaux used sponge, Wiley and Johnson used asbestos as absorbing agents in their adaptations of the Adams method. Fernandez-King and Hampe dried their milk samples by use of anhydrous sodium sulphate in conjunction with dried Kaolin. The anhydrous product was extracted directly with 25 ml. of ether, and an aliquot portion taken for analysis. Froidevaux precipitated the protein and fat by use of a calcium phosphate solution containing acetic acid. The fat is extracted from this precipitate as in the Ritthausen process.

Wet Extraction Procedures Involving Ether

The earliest method using the wet extraction technique was originated by Horsley (29) and was known as the Meigs method (46). The procedure is as follows: ten ml. of milk are weighed and poured into a 100 ml. graduated cylinder, using 20 ml. of water to wash the weighing dish and dilute the sample. Twenty ml. of ether are added and the cylinder shaken violently for five minutes. Twenty ml. of alcohol are then placed in the cylinder and the whole shaken again for five minutes. On standing, the ethereal layer separates and is drawn off with a pipette. Several five ml. portions of ether are shaken in and removed to wash out any remaining fat. The ether is boiled off and the fat is weighed. Meigs states that this method gives distinctly higher results than the dry extraction methods, and a determination may be conducted in less than one hour.

Croll (12) made numerous comparisons of Meigs' method with the Soxhlet method and found an average deviation of 0.023 per cent in 24 samples. He further proceeded to modify Meigs' method by adapting a new type of mixing

cylinder and ether layer removing tube which resembles the presently used wash bottles. His results show that this new apparatus shortened the length of time necessary to complete the extraction without incurring any greater error than the original Meigs method.

Fehling Solution as Precipitant: Fehling's solution has been used to precipitate the proteids in milk with the precipitate trapping the fat. (Gudeman (21), Ritthausen (59), Broeman (8), Rieter (61), Sutton (74), and Szilasi (75)). Broeman (8) and Sutton (74) treated the precipitate with hydrochloric acid whereas Szilasi (75) treated the precipitate with NaOH. However, Gudeman (21), Reiter (61) and Ritthausen (59) extracted the precipitate directly using ether and alcohol. Gudeman (21) made extensive investigations on the efficiency of this technique and concluded that in a high fat product, a preliminary extraction with petroleum ether should be conducted. Richmond (59) quotes workers as stating that the Ritthausen method cannot be used for the estimation of fat in sterilized or condensed milk, but Broeman (8), Sutton (74), and Szilasi (75) found their modifications to give excellent results when used on concentrated milks.

Acid Methods: Various acids, notably sulphuric, hydrochloric and acetic, have been used as agents to facilitate extraction. The Richmond and Rosier method (60) was developed to provide a more rapid extraction method which substituted petroleum ether for methylated ether because of the undesirable water soluble material often carried over by the latter. The procedure is as follows: Nine ml. concentrated sulphuric acid are placed in a 50 ml. tube having a constriction at the 20 ml. mark. Ten grams of milk are added being careful to prevent mixing with the acid. This is followed by the addition of 0.9 ml. of amyl alcohol and the mixture is then thoroughly shaken. After cooling to about 25° C, 20 ml. of petroleum ether (b. p. 60° C.) are added and the sample

again thoroughly shaken. When the layers separate, an additional shaking is made. The separation and shaking are repeated the second time and the ether layer then decanted into a beaker containing 20 ml. water. After separation from the H_2O , the ethereal layer is removed to a tared flask where the fat is dried and weighed. Richmond explains the function of amyl alcohol as causing the surface energies of the surface of the fat globule to be broken down, allowing the ether to penetrate to the fat globule. Richmond and Rosier's data show good agreement with those secured with the Storch and Ritthausen methods, but according to Richmond (59) the good results are due to a compensation of errors, since the fat is affected by the sulphuric acid even as it is in the Gerber test from which this test was adapted.

Cochrane (10) developed a modification of the Babcock test similar to Richmond and Rosier's (60) modification of the Gerber test. Cochrane's method followed the usual Babcock method until the centrifuging step, at which point he added ether for dissolving the fat freed by the action of the acid. The ether layer is drawn off, and the fat determined. This method is not extremely accurate because of the action of sulphuric acid on ether, as explained by Richmond (59).

A test devised by Cobleigh (9) is very similar to Cochrane's, and is especially adapted to homogenized milk.

Richmond and Musgrave (57), finding that all other available methods failed in the analysis of malted milk, developed their own method which is as follows: one gram malted milk powder is stirred with 25 ml. of water till homogeneous, acidified with five ml. of one per cent acetic acid and then heated on steam bath until precipitation is complete. The precipitate is transferred to a Gooch crucible where it is extracted in a continuous extractor using petroleum ether as a solvent. The fat is determined by loss of

weight in the crucible. They describe a similar method developed by Trillat and Sauton wherein acetone is utilized instead of petroleum ether for extraction. Richmond and Musgrave (57) found their method to agree closely with Cochrane's (10) modified Babcock test, which they consider to be a valuable, accurate method.

The Werner-Schmid method is described by Richmond (59) and McLellan (44). The original method was applied to milk, and was adapted to cheese by Bondzynski, resulting in the Schmid-Bondzynski Method (52, 72). Werner-Schmid's procedure is as follows: ten ml. of milk are placed in a graduated tube of 50 ml. capacity. Ten ml. of hydrochloric acid are added and the mixture boiled until dark brown. After the sample is cooled, 30 ml. of ether are added and shaken. After standing, the volume of the ethereal solution is measured and ten ml. are removed with a pipette. The ether is evaporated and the fat dried and weighed. Woosnam (87) developed an apparatus for use with this method which consisted of a boiling flask equipped with a ground glass top and a stopcock. A graduated cylinder, also equipped with stopcocks for withdrawing the sample, was fitted into the flask after boiling, and the solvent added. When inverted, this apparatus could remove as much of the ethereal layer as desired.

Van Lennep and Ruys (79) modified the Werner-Schmid method by substituting trichlorethylene for the ether, and by filtering the aliquot before evaporation. They employed a correction factor as follows:

$$\frac{\text{Per cent Fat}}{500 \times .993 - \text{Per cent Fat}}.$$
 The authors found this to give perfect agreement

with the Rose-Gottlieb test.

Grossfeld (20) modified the Werner-Schmid method for the analysis of cheese by substituting chloroform for the ether as extraction agent and refluxing the mixture to attain better dissolving. He also used a correction

factor secured by the following equation: $\frac{92 \times \text{dry residue}}{32 - \text{dry residue}} = \text{Per cent fat.}$

Sutton (74) modified the Werner-Schmid method as applied to condensed milk to eliminate the charring of the sugar. His method requires the precipitation of proteins by copper sulphate and washing this precipitate to free it of sugars. The hydrochloric acid is then added and the procedure continued as in the Werner-Schmid method.

Weibull and Smetham, according to Scheringa (68) and Richmond (59), devised a special extractor for extracting the fat from the acid-treated milk. Scheringa (68) advised against dilution of the milk as it caused inconsistent results.

Several workers have studied the Werner-Schmid method from a critical point of view with the objections being centered on the action of the solvent on the non-fat constituents. (Dyer (13), Fleming (16), Hals and Klykksen (24), Richmond (58), McLellan (44), and Ardenghi (2)).

The Werner-Schmid method was criticized by Dyer (13) for not extracting the fat completely, but this error was balanced by the inclusion of non-fat substances in the residue. However, in the case of skim milk and condensed products the error was magnified, giving high results. Fleming (16) also criticized the Werner-Schmid method because of the impure fat residue.

Hals and Klykksen (24) in a series of extensive comparisons of methods of analysis found that the Werner-Schmid test gave higher results than the Adams or Rose-Gottlieb methods. Richmond (58) considers the Werner-Schmid test as an accurate, but not particularly rapid method.

McLellan (44) conducted a comparison of methods of analysis for fat in dried milks. He found the Werner-Schmid method gave results which were superior to other methods tried. His work includes a description of the Werner-Schmid method with data showing its superiority in this type of analysis.

Ardenghi (2) after a review of the current standard methods for fat determination decides that the Werner-Schmid is most suitable for laboratory control work. Richmond (59) discusses additional modifications of the Werner-Schmid method including weighing of the sample and withdrawal of the aliquot for determination.

The Schmid-Bondzynski method was adopted as the official method for analysis of cheese by the Association of Official Agricultural Chemists (31).

Another acid method making use of acetic acid for dissolving the protein was devised by Harding and Parkin (25) for the analysis of evaporated milk and milk powders. The method consists of treating a small sample with 25 per cent acetic acid to dissolve protein material, then shaking it with alcohol and carbon tetrachloride to suspend the fat and finally, extracting the fat with petroleum ether. Three extractions are used, and the fat is dried and weighed. A Nessler jar with a blow-off tube similar to Croll's (12) is employed in removing the ether.

Data presented by Harding and Parkin (25) average 0.43 per cent higher than those secured by the Rose-Gottlieb method and average 0.166 per cent higher than results secured by Richmond's modification of the Rose-Gottlieb (58). On trials using purified milk fat, their extractions were 99.9 per cent efficient. Harding and Parkin (26) further adapted the method to ice cream. Their data average 0.11 per cent higher than results by the Rose-Gottlieb method. Hortvett (32) recommended that the Association of Official Agricultural Chemists tentatively adopt the Harding Parkin method for analysis of ice cream.

Alkali Methods: An entirely different principle is employed in an alkali method proposed by Rose (65). In this method the sample is shaken with ammonia in contrast to the acids of previous techniques. His procedure is as

follows: About 20 g. of milk are mixed with two ml. of ammonia, 45 ml. of alcohol and 120 ml. of mixed ethyl and petroleum ethers are added, and the solutions mixed by shaking in a 230 ml. graduated burette. The ethereal volume is measured, 25 ml. taken, and the fat in this amount is determined. He found that it was necessary to add 0.015 per cent to account for the fat remaining in the aqueous layer.

Schreib (69) describes extensive comparisons which show the Rose method to be as accurate as any existing method.

The Rose method was studied and modified by several workers, with important modifications being proposed by Popp (53) and Gottlieb (18). Adaptations of the method were applied to all types of dairy products.

Popp (53) modified the Rose method by weighing the milk in accurately, and by changing the extraction tube. He found no saponification of the fat to be caused by the ammonia.

Gottlieb (18) further modified the Rose method by changing the quantities of reagents used. This modification became known as the Rose-Gottlieb method. The revised procedure was as follows: ten g. of milk are weighed into a cylinder graduated to 0.5 ml. The sample is then shaken, in turn, with one ml. of 10 per cent ammonia, 10 ml. of ethyl alcohol, 25 ml. of ethyl ether, and 25 ml. of petroleum ether. The mixture is allowed to stand for six hours to complete separation of the aqueous and ethereal fractions. The upper layer is then completely removed, and the fat obtained from it in the usual manner. Gottlieb states that making the volumes of milk and alcohol equal causes a more rapid separation of the ethereal and aqueous layers because of the increased density of the aqueous layer.

Gottlieb (18) later presented a more rapid adaptation which utilized pipettes for placing the sample in the tube, for withdrawing the ethereal

layer from the tube. He states that this increase in rapidity is gained without loss of accuracy, and his analyses show the fat to be pure, and not contaminated with other ether soluble substances.

In early work, McLellan (44) found the Rose-Gottlieb method to be inferior to the Werner-Schmid method in the analysis of dried milks. Fleming (16), on the other hand, criticized the Werner-Schmid method as giving an impure fat residue, whereas the Rose-Gottlieb method gave a pure fat, but was a more tedious procedure. Bigelow and Fitzgerald (6) found the Rose-Gottlieb technique to be the only method which gave good results on concentrated milk products, when compared to dry extraction methods and rapid volumetric methods.

Many early workers used the Rose-Gottlieb test as a control when conducting comparisons of other methods. (Hals and Klykksen (24), Notbohm and Angerhausen (49), Rammstedt (55), Windisch (85), and Olson (50)).

Weibull (83), Kuhn (40), Popp and Siegfeld (54), and Gottlieb (18) showed the Rose-Gottlieb test to be applicable to skimmed milk, buttermilk, cream, butter and cheese.

Eichloff (14) and Eichloff and Grimmer (15) devised adaptations of the Rose-Gottlieb test for use in the analyses of cream, butter, cheese, and dried milks. Hesse (28) developed an adaptation for use in butter analysis. Richmond (58) outlines an adaptation of the Rose-Gottlieb method for analysis of dried milk in which the amount of all reagents used were reduced 50 per cent. A micro-analytical method was developed by Kurz (41) which employed all the reagents used in the Rose-Gottlieb method and in similar proportions.

Improved extraction tubes were developed by Rohrig (63) and Rieter (61), and Adorjan (1) developed a burette for drawing off the ethereal layer. This burette, graduated in 0.1 ml., was equipped with a ground glass stopcock, and was attached to the extraction tube in such a way that the junction of the

ethereal layer and the aqueous layer was level with it.

Biesterfeld and Evanson (5) modified the Rose-Gottlieb method by making use of acid in conjunction with the ammonia. They found this liberated a slightly greater amount of fat in the case of malted milk. Roop (64) replaced all the ammonium hydroxide with one ml. of 1:1 sulphuric acid. He agreed with Biesterfeld and Evanson that this use of acid gave a true picture of the fat content of the milk.

The Rose-Gottlieb method and its adaptations for various dairy products received much attention from the referees of the Association of Official Agricultural Chemists. In 1906, after comparisons of all available methods for the analysis of cheese, Olson (50) states that the true picture is shown by the Gottlieb method. Also in 1906 Woll (86) reports that the Gottlieb results were higher, but believed more accurate than other methods, and recommends that the Gottlieb test be made provisional by the Association. In 1915, Hortvett (30) recommended that the Association adopt provisionally the continuous method of Paul. (The method of Paul is described by Bigelow and Fitzgerald (6) as being a dry extraction method very similar to the Adams method.) The procedure gave higher results than the Rose-Gottlieb test. However, in 1917, this investigator recommended the adoption of the Rose-Gottlieb method for use on milk, sweetened and unsweetened condensed milk, and later (32) recommended the adoption of the Rose-Gottlieb method for the analysis of ice cream and malted milk. Keister (36) reports that the acid medium modification of Roop (64) gave good results if the procedure was followed carefully.

Rupp and Müller (66) further modified the Rose-Gottlieb method by the addition of 0.4 g. of gum tragacanth after the ethereal additions, a treatment based on earlier findings of Rusting (8). This procedure gelatinized the aqueous layer, causing a more rapid and clearer separation of the two layers.

Oven (51) found that less tragacanth could be used with equal benefits, and studied the method in comparison to others. His claim is that the Rupp-Müller modification is just as accurate as the Gottlieb test, and is more rapid. Kropat (39) applied the Rupp-Müller modification to cheese, cream and butter analysis. Koenig (37) found that in butter analysis, more tragacanth was necessary, and he advised three washings with petroleum ether after decantation.

The Use of Solvents other than Ether

Various solvents have been suggested to replace ether in fat extraction methods. Certain workers (13, 16, 60) have shown that errors are encountered when petroleum ether or methyl ether were used as solvents. Broderick and Pittard (7) have also found that petroleum ether dissolves the fatty acids with greater difficulty than ethyl ether.

The use of carbon tetrachloride as a solvent in fat extraction has been the object of much study. Volrath (81) mentions that carbon tetrachloride is superior to ether, benzene, and carbon disulphide as a solvent, due to the fact that it carries no water on condensation. Workers for the Greishm Electrochemical Works (19) found that carbon tetrachloride was superior to gasoline as a solvent, and was less dangerous to use. Rammstedt (55) ran extensive comparisons of the existing ether extraction and carbon tetrachloride methods, and found the results obtained by using carbon tetrachloride were about four per cent higher than by the ether extraction method. For this reason he did not recommend the use of carbon tetrachloride.

Carbon tetrachloride was used as solvent in Leithe's pycnometer method for the determination of fat in dairy products (43), and in Hackman's method for determination of fat in cheese by measurement of the specific gravity of the solvent before and after treatment of the cheese (22).

The Consortium für Electrochemische Industrie (11) compared the chlorine derivatives of ethane and ethylene with benzene, carbon tetrachloride and carbon disulphide. A preference was indicated for the derivatives because of their freedom from toxic effects and explosion. The belief was expressed that dichlorethylene is an efficient solvent and can be used as a substitute for ether.

Taufel and Standigl (76) conducted a comparison of trichlorethylene, benzene, carbon disulphide, acetone, ethyl ether, and chloroform. They state that the ether and benzene gave clear, light-colored extracts whereas the other solvents yielded darker extracts. The trichlorethylene extracted the fat slowly, but gave the greatest yield in the end. The composition of the fatty residues varied only slightly, the acetone having extracted a greater amount of phosphatides. The authors believe this increase in phosphatide content is due to a higher percentage of lecithin in the acetone extract. Acetone was used by Beida (4) and obtained French patent rights on his use of this solvent.

Other fat solvents have been used to replace ether by still more workers. Harris (27) describes a method in which ortho-dichloro benzene is used as the solvent, and the fat percentage found by the change in specific gravity of the solvent.

DeWaal (82) reports that the petroleum ether in the Rose-Gottlieb test can be replaced by gasoline without any loss of accuracy in the method. Gasoline has also been used along with petroleum ether by Kohman for butter analysis (38) and Mandal substituted benzene for petroleum ether as a solvent without loss of efficiency. Carbon disulphide was preferred to ethyl and petroleum ethers by Visern and Guillot (80).

Jaffe (34) describes monoethyl ether of ethylene glycoll as being a very good solvent, but the work was not conducted on butterfat.

Various alcohols have also received attention in fat extraction studies. Gerber's (17) patented the process of extracting fat by use of ketones as methylethyl ketone alone or combined with alcohols of the fatty series - e.g. amyl, butyl, or methyl alcohol. Lampestrasse (42) received patent rights on the use of a butyl alcohol fat-dissolving agent in combination with a fat-soluble coloring agent. Wendler (84) used butyl alcohol with sodium salicylate, and Sichler (7) used isobutyl alcohol with a fat-soluble coloring agent in addition to alkali salts of pyrotartaric acid.

The Mojonnier Method

The Mojonnier method of fat extraction (48) has in recent years been accepted by the dairy industry as being the standard method for laboratory control work. The extraction principles are exactly the same as in the original Rose-Gottlieb method, the changes being made only in the apparatus used. Hortvett (33) states, "The Mojonnier method is nothing more or less than the Rose-Gottlieb method. The term relates specifically to an apparatus or machine designed especially for the purpose of shortening the time required for carrying out a determination." The Mojonnier apparatus consists of a compact piece of equipment containing an analytical balance, vacuum drying ovens, hot plates, dessicators, a centrifuge, reagent burettes, extraction flasks and tared evaporating pans. Mojonnier and Troy (48) and Supplee and Bellis (73) made exhaustive studies as to the accuracy of this method as compared with the official Rose-Gottlieb method. Their data show rather conclusively that there is no significant difference in the results obtained by using either method, but the Mojonnier method required only about one-sixth to one-seventh the time necessary to conduct the Rose-Gottlieb test.

Mojonnier and Troy (48) also present data showing the effect of varying the amounts of reagents. A portion of their results are shown in table I.

Table I. The Effect of Varying Amounts of Solvents on the
Efficiency of Fat Extraction

Product	Quantity of Solvent	%Fat	Remarks
Milk	Normal Amount	3.13	Normal
	50% Reduction	3.07	Gell formed on adding ether
	50% Increase	3.12	No advantage
Evaporated Milk	Normal Amount	8.09	Normal
	40% Reduction	8.07	Extraction incomplete
	20% Increase	8.09	No advantage
Ice Cream Mix	Normal Amount	13.51	Normal
	40% Reduction	13.50	Extraction incomplete
	20% Increase	13.53	No advantage

Table I shows that reducing the quantities of the solvents resulted in incomplete extraction of the fat, whereas increasing the amounts failed to improve the extraction. These investigators further show that in some cases large amounts of ethyl ether lowered the dividing line, whereas large amounts of petroleum ether and reduced quantities of ethyl ether had the opposite effect. In summarizing their work, Mojonnier and Troy (48) state that variations in the quantities of water and alcohol have a greater effect on the accuracy of the method than do variations in the other reagents.

Since the development of the Mojonnier tester only one modification has been proposed, - that being by Johnson (35). This modification replaces

both the ethyl and petroleum ethers with isopropyl ether. The method involves the dilution of the sample with water, the use of ammonia and alcohol much as in the original method, but replaces the 50 ml. of solvents by 25 ml. of isopropyl ether. The data average 0.02 per cent higher on all products than the Mojonnier test. In many products the tests were in perfect agreement, but in evaporated milk, the Mojonnier test is nearly 0.10 per cent lower than the modification. Johnson (35) states that isopropyl ether is advantageous over ethyl ether because of its higher boiling point which reduces evaporation losses. He states further that isopropyl ether is free of non-volatile residue, is an excellent solvent and does not absorb water, or carry over milk solids-not-fat. The approximate cost is stated to be about 60¢ per gallon, with no drum deposit required. It has the disadvantages of forming peroxides more readily than ethyl and petroleum ethers, and also tends to form an emulsion with water, necessitating the use of 50 per cent alcohol in raising the volume of the lower layer after the final extraction.

Johnson concludes that the replacement of ethyl ether and petroleum ether by isopropyl ether results in a method which is more rapid, more convenient and cheaper than the standard Mojonnier method.

SCOPE OF INVESTIGATION

This study was conducted with the following intentions:

1. To investigate the possibility of replacing the ethyl and petroleum ethers, either wholly or in part, by some less expensive and more easily obtainable solvent. The greater part of the investigation was devoted to this phase of the study.
2. To ascertain the efficiency of fat extraction as influenced by variations in the quantity of the reagents. The chief consideration in this connection pertains to the use of reduced amounts of solvents.
3. To gain further knowledge of the interactions of the several reagents used in the Mojonnier procedure.

EXPERIMENTAL PROCEDURE

General: Determinations in this study were made using whole milk, homogenized milk, ice cream mix, evaporated milk, skimmilk, and churned buttermilk. The milk, skimmilk, and buttermilk were obtained from the College creamery. The homogenized milk was processed at 2,500 pounds using a piston-type viscolizer. Ice cream samples were obtained partly from commercial dairies and partly from the College creamery. Commercial samples of evaporated milk were obtained for the trials devoted to their analyses.

All products were warmed to room temperature before the samples were weighed and each sample was well mixed by pouring from one container to another immediately prior to weighing. When partial churning was observed, or the sample was otherwise found to be non-homogeneous, it was discarded.

Control procedure: The Mojonnier method, conducted under carefully controlled conditions and in accordance with the directions of Mojonnier and Troy (48), served as the control method. In the case of milk, homogenized milk, skimmilk and butter milk, the general procedure is as follows: An accurately weighed ten gram sample is treated successively with 1.5 ml. ammonium hydroxide, 10 ml. ethyl alcohol, 25 ml. ethyl ether, and 25 ml. petroleum ether, being shaken for 30 seconds after each of the additions. The mixture is then centrifuged and the ether layer decanted into tared pans. A second extraction is made consisting of 5 ml. ethyl alcohol, 15 ml. ethyl ether and 15 ml. petroleum ether. After complete decantation of the second ethereal layer, the ether is volatilized, the fat dried at 135° C. under 20 inches of vacuum for 5 minutes, cooled in a dessicator to room temperature, and the fat determined by weighing.

For the analysis of evaporated milk and ice cream mix the procedure was varied slightly by using a 5 gram sample, and diluting this with 4 ml. of

water for evaporated milk and 5 ml. of water for ice cream mix. These two products also received 25 ml. of each ether on the second extraction instead of the 15 ml. used for the analysis of the other products.

Procedure for the replacement of solvents: For the purpose of substitution for the ethyl and petroleum ethers, two petroleum naphthas were obtained from the Skelly Oil Company, Lyman, Oklahoma. These naphthas were Skellysolve "F" and Skellysolve "A".

The first of these, Skellysolve "F", is described by the Skelly Oil Company as being essentially petroleum ether, having a boiling range of 30° - 60° C, a non-volatile residue of 0.0015 per cent, and A. P. I. gravity of 90⁰ at 15.7° C, and a Reid vapor pressure of 14.2 pounds at 37.9° C. The actual boiling point was determined at 39.0 - 48° C. This material was used experimentally to replace the petroleum ether which was found to have a boiling point of 48 - 57° C.

The second of these naphthas, Skellysolve "A", was used to replace the ethyl ether in proportions of 25 per cent, 40 per cent, 50 per cent and 100 per cent respectively. This reagent is described by the Skelly Oil Company as being essentially normal pentane with a boiling range of 28.5° - 38° C, an A. P. I. gravity of 91.7 at 15.7° C, a Reid vapor pressure of 14.8 pounds at 37.9° C, and a non-volatile residue of 0.0013 per cent. The actual boiling point of this material was found to be 33.0 - 35.5° C.

These solvents were used in the same amounts and manner as the materials they replaced in the standard Mojonnier procedure.

Procedure for reducing the quantity of ethyl ether: In order to find the efficiency of Skellysolve "A" when used in the ethyl ether mixtures, two experiments were conducted in which reduced amounts of ethyl ether alone were used as the first solvent. In the first of these trials, 50 per cent of the normal amount of ether was used, and in the second series 75 per cent of the

normal amount was used. Both of these series were conducted in two sets of experiments, one set utilizing petroleum ether as the second solvent, the other set using Skellysolve "A" as the second solvent. Except for necessary reductions in the quantity of alcohol used in the second extraction, no other changes were made in the original method.

Procedure for studying the interactions of the various reagents: Preliminary trials in which the ethyl ether was replaced, either partially or entirely, by Skellysolve showed a distinct difference in the volume of the lower layer of liquid in the extraction flask when compared with the control procedure. It was found that a smaller quantity of alcohol, or none at all, could be added in the second extraction because of the high dividing line which prevented efficient decantation of the ether layer. It was also observed that in all trials in which ethyl ether was replaced by Skellysolve, there appeared a flocculant yellowish layer of fat-like appearance at the junction of the two layers in the flask. Because of these observations, an experiment was conducted for the purpose of determining (a) the miscibilities of each of the reagents in various combinations and concentrations, and (b) the effect of each reagent on the volume and appearance of the lower layer.

Several 100 ml. cylinders, graduated to 1.0 ml. were equipped with tightly fitting stoppers, and into certain of these were placed the reagents used in the Skellysolve method. Control cylinders contained the Mojonnier reagents. Shaking treatment was administered as in other trials. Observations were made on the variation in the volume of the lower layer, as affected by changes in the concentrations of the various reagents. The color and appearance of the lower layer was observed, and changes in the flocculant, fat-like mass, occurring at the junction of the two layers, were noted.

Statistical treatment of the data: The data in these experiments were subjected to a statistical analysis wherever possible. The standard error, which measures the dispersion of the cases about the mean, was determined for each series of experiments.

As a note of explanation concerning the standard error, it should be pointed out that approximately 67 per cent of all cases will fall within the range of one standard error of the mean; therefore, a small standard error indicates a narrow range of dispersion. In data such as these, where an experimental method is compared to a standard control method, a small standard error indicates that the difference between the methods is constant, and a similar difference would be obtained in 67 per cent of the cases if the work were to be repeated. In contrast, a large standard error indicates a wider range of differences as obtained by comparison of the two methods.

The significance of the average difference between the two methods was found by use of the following formula:

$$\text{Significance} = \frac{\text{Actual difference between the methods} - \text{zero}}{\text{Standard error of the difference between the methods}^*}$$

The "significance" thus found is compared to a tabular value which corresponds to the number of trials on which the observation was based. This tabular value is called "t". If the "significance" is greater than the corresponding "t", the difference between the methods is significantly different from zero, and it can be said that the difference obtained is not due to error in obtaining samples, but due to the methods themselves. Conversely, a value smaller than the corresponding "t" means that the methods are not significantly different.

For the purpose of estimating the actual amount of fat which is pres-

*Baten, W. D. Mathematical Statistics, p. 220, John Wiley & Sons, New York.

ent when only the modified procedure is known, the regression curve was used. Through this procedure, the Mojonnier value may be calculated from the value obtained by the modified method by use of the equation $y = a + bx$ where y represents the Mojonnier control value, x represents the value as determined by the modified procedure and a and b are constant values which have been determined for each method by use of the regression curve.

The curve is constructed by plotting a graph of the results of a series of trials using both the Mojonnier and a modified procedure. The Mojonnier value is plotted along the ordinate and the values obtained by the modified method are plotted along the abscissa. A straight line may be drawn through the points thus determined which will intercept the abscissa near the origin. The values a and b are found from this line, a being the slope of the line and b being the point of intersection with the y axis.

RESULTS

Replacement of Solvents in the Mojonnier Method

Complete replacement by Skellysolve products: 1. Substitution of Skellysolve "F" for petroleum ether.

Results obtained by complete replacement of the petroleum ether of the Mojonnier procedure by the same quantity of Skellysolve "F" are presented in Table 2. These results were obtained on samples secured during January, February and July.

These data show excellent agreement between the two methods and between their respective duplicate determinations. In general, the Mojonnier control procedure extracted slightly more fat than the modified experimental method, although in the four trials conducted on evaporated milk the modified method gave somewhat higher values than the Mojonnier procedure. However, the differ-

Table 2. Total Replacement of Petroleum Ether by Skellysolve "F" Using Ethyl Ether as the First Solvent*

Product	No. of Trials	Fat Obtained		Differences			
		Mojonnier Modified		Between Methods		Between Duplicates	
		(%)	(%)	(%)	(%)	(%)	(%)
Milk	14	3.5434	3.5440	0.0044 ± 0.0020		0.0090	0.0109
Homo. Milk	15	3.9389	3.9338	0.0051 ± 0.0020		0.0105	0.0077
Evap. Milk	4	7.8927	7.9327	0.0300	-	0.0300	0.0088
Ice cream	9	9.5948	9.5907	0.0141 ± 0.0122		0.0191	0.0243

*Complete data found in Appendix Table II.

ence in extraction efficiency between these two methods is well within the range of experimental error and is statistically insignificant.

There is some indication that considerable more laxity in the shaking of the samples in the extraction is allowable in the case of petroleum ether than when Skellysolve is used with^{out} affecting the results. In several trials in which the shaking was limited to a slow mixing rather than the prescribed vigorous horizontal shaking, the Skellysolve gave results which were considerably lower than the Mojonnier control method, and triplicate samples showed poor agreement. The detailed presentation of these data may be found in Table I of the appendix.

2. Substitution of Skellysolve "A" for ethyl ether. Effect of reducing the quantity of alcohol.

In trials in which the ethyl ether was replaced by the same quantity of Skellysolve "A" it was found necessary to use reduced quantities of alcohol in the second extraction, since the addition of the usual five milliliters increased the volume of the lower layer to such an extent that efficient decantation of the ethereal layer was impossible. To find the effect of this reduction in the quantity of alcohol on the extraction efficiency, trials were conducted in which 2.5 ml. of alcohol were used in the second extraction of

the Mojonnier method, instead of the standard five milliliter quantity. No other change in the procedure was made. The results of these studies are shown in Table 3.

Table 3. The effect of Reducing the Quantity of Alcohol Used in the Second Extraction of the Mojonnier Method*

Amount of Alcohol ml.	No. of Trials	Fat Obtained (%)	Differences	
			Between Methods (%)	Between Duplicates (%)
5	5	4.0821	-	0.0136
2.5	5	4.0738	0.0083	0.0227

*Complete data found in Appendix Table IV.

These data indicate that no appreciable extraction efficiency is lost by reducing the quantity of alcohol used in the second extraction of the Mojonnier method.

Efficiency of Skellysolve "A" when used in place of ethyl ether.

Trials were conducted in which the ethyl ether was replaced by the same amount of Skellysolve "A". These trials were conducted on samples obtained during the months of February and March, and the data are presented in Table 4.

In the analysis of milk, the modified procedure extracted 0.2218 ± 0.0094 per cent less fat than the Mojonnier control method, a statistically significant difference. It was also found that wide variations occurred between triplicate determinations when this modified procedure was used. In three trials conducted on ice cream mix, the modified method was found to extract an average of only 0.0665 per cent fat as contrasted to the 12.4217 per cent average obtained by the Mojonnier method.

It may be concluded from these data that this modified procedure not only extracted much less fat than the control procedure, but resulted in poor agreement between triplicate determinations. For these reasons, such a modification is unsatisfactory for laboratory control work.

Table 4. Total Replacement of Ethyl Ether by Skellysolve "A" Using Petroleum Ether as the Second Solvent*

Product	No. of Trials	Fat Obtained		Differences			
		Mojonnier	Modified	Between Methods	Between Triplicates		
		(%)	(%)	(%)	Mojonnier Modified	(%)	(%)
Milk	31	4.4913	4.2695	0.2218 ± .0094	0.0125	0.0592	
Ice Cream	3	12.4212	0.0665	11.7576	0.0125	0.0911	

*Data found in Appendix Table III.

Miscibility of reagents:

As noted in the previous trials involving replacement of the ethyl ether with Skellysolve "A", only a small amount of alcohol could be added in the second extraction without resulting in a high dividing line. It was further noticed that in these trials a yellow, flocculent fat-like layer appeared at the junction of the two liquids in the extraction flask. The volume of this colored layer was reduced by the second extraction but did not entirely disappear. The variation in volume of the lower layer encountered when using Skellysolve was believed to be due to the immiscibility of alcohol and Skellysolve "A". Accordingly, trials were conducted to find the extent of this immiscibility, and the effect of other Mojonnier reagents upon it.

The results of preliminary determinations showed that: a) Ethyl alcohol and ethyl ether are perfectly miscible in Mojonnier proportions. b) Ethyl ether and Skellysolve "A" are perfectly miscible in Mojonnier proportions. c) Ethyl alcohol and Skellysolve "A" are not miscible in Mojonnier proportions. d) The addition of ethyl ether to a Skellysolve-ethyl alcohol mixture will increase the miscibility of the system.

Trials were conducted for the purpose of determining the amounts of ethyl ether required to bring about miscibility in a three-component system of alcohol, Skellysolve "A" and ethyl ether, under conditions of varying

quantities of ethyl alcohol and Skellysolve "A". The results of these trials are shown in Table 5.

Table 5. Effect of Ethyl Ether on the Miscibility of an Ethyl Alcohol-Skellysolve "A" Mixture when the Quantities of Alcohol and Skellysolve Are Varied

Series No.	Ethyl Alcohol (ml.)	Skellysolve "A" (ml.)	Ethyl Ether Required to Bring about Miscibility	
			(ml.)	(%)
I.	10	20	4	13.3
	15	20	4	11.4
	15	35	6	12.0
	15	40	10	18.1
	15	55	16	22.8
II.	10	20	4	13.3
	15	20	4	11.4
	15	35	8	16.0
	15	50	13	20.0
III.	1	10	2.0	18.1
	2	10	1.0	8.3
	3	10	.5	3.8
	4	10	.0	0.0
	5	10	.0	0.0

These trials show that increasing the amount of Skellysolve in the system necessitates an increased amount of ethyl ether to bring about miscibility. In contrast, increases in the amount of ethyl alcohol do not require increased amounts of ethyl ether to bring about miscibility. These data also indicate that when 20 per cent of the system is ethyl ether, the system is entirely miscible despite variations in the amounts of ethyl alcohol.

The addition of water to the above mixture resulted in a four-component system which responded to the addition of ethyl ether in a manner somewhat similar to the three-component system. Additions of ethyl ether lowered the demarcation line until it was at the level of the amount of water present. It was found that this immiscible layer persisted at the same vol-

ume as that of the water added, despite large additions of ethyl ether. The quantity of ethyl ether necessary to bring the line down to the level of the volume of water in the system was increased in this four-component system to 60 per cent when reagents are used in Mojonnier proportions, compared to the 20 per cent required when water is absent. It was likewise noticed that when larger amounts of water are present, more ethyl ether is required to bring about miscibility. When larger amounts of Skellysolve are present, less ethyl ether is required.

As noted previously, a considerable difference exists between the volumes of the lower layers of the Mojonnier extracts and the extracts in the modified method in which Skellysolve "A" is used in place of ethyl ether. This may not be entirely accounted for by the difference in miscibility of the two materials. In order to study other effects on the volume of the lower layer, several trials were conducted in which the volumes obtained when using ether were compared with volumes obtained using Skellysolve "A".

Mixtures of Skellysolve and ethyl ether were prepared which contained 0, 20, 40, 60, 80, and 100 per cent ethyl ether respectively. Forty milliliters of the desired mixture were combined with all other Mojonnier reagents except petroleum ether, with the milk being replaced by water in the first trial and skim milk in the second trial. These results are shown in Table 6.

Table 6. Variation in Volume of the Lower Layer as Affected by Varying Composition of Ether-Skellysolve Mixture when 1.5 ml. Ammonia, 15 ml. Alcohol, and 40 ml. of the Ether-Skellysolve Mixture Are Present*

			Volume of Lower Layer when the Following Percentage of Ethyl Ether Is Used					
Water	Skimmilk	Milk	0	20	40	60	80	100
(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
10	0	-	26	26	25	23	19.5	10.5
-	10	-	26	26	25	22.5	17.0	10.0
-	-	10	26	26	26	22	16.5	9.0

*Average of four trials.

These data show that in the presence of all Mojonnier reagents except petroleum ether, the volume of the lower layer is roughly proportional to the amount of Skellysolve in the system.

These trials were repeated, with the exception that petroleum ether was included in its regular amount. The same mixtures of ethyl ether and Skellysolve "A" were used, and no other changes were made. The results of these trials are shown in Table 7.

Table 7. Variation in Volume of the Lower Layer as Affected by Varying Composition of Ether-Skellysolve Mixture when 1.5 ml. Ammonia, 15 ml. Alcohol, 40 ml. of the Ether-Skellysolve Mixture, and 40 ml. of Petroleum Ether are Present*

Water (ml.)	Skimmilk (ml.)	Milk (ml.)	Volume of Lower Layer when the Following Percentage of Ethyl Ether Is Used					
			0 (ml.)	20 (ml.)	40 (ml.)	60 (ml.)	80 (ml.)	100 (ml.)
10	-	-	25.0	25.0	23.5	23.5	22.0	21.0
-	10	-	25.0	25.0	25.0	23.0	22.0	20.5
-	-	10	26.0	25.0	23.5	23.0	21.0	19.5

*Average of three trials.

These data indicate that the volume of the lower layer is increased with increased amounts of Skellysolve in the system. However, the difference in volume between the extracts of 0 per cent ethyl ether and the 100 per cent ethyl ether is decreased in these trials due to the presence of petroleum ether. The volume change in the lower layer resulting from the use of petroleum ether amounts to approximately 11 ml. (66 per cent), and is caused by the removal of alcohol or water from the ethyl ether.

Table 8 shows the results obtained when the petroleum ether was replaced by the same quantity of Skellysolve "F". All other reagents were left unchanged.

Table 8. Variation in Volume of the Lower Layer as Affected by Varying Composition of Ether-Skellysolve Mixture when 1.5 ml. Ammonia, 15 ml. Alcohol, 40 ml. of the Ether-Skellysolve Mixture, and 40 ml. of Skellysolve "F" are Present*

Water (ml.)	Skimmilk (ml.)	Milk (ml.)	Volume of the Lower Layer when the Following Percentage of Ethyl Ether Is Used					
			0 (ml.)	20 (ml.)	40 (ml.)	60 (ml.)	80 (ml.)	100 (ml.)
10	-	-	24.0	24.0	23.5	23.0	22.0	22.0
-	10	-	24.2	24.4	24.0	23.0	21.5	20.0
-	-	10	24.8	26.0	24.0	23.7	22.0	20.0

*Average of three trials.

These results indicate that there is little difference between the effects of petroleum ether and Skellysolve "F" in the respect of regulating the volume of the lower layer. In these trials also, the sample with the greater amount of Skellysolve "A" and with less ethyl ether showed a greater volume in the lower layer than when ethyl ether was used alone. These data show that in following the Mojonnier procedure, 3 to 5 ml. of alcohol are carried into the ether layer, while little or none remains in the ether layer when the modified procedure is used.

Along with changes in the volume of the lower layer, noticeable changes also occurred in the color and opacity of the lower layers as the ethyl ether content was increased from 0 per cent to 100 per cent. In the trials in which no second solvent was used (Table 6) trials conducted on whole milk showed the yellowish (fat-like) layer between the ether layer and lower layer in all mixtures, but was not observed when only ethyl ether was used. The lower layers of the 0, 20, 40, and 60 per cent mixtures showed a white opaque color, which was not present to such an extent in the 80 and 100 per cent mixtures. The volume of the fat-like layer was the greatest (1.5 ml.) when the 20 per cent mixture was used.

The fat-like layer was present only in the 0 per cent, 20 per cent and 40 per cent mixtures in the trials where petroleum ether was used (Table 8).

The colors of the lower layers in these three mixtures were less clear than in the 60 per cent, 80 per cent, and 100 per cent mixtures which showed no yellow, flocculent layers between the ether layer and lower layer. The volume and appearance of this yellowish layer and the lower layer did not change on standing 24 hours. Similar results were obtained when comparing the mixtures in which the petroleum ether was replaced by Skellysolve "F".

Partial Replacement by Skellysolve Products:

Results of these previous investigations indicate that when the 60 per cent mixture of ethyl ether and Skellysolve "A" was used, the alcohol and Skellysolve were miscible, the volume of the lower layer was not too great to permit a second extraction, the color of the lower layer was satisfactory, and there was no evidence of the flocculent, fat-like layer. A 50 per cent mixture was also prepared and this was found to exhibit the same physical characteristics as the 60 per cent mixture.

On the basis of these preliminary observations, fat determinations were made using mixtures of 50 per cent, 60 per cent and 75 per cent ethyl ether in Skellysolve "A". These mixtures were used as substitutes for ethyl ether in the modified experimental procedures.

1. Use of a 1:1 mixture of Skellysolve "A" and ethyl ether:

The original trials conducted during July using this 1:1 mixture of ethyl ether and Skellysolve "A" were conducted using Skellysolve "F" as the second solvent. Later work was done in August with the same mixture, using Skellysolve "A" as the second solvent. The results of these trials are presented in Table 9.

These data show that the use of the ethyl ether Skellysolve "A" mixture results in lower values than were obtained by the Mojonnier control method. When Skellysolve "F" was used as second solvent the differences were as follows: milk, 0.0495 ± 0.0030 per cent; homogenized milk, 0.1032 ± 0.0260

Table 9. Comparison of the Mojonnier Method with a Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A"*

Product	No. of Trials	Fat Obtained		Differences		
		Mojonnier Modified		Between Methods	Between Duplicates	
		(%)	(%)	(%)	Mojonnier	Modified
					(%)	(%)
Skellysolve "F" as Second Solvent						
Milk	38	3.8604	3.8109	0.0495 ± 0.0030	0.0089	0.0244
Homo. Milk	8	4.1162	4.0130	0.1032 ± 0.0260	0.0077	0.0186
Evap. Milk	6	8.1420	8.0475	0.0945 -	0.0228	0.0180
Ice Cream	5	11.0627	10.8080	0.2547 -	0.0263	0.0343
Skellysolve "A" as the Second Solvent						
Milk	9	3.9408	3.8960	0.0448 ± 0.0060	0.0091	0.0179
Homo. Milk	10	3.8454	3.7741	0.0713 ± 0.0036	0.0084	0.0278
Evap. Milk	5	7.9775	7.6992	0.2783 -	0.0196	0.1312
Ice Cream	9	11.1424	10.9567	0.1857 ± 0.0307	0.0235	0.0447

*Complete data appears in Appendix Tables V and IX.

per cent; evaporated milk, 0.0945 per cent; ice cream, 0.2547 per cent. When Skellysolve "A" was used as the second solvent the differences were: milk, 0.0448 ± 0.0060 per cent; homogenized milk, 0.0713 ± 0.0036 per cent; evaporated milk, 0.2783 per cent; ice cream, 0.1857 ± 0.0307 per cent. These trials indicate that except in the case of evaporated milk, the Skellysolve "A" results in values which are closer to the Mojonnier control values.

Since this method involving a 1:1 mixture of Skellysolve "A" and ethyl ether results in incomplete extraction, it was considered advisable to study the effect of a third extraction. Consequently a third extraction was utilized which consisted of mixing the residue of the second extraction with a 15 ml. portion of the 1:1 mixture of ethyl ether and Skellysolve "A" and a 15 ml. portion of Skellysolve "F". The results of using this third extraction are shown in Table 10. These trials were conducted on samples obtained during July.

Table 10. Comparison of the Mojonnier Method with a Modified Method in which three Extractions are Made with a 1:1 Mixture of Ethyl Ether and Skellysolve "A"*

Method	No. of Trials	Fat Obtained (%)	Differences	
			Between Methods (%)	Between Duplicates (%)
Mojonnier	9	3.9037	-	0.0108
Modified (2 extractions)	9	3.8458	0.0579	0.0343
Modified (3 extractions)	9	3.8777	0.0326	0.0374

*Complete data found in Appendix Table VI.

The third extraction reduced the difference between the modified method and the control method from 0.0579 per cent to 0.0326 per cent, a reduction of 44 per cent. The data in Tables 9 and 10 show the agreement between duplicate determinations is more satisfactory when the Mojonnier procedure is used. However, the agreement between duplicates is satisfactory, and within range of normal experimental error in all cases with the exception of the 5 samples conducted on evaporated milk in which Skellysolve "A" is used as the second solvent. (See Table 9)

2. Using a 3:2 mixture of Skellysolve "A" and ethyl ether with Skellysolve "F" as the second solvent. Table 11 shows the results of trials conducted using a mixture of ethyl ether and Skellysolve "A" in which the ether makes up 60 per cent of the solution, or a 3:2 proportion. Skellysolve "F" was again used as the second solvent, and no other changes were made in the procedure. The determinations were conducted on samples obtained during July.

These trials indicate that the use of this method also results in values lower than those obtained by using the Mojonnier method. The differences between the two methods are: milk, 0.0298 ± 0.0025 per cent; homogenized milk, 0.0452 ± 0.0141 per cent; evaporated milk, 0.0615 ± 0.0375 per cent; ice cream, 0.1289 ± 0.0496 per cent.

Table 11. Comparison of the Mojonnier Method and a Modified Method which Utilizes a 3:2 Proportion of Ethyl Ether and Skellysolve "A"

Product	No. of Trials	Fat Obtained		Differences		
		Mojonnier	Modified	Between Methods	Between Duplicates	
		(%)	(%)	(%)	Mojonnier	Modified
					(%)	(%)
Milk	15	3.6786	3.6488	0.0298 ± 0.0025	0.0121	0.0160
Homo. Milk	6	3.9378	3.7926	0.0452 ± 0.0141	0.0082	0.0235
Evap. Milk	6	7.9684	7.9069	0.0615 ± 0.0375	0.0211	0.0317
Ice cream	6	11.2093	11.0804	0.1289 ± 0.0496	0.0267	0.0451

*Complete data shown in Appendix Table VII.

These data also show that the use of a 3:2 mixture of ethyl ether and Skellysolve "A" results in an improvement in extraction efficiency over the 1:1 mixture. The agreement between duplicate determinations is also greater when this 3:2 mixture is used. In this procedure, as in the preceding ones, it is interesting to note that in nearly all cases the extraction efficiency of the modified procedure is poorest in the trials conducted on high fat samples such as ice cream mix and evaporated milk.

3. The use of a 3:1 mixture of ethyl ether and Skellysolve "A", using Skellysolve "F" as the second solvent. Trials were conducted in which the per cent ethyl ether in the mixture was 75 per cent, resulting in a 3:1 mixture of ethyl ether and Skellysolve "A". This mixture was used to replace the ethyl ether as in previous trials. Skellysolve "F" was again used as the second solvent. The samples were obtained during August. Table 12 shows the results of these trials.

These data show that in the analysis of milk the modified procedure extracts 0.0245 ± 0.0038 per cent less fat than the Mojonnier control method. In homogenized milk this difference amounts to 0.0165 ± 0.0037 per cent and in evaporated milk and ice cream the differences are 0.0461 per cent and 0.0641 per cent respectively.

Table 12. Comparison of the Mojonnier Method with a Modified Method which Utilizes a 1:3 Proportion of Ethyl Ether and Skellysolve "A"

Product	No. of Trials	Fat Obtained		Differences			
		Mojonnier	Modified	Between Methods	Between Duplicates		
		(%)	(%)	(%)	Mojonnier	Modified	
					(%)	(%)	
Milk	10	4.1066	4.0821	0.0245 ± 0.0038	0.0087	0.0108	
Homo. Milk	8	4.6092	4.5927	0.0165 ± 0.0037	0.0087	0.0183	
Evap. Milk	3	7.9316	7.8855	0.0461 -	0.0227	0.0251	
Ice Cream	3	10.4008	10.3367	0.0641 -	0.0162	0.0428	

*Complete data found in Table VIII of the Appendix.

These results also indicate that the 3:1 proportion of ethyl ether and Skellysolve "A" is the most efficient extractor of all the mixtures tried, but does not equal the extraction ability of ethyl ether alone.

Reduction in the Amount of Ethyl Ether

1. The use of 50 per cent of the normal amount of ethyl ether. In order to determine the amount of extraction accomplished by the Skellysolve "A" in the 1:1 mixture with ethyl ether, determinations were conducted in which the Skellysolve "A" was omitted, and the ethyl ether was used to the extent of 50 per cent of the normal amount. For the purpose of comparison, trials were conducted using both petroleum ether and Skellysolve "A" as the second solvent. The amounts of all other reagents were unchanged and these studies were conducted during August. Table 13 shows the results of these trials.

These data show that a 50 per cent reduction in the amount of ethyl ether used in the extraction results in incomplete extraction of the fat when compared to the Mojonnier control method. When petroleum ether is used as the second solvent, the differences between the control and modified methods are: milk, 0.0335 ± 0.0034 per cent; homogenized milk, 0.0365 ± 0.0072 per cent; evaporated milk, 0.0549 per cent; ice cream, 0.0354 per cent. When Skellysolve "A" is used as the second solvent, the differences between the two methods are:

Table 13. Comparison of the Mojonnier Method with a Modified Method which Utilizes 50 per cent of the Normal Amount of Ethyl Ether*

Product	No. of Trials	Fat Obtained		Differences			
		Mojonnier	Modified	Between Methods	Between Duplicates	Mojonnier	Modified
		(%)	(%)	(%)	(%)	(%)	(%)
Petroleum Ether as Second Solvent							
Milk	9	3.5351	3.5016	0.0335 ± 0.0034	0.0068	0.0263	
Homo. Milk	9	3.9354	3.8989	0.0365 ± 0.0072	0.0086	0.0260	
Evap. Milk	3	7.8909	7.8360	0.0549 -	0.0219	0.0183	
Ice Cream	3	10.7204	10.6850	0.0354 -	0.0336	0.0365	
Skellysolve "A" as Second Solvent							
Milk	8	4.2154	4.1960	0.0194 ± 0.0040	0.0124	0.0078	
Homo. Milk	8	4.3186	4.2927	0.0259 ± 0.0019	0.0145	0.0227	
Evap. Milk	3	7.9922	7.8913	0.1009 -	0.0217	0.0332	
Ice Cream	3	10.5433	10.3771	0.1662 -	0.0218	0.0365	

*Complete data found in Tables XI and XIII of the Appendix.

milk, 0.0194 ± 0.0040 per cent; homogenized milk, 0.0259 ± 0.0018 per cent; evaporated milk, 0.1009 and ice cream, 0.1662 per cent.

These data show that in the analysis of milk and homogenized milk, the use of Skellysolve "A" results in more efficient extraction, while the limited data for evaporated milk and ice cream indicate petroleum ether is somewhat superior.

A comparison of Table 13 with Table 9 will show that the modified procedure which used 50 per cent of the normal amount of ethyl ether is more effective in fat extraction than is the 1:1 mixture of ethyl ether and Skellysolve "A". For example, in the case of milk, the use of a 1:1 mixture resulted in a difference of 0.0495 per cent, while this modification which uses reduced quantities resulted in an average difference of 0.0264 per cent. This would indicate that the Skellysolve "A" itself is not efficient in extracting fat when it is used in mixture with ethyl ether. In fact, these data show that the Skellysolve "A" in mixture with ethyl ether has a detrimental effect on the extraction efficiency of the ethyl ether.

2. The use of 75 per cent of the normal amount of ethyl ether: In another series of trials, 75 per cent of the normal amount of ethyl ether was used as the first solvent with normal amounts of either petroleum ether or Skellysolve "A" being used as the second solvent. Table 14 shows the results of these trials which were conducted during August.

Table 14. Comparison of the Mojonnier Method with a Modified Method which Utilizes 75 per cent of the Normal Amount of Ethyl Ether*

Product	No. of Trials	Fat Obtained		Differences		
		Mojonnier	Modified	Between Methods	Between Duplicates	
		(%)	(%)	(%)	Mojonnier Modified	(%)
Petroleum Ether as Second Solvent						
Milk	4	3.7821	3.7715	0.0106	0.0059	0.0188
Ice Cream	3	10.2645	10.2409	0.0236	0.0187	0.0210
Skellysolve "A" as Second Solvent						
Milk	4	4.3435	4.3333	0.0102	0.0074	0.0071
Homo. Milk	10	3.8529	3.8365	0.0164 ± 0.0026	0.0137	0.0098
Evap. Milk	3	7.9464	7.9111	0.0353	0.0067	0.0102
Ice Cream	3	10.3947	10.3249	0.0698	0.0303	0.0488

*Complete data found in Appendix Tables XII and XIV.

The use of 75 per cent of the normal amount of ethyl ether gives results which are close to, but significantly lower than the results obtained by the Mojonnier method. When petroleum ether is used as the second solvent the difference between the two methods in the analysis of milk is 0.0106 per cent, and in the analysis of ice cream 0.0236 per cent. The use of Skellysolve "A" as the second solvent results in the following differences: milk, 0.0102 per cent; homogenized milk, 0.0164 ± 0.0036 per cent; evaporated milk, 0.0353 per cent; ice cream, 0.0698 per cent. These data substantiate the findings in Table 13 that the petroleum ether is more effective as a second solvent than is the Skellysolve "A" in the analysis of evaporated milk and ice cream.

A comparison of these results with the data presented in Table 12 again reveals that the Skellysolve "A" itself does not contribute towards increased efficiency in fat extraction when mixed with ethyl ether.

Possible Influence of Seasons

Previous studies involving the replacement of ethyl ether were conducted during the months of June to September with no attention being given to possible seasonal influences. However, in later experiments dealing with the influence of certain factors on the efficiency of extraction by the various modified procedures, it was observed that the differences between the results obtained by the modified and Mojonnier methods were appreciably greater than had been found in the earlier work. This change was noted in both the mixture and reduction modification. These later experiments were conducted during November and December and general observations were made as follows:

(a) The average discrepancies between results obtained by use of the Mojonnier and the modified methods is significantly higher than differences obtained in the summer months. (b) The differences between the methods covered a wider range than noted previously, certain samples resulting in smaller differences and other samples resulting in greater differences. (c) The color of the lower layer of liquid in the extraction flasks was noticeably more opaque and gelatinous when the modified procedures were used on samples of milk obtained during the winter. This characteristic was especially obvious after the second centrifuging. (d) These larger discrepancies are apparently not caused by minor changes in technique or reagents, since changing and purification of the reagents and careful scrutinization of technique resulted in no appreciable change.

Table 15 presents results obtained by comparing the Mojonnier method with the modified method which employs a 1:1 mixture of ethyl ether and Skelly-

solve "A" and that which utilizes reduced quantities of ethyl ether in the analysis of milk produced under winter conditions. For the purpose of comparison, the data from tables 9 and 13 on the analysis of milk under summer conditions are also shown.

Table 15. Comparison of the Mojonnier Method with a Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A" and a Method which Employs 50 per cent of the Normal Quantity of Ethyl Ether in the Analysis of Milk Produced under Varying Seasonal Conditions*

Modification	No. of Trials	Fat Obtained		Differences	
		Mojonnier (%)	Modified (%)	Between Methods (%)	Range (%)
1:1 Mixture					
Winter	33	3.7867	3.6971	0.0896 ± 0.0049	0.0491 - 0.1522
Summer	47	3.9446	3.8960	0.0486 ± 0.0040	0.0133- 0.0960
50% Reduction					
Winter	25	3.9531	3.8870	0.0661 ± 0.0073	0.0078 - 0.1207
Summer	17	4.2209	4.1960	0.0249 ± 0.0037	-0.0060 - 0.0492

*Complete data found in Appendix Table XV.

These comparisons indicate that a definite change has occurred in the relationship of these modified procedures to the Mojonnier procedure over a period of a few months. The difference between the Mojonnier results and the results obtained using the 1:1 mixture has increased from 0.0496 per cent to 0.0896 per cent, an increase of 0.0400 per cent. A corresponding increase of 0.0412 per cent is noted in the relationship between the Mojonnier method and the modification which makes use of reduced quantities of ether. The range of differences obtained by using the 1:1 mixture is increased from 0.0827 per cent to 0.1031 per cent, with minimum and maximum values both being higher for the winter analyses than for the corresponding summer values. In the results obtained by use of the 50 per cent reduced quantity method, the range of differences is increased from 0.0552 per cent to 0.1129 per cent. This change, itself, is minor, but it is in line with other findings.

The lower layer of liquid in the extraction flasks containing the winter milk showed an opaque color and a gelatinous nature in all trials conducted using the modified procedures. These characteristics were not present during the summer. The appearance of these lower layers suggested incomplete dissolving of the protein material.

Effect of Various Factors on the Efficiency of the Modified Methods

1. Effect of increased shaking on the efficiency of fat extraction:

In an attempt to bring about closer agreement between the Mojonnier method and the modified methods, the milk being analyzed by use of the modified procedures was subjected to a 60 second shaking interval after the addition of each solvent, instead of the regular 30 second shaking time. This variation was administered to both the modification which uses the 1:1 mixture of ethyl ether and Skellysolve "A" and the modification which utilizes 50 per cent of the standard quantity of ethyl ether. The amounts of reagents used, and other techniques remained the same, and these determinations were conducted during January Table 16 shows the results of these trials.

Table 16. Effect of Lengthening the Shaking Period on the Efficiency of the Modification Using the 1:1 Mixture and Reduced Quantities of Ethyl Ether as Compared to the Mojonnier Method in the Analysis of Winter Milk*

Method	No. of Trials	Fat Obtained			:Deviation from Mojonnier	
		Mojonnier (%)	Modified Regular (%)	Modified 60 sec. shaking (%)	Modified Regular (%)	Modified 60 sec. shaking (%)
1:1 Mixture	11	3.5446	3.4594	3.5037	0.0852±0.0091	0.0436±0.0060
50% Reduced	6	3.7643	3.7262	3.7368	0.0380	0.0275

*Complete data found in Appendix Tables XVI and XVII.

These data show that increased shaking causes the modified methods to extract more fat than is accomplished by normal shaking. The difference between the 1:1 mixture procedure and the Mojonnier control is reduced from

0.0352 ± 0.0091 per cent to 0.0436 ± 0.0060 per cent due to the effect of additional shaking with the solvents. This difference of 0.0436 ± 0.0060 is well within the established range of differences which were obtained by use of this modified method during the summer months. This technique of increasing the shaking period from 30 seconds to 60 seconds when applied to the method which uses 50 per cent of the normal amount ethyl ether reduces the difference between this modification due the Mojonnier from 0.0380 per cent to 0.0275 per cent.

This limited number of trials indicates that the extra shaking period affects this modification much as it does the modification utilizing the mixture of ethyl and Skellysolve "A", although the increase in efficiency is less pronounced.

2. Extraction efficiency of the method which uses a 1:1 mixture when used for the analysis of milk obtained from animals on fat feeding trials.

To ascertain if the change in character of the fat as caused by seasonal variation may be a factor in the change in extraction efficiency observed during the seasons, feeding trials utilizing linseed oil were conducted during November and the milk then analyzed for fat. In this feeding trial, conducted with the view of obtaining a softer, lower melting-point fat, cow No. A 24 from the College Experimental herd was selected and fed linseed oil at the rate of 0.7 lbs. per day, the oil being blended with silage to insure complete assimilation. Cow No. 77 was used as a control, and received a normal winter ration supplemented with corn. Samples for analysis were obtained before feeding the oil and after 4 and 7 days respectively. These samples were analyzed by the modified method which utilizes a 1:1 mixture of ethyl ether and Skellysolve "A". This work was conducted during November and the results are shown in Table 17.

Table 17. Comparison of the Mojonier Method with the Modified Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A" in the Analysis of Milk Obtained when Feeding Linseed Oil to the Cow

Days of oil feeding	Fat Obtained				Difference between Methods	
	Mojonnier		Modified			
	Cow A24 (%)	Cow #77 (%)	Cow A24 (%)	Cow #77 (%)	Cow A24 (%)	Cow #77 (%)
0 days	3.3009	5.1156	3.6753	4.9917	0.1256	0.1239
After 4 days	4.3559	-	4.2233	-	0.1326	-
After 7 days	4.7186	7.5092	3.7522	7.2665	0.9664	0.2427

These trials show that softening the fat by means of feeding linseed oil does not result in improved efficiency in the modified procedure. In fact the reverse situation prevailed. The large increase in difference between the control and modified methods from 0.1256 to 0.9664 is due to the change in ration, and shows that significant increases in the difference between the methods can be caused by changes in feeding. The large increase in fat content exhibited by control cow No. 77 is an occurrence which is not ascribed to its ration.

Another observation was that in the flasks containing the modified reagents, a white gelatinous emulsion was present particularly after the second extraction, although all reagents were used properly. This characteristic was noted in the milk from both cows.

3. Effect of low temperature storage on efficiency of the modified procedure which employs the 1:1 mixture of ethyl ether and Skellysolve "A".

In other trials, the fat hardening as affected by holding for long periods at a low temperature was studied to find its effect on the extraction efficiency of this modified procedure. Pasteurized samples were obtained from the creamery immediately after cooling, and raw samples obtained directly from the pail at the time of milking for the 0 hour samples. The samples were examined immediately, the raw samples were still warm, and were then aged at

40° C for varying periods up to 72 hours, when each sample was again subjected to analysis. The results of these determinations which were obtained during November and January are shown in Table 18.

Table 18. Effect of Storing Milk at Low Temperature on the Comparison between the Mojonnier Method and the Modified Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A"

Sample No.	Fat Obtained		Difference (%)	History of Sample
	Mojonnier (%)	Modified (%)		
1	3.8861	3.9184	0.0677	0 hours from bottler
	3.8604	3.7922	0.0682	24 hours from bottler
	3.8640	3.7698	0.0942	48 hours from bottler
2	3.7385	3.6633	0.0752	0 hours from bottler
	3.7556	3.6741	0.0815	24 hours from bottler
	3.7541	3.6889	0.0652	72 hours from bottler
3	3.4030	3.3523	0.0507	0 hours from cow
	3.3906	3.3315	0.0491	48 hours from cow
4	3.9283	3.8634	0.0649	0 hours from cow
	3.8932	3.7888	0.1044	24 hours from cow
5	5.6465	5.4819	0.1646	0 hours from cow
	5.6774	5.5336	0.1438	24 hours from cow
6	5.0725	4.9206	0.1519	0 hours from cow
	5.0502	5.9149	0.1353	24 hours from cow
7	3.4995	3.3703	0.1292	0 hours from cow
	3.4590	3.3334	0.1256	72 hours from cow

These data indicate that the difference between the Mojonnier method and the modified mixture procedure is not significantly affected by low temperature storage. Sample No. 4 is the only sample showing a significant increase in the difference; this increase amounting to 0.0395 per cent. Other samples show slight decreases in the difference between the two methods after storage.

Low temperature storage slightly decreased the results secured by the Mojonnier method in the majority of the trials. Each sample in this series

shows a decrease of 0.025 per cent to 0.040 per cent except sample No. 2 which shows an increase of 0.017 per cent after storage.

4. Effect of heating the sample to 100° F immediately before analysis

In another experiment, the fat was softened by heating the milk to 100° F for a few minutes just prior to weighing the sample into the extraction flask. As a control, analyses by the Mojonnier procedure were made on samples which did not receive the heat treatment. The modified procedure used here employed a 1:1 mixture of ethyl ether and Skellysolve "A". These trials were conducted during December, and the results are presented in Table 19.

Table 19. Effect of Heating Milk to 100° F Immediately before Testing on the Efficiency of the Method Utilizing the 1:1 Mixture of Ethyl Ether and Skellysolve "A"*

No. of Trials	Fat Obtained			Deviation from Mojonnier	
	Mojonnier	Regular Modified	Modified plus Heat	Regular Modified	Modified plus Heat
	(%)	(%)	(%)	(%)	(%)
8	3.7365	3.6583	3.6778	0.0782 ± 0.0088	0.0587 0.0098

*Complete data found in Table XVIII of the Appendix.

These data show that warming the milk to 100° F just before the analysis is made, reduces the difference between the modified and control procedures by 0.0195 per cent. This slight increase in efficiency did not occur in each case as demonstrated by sample No. 4 (Appendix Table 18). In this case this technique resulted in an increase of 0.0511 per cent in the difference between the Mojonnier method and the modified procedure - an unexplainable abnormal variation.

The average increase in efficiency found here, 0.0195 per cent, is of doubtful significance and does not account for the seasonal difference which has been noted.

5. Effect of variation in the amounts of ammonia and alcohol on the efficiency of the modified procedures. In order to decrease the difference between the results obtained by the Mojonnier control method and the reduced modification and mixture modification procedures, and thereby obtain a method which resembles the Mojonnier more closely, certain variations in alcohol and ammonia were conducted.

As stated previously, a gelatinous condition exists in the flasks containing the modified reagents in the trials conducted during the winter. This white, gelatinous texture in the lower layer is believed to be due to undissolved protein, and inferior extraction efficiency is caused by the trapping of fat in this gelatinous layer.

Results obtained by use of these variations in the quantities of ammonia and alcohol are shown in Tables 20 and 21. All these trials were conducted during January.

Table 20. Effect of Variations in the Quantities of Ammonia and Alcohol on the Efficiency of the Modified Procedure which Employs a 1:1 Mixture of Ethyl Ether and Skellysolve

Trial No.	Fat Obtained				Deviation from Mojonnier		
	Mojonnier (%)	Var. I* (%)	Var. II (%)	Var. III (%)	Var. I (%)	Var. II (%)	Var. III (%)
1	3.7385	3.6633	3.6464	3.6574	0.0752	0.0921	0.0811
2	3.7556	3.6741	3.6400	3.6478	0.0815	0.1156	0.1078
3	3.4030	3.3523	3.3473	3.3290	0.0507	0.0554	0.0740
4	3.3806	3.3315	3.3075	3.2836	0.0491	0.0731	0.0970
5	3.9283	3.8634	3.8504	3.8554	0.0649	0.7790	0.0729
Ave.	3.6412	3.5769	3.5583	3.5546	0.0643	0.0829	0.0866

*Variation I consisted of the regular modification which makes use of a 1:1 mixture of ethyl ether and Skellysolve "A".

Variation II consisted of using 1 ml. ammonia and no alcohol in the second extraction together with the mixture procedure.

Variation III consisted of using 1 ml. ammonia and $2\frac{1}{2}$ ml. alcohol in the second extraction together with the mixture procedure.

These variations in alcohol and ammonia served to increase the average difference between the Mojonnier results and the regular modified results by 0.0168 and 0.0223 per cent respectively. For this reason these variations are not practical in increasing the efficiency of the regular modified procedure.

Table 21 shows the results of still another variation in the quantity of ammonia. This variation is accompanied by the use of heat, which has previously been shown to have some effect in increasing the efficiency of the modified methods. These determinations were conducted using both the mixture and reduced quantity modifications, and are compared to the Mojonnier method and the regular modified procedures.

These data show that heating the sample to 100° F just before analysis combined with the use of additional ammonia in the second extraction serves to decrease the difference between the Mojonnier procedure and both of the two modified methods. The application of these techniques to the method which uses the 1:1 mixture of ethyl ether and Skellysolve "A" resulted in an increase of 0.0384 per cent in extraction efficiency, leaving a difference of only 0.0581 ± 0.0085 per cent between this technique and the Mojonnier in the fourteen trials conducted. This treatment increased the efficiency of the reduced method by 0.0319 per cent, resulting in an average value for six trials of 0.0584 per cent lower than results obtained by the Mojonnier method. In the six analyses in which both modified procedures were used, the modification III resulted in an average of 0.0761 per cent below the Mojonnier results, while modification IV gave a value which was 0.0584 per cent lower. This relationship between these two modifications is in line with earlier findings.

Comparison of these results with the data presented in table 9 shows that this application of heat and extra ammonia causes the results obtained

Table 21. Effect of Variation in the Amount of Ammonia on the Efficiency of Two Modified Procedures, as Compared to the Mojonnier Method

Trial No.	Fat Obtained				Deviation from Mojonnier				
	Mojonnier (%)	Mod. I* (%)	Mod. II (%)	Mod. III (%)	Mod. IV (%)	Mod. I (%)	Mod. II (%)	Mod. III (%)	Mod. IV (%)
1	3.7385	3.6633		3.6730		0.0752		0.0655	
2	3.7556	3.6741		3.6936		0.0815		0.0620	
3	3.4030	3.3523		3.3978		0.0507		0.0052	
4	3.3806	3.3315		3.3190		0.0491		0.0616	
5	3.9283	3.8634		3.9230		0.0649		0.0053	
6	3.8932	3.7888		3.8571		0.1044		0.0361	
7	3.9206	3.8004		3.8493		0.1202		0.0713	
8	3.8718	3.7924		3.8219		0.0794		0.0499	
9	5.6465	5.4819	5.5926	5.6109	5.6045	0.1646	0.0539	0.0356	0.0420
10	5.0725	4.9206	4.9798	4.9530	4.9911	0.1519	0.1073	0.1195	0.0614
11	3.5826	3.4659	3.4980	3.4893	3.5004	0.1167	0.0846	0.0933	0.0822
12	3.4995	3.3703	3.4189	3.4060	3.4263	0.1292	0.0806	0.0935	0.0732
13	3.6391	3.5471	3.5302	3.5778	3.5893	0.0920	0.1089	0.0613	0.0498
14	5.5798	5.5077	5.4596	5.5262	5.5578	0.0721	0.1202	0.0536	0.0220
Ave.	4.0651	3.9686	4.4130	4.0070	4.4449	0.0965±0.0120	0.0903	0.0581±0.0085	0.0584

*Modification I consists of the regular modified method using the 1:1 mixture.

Modification II consists of the regular modified method employing 50 per cent of the standard quantity of ethyl ether.

Modification III consists of proceeding as in Modification I with the exceptions that the sample is heated to 1000 F before analysis, and 1 ml. of ammonia is added during the second extraction.

Modification IV consists of Modification II similarly varied by use of heat and additional ammonia.

by use of the modified method which uses the 1:1 mixture to fall within the range of values established for this method in the analysis of summer milk. The efficiency of the modified method which uses reduced quantities of ethyl ether was not increased by this treatment to a point where it was equivalent to the results obtained during the summer months.

The combination of heat treatment and extra ammonia also served to destroy the opaque, gelatinous appearance which the lower layer of liquid in the extraction flasks had developed during the winter months.

Attention is called to the differences obtained by using modification III on samples 3 and 5. These samples were analyzed immediately after being drawn from the cow, and the combination of heat and added ammonia in the second extraction resulted in differences of 0.0052 and 0.0053 per cent respectively from the Mojonnier control method. These remarkably slight differences were not obtained by this procedure when the milk was allowed to stand for 24 hours at 4° C, as is shown by samples 4 and 6 which are the same respective samples after being held for this length of time. Similarly other samples obtained directly from the cow did not give similar results as is shown by trials 9-14, all of which were analyzed directly after being drawn from the cow.

In two separate preliminary trials the combined effects of heat treatment and extra ammonia on the efficiency of the modified method which used reduced quantities of ethyl ether were studied. These preliminary studies gave evidence that this added technique resulted in values which were 0.0346 and 0.0299 per cent higher than the Mojonnier control results. The normal reduced procedure gave results in these studies which were typical of other values obtained in the winter, their average being 0.0492 per cent lower than the Mojonnier value. As is shown by trials 9-14 of Table 21, these results

could not be repeated, leaving the explanation of these results and those obtained in trials 3 and 5 a matter of conjecture.

6. The effect of milk preservatives on the efficiency of the modified method which uses reduced quantities of ethyl ether. To study the effect of milk preservatives on the extraction efficiency of the reduced modified method, trials were conducted in which this method was compared to the Mojonnier method in the analysis of milk which had been preserved by 0.0017 per cent of corrosive sublimate in 250 ml. of milk, and held for 7 days at 4° C. For each sample treated with corrosive sublimate a duplicate sample was carried along for a control which contained no preservative.

The data which appear in Table 22 were obtained during January. These data show that, in general, the relationship between the Mojonnier method and the modified method which utilizes 50 per cent of the standard amount of ethyl ether is not affected by the use of such a preservative as corrosive sublimate. The average difference between those two methods at 0 days is 0.0822 per cent, and after 7 days of holding both with and without the presence of corrosive sublimate the difference between the results obtained by the two methods is 0.0806 per cent.

Although the relationship between the reduced method and the Mojonnier is unchanged through this treatment, it can be seen that both methods extracted less fat in the samples, both preserved and unpreserved, which had been held 7 days than in the same samples at 0 days. This decrease in apparent fat content of the samples may be due to a development of rancidity. Samples 6 and 7 were the only pasteurized samples, and these show only a slight change in fat content due to aging, whereas sample 10 had developed a strong rancid flavor after holding and this sample shows the greatest decrease in fat after storage, a decrease of 0.1463 per cent.

Table 22. Comparison of the Mojonnier Method with the Modified Procedure which Employs Reduced Quantities of Ethyl Ether in the Analysis of Milk which Has Been Preserved by Corrosive Sublimate

Trial No.	Difference between				Fat Obtained - 7 da.			
	Fat Obtained - 0 da.		Preserved Sample		Unpreserved Sample		Difference between	
	Mojonnier Modified (%)	Methods (%)	Mojonnier Modified (%)	Methods (%)	Mojonnier Modified (%)	Methods (%)	Mojonnier Modified (%)	Methods (%)
1	4.9167	4.8127	0.1043	4.6889	4.5806	0.1083	4.8259	
2	4.4620	4.4047	0.0573	4.3300	4.2200	0.1100	4.3922	
3	4.0344	3.9761	0.0583	3.8457	3.7504	0.0953	3.9377	
4	3.5938	3.5508	0.0430	3.4441	3.3769	0.0672	3.5272	
5	3.3943	3.3248	0.0695	3.2342	3.1511	0.0831	3.3172	
6	1.9704	1.9281	0.0423	1.9733	1.9063	0.0670	1.9536	
7	3.8086	3.6879	0.1207	3.7997	3.7130	0.0867	3.8011	0.1298
8	3.8988	3.7973	0.1015	3.8216	3.7527	0.0689	3.8359	0.0862
9	3.7549	3.6477	0.1072	3.6791	3.6294	0.0497	3.6974	0.0477
10	3.5943	3.4869	0.1179	3.4485	3.3831	0.0654	3.4970	0.0633
Ave.	3.7429	3.6617	0.0812	3.6265	3.5464	0.0801	3.7079	0.0818

*Average of last four trials.

Other trials were conducted in which a 3:2 mixture of ethyl ether and Skellysolve "A" was compared to the Mojonnier method in the analysis of milk which had been preserved by the use of 0.0017 per cent corrosive sublimate. These data are presented in Appendix Table X and indicate that the preservative together with 7 days of aging resulted in a difference of 0.0621 per cent between this modification and the Mojonnier method.

The Use of the Modified Methods in the Analysis of Low-Fat Products

In order to find the comparative efficiency of the two modified procedures in the analysis of products which contain small amounts of fat, several trials were conducted in which the modified methods were compared to the Mojonnier in the analysis of skimmilk and churned buttermilk. The two modified procedures used here are those which (a) utilize reduced quantities (50 per cent) of ethyl ether and (b) employ a 1:1 mixture of ethyl ether and Skellysolve "A".

Table 23 shows the results of these trials which were conducted during August and January.

From these data it can be seen that in the analysis of churned buttermilk the method which uses only 50 per cent of the standard quantity of ethyl ether extracted only 0.0152 per cent less fat than the Mojonnier method. However, the method which utilizes the 1:1 mixture of ethyl ether and Skellysolve "A" is much less efficient in the analysis of buttermilk, extracting less than 55 per cent as much as the Mojonnier technique, the difference between these methods amounting to 0.2711 per cent. In the case of skimmilk, however, the inefficiency of this procedure is less pronounced, the difference between it and the Mojonnier control amounting to only 0.0130 per cent. This represents ^{97.5}~~92.15~~ per cent efficiency when compared to the results obtained by the Mojonnier method.

Table 23. Comparison of the Mojonnier Method with Two Modified Methods which Use Reduced Quantities of Ethyl Ether and a 1:1 Mixture of Ethyl Ether and Skellysolve "A" in the Analysis of Low-Fat Products*

Product	No. of Trials	Fat Obtained			Deviation from Mojonnier	
		Mojonnier (%)	Mixture (%)	Reduced 50% (%)	Mixture (%)	Reduced 50% (%)
Buttermilk	3	0.6345		0.6193		0.0152
Buttermilk	3	0.6014	0.3303		0.2711	
Skimmilk	3	0.1411	0.1231		0.0130	
Powdered Buttermilk	2	5.9115	4.8405	5.5105	1.0710	0.4010
Powdered Skimmilk	2	0.7707	0.4968	0.4883	0.2739	0.2824

When these same modified procedures are compared to the Mojonnier technique in the analysis of powdered skimmilk, they give somewhat similar results. In these trials the 1:1 mixture gave values which were 0.2739 per cent lower than the Mojonnier, representing 64.5 per cent efficiency, while the reduced quantity procedure resulted in a difference of 0.2824 per cent from the Mojonnier, an efficiency of 63.4 per cent. The two modified methods give dissimilar results when powdered buttermilk is analyzed. In these trials the reduced quantity procedure resulted in a difference of 0.4010 per cent from the Mojonnier, representing an efficiency of 93.22 per cent, while the mixture procedure resulted in a difference of 1.0710 per cent, an efficiency of 81.88 per cent.

*Complete data found in Appendix Table XIX.

DISCUSSION

Of primary importance in evaluating a method is a demonstration of its accuracy when compared to a well-known standard procedure. In this particular study involving fat determination in dairy products, the control method utilized is the Mojonnier technique. Results obtained by the modified methods are compared thereto to ascertain the comparative accuracies of these procedures. To demonstrate the relationship between the Mojonnier and modified methods, statistical treatment of the data involving the use of regression curves may be used satisfactorily. Such treatment has been administered in this study wherever sufficient data were available. By an application of such curves, the per cent fat extracted by the modified method may be used to estimate the per cent fat which would be extracted by the Mojonnier method. This calculation is accomplished by the use of the equation $y = a + bx$ where y represents the unknown Mojonnier value, x stands for the value obtained by use of the modified procedure, and a and b are constant values representing the slope and intercept of the regression curve.

Results obtained in these studies of the various modifications of the Mojonnier method indicate that the only modification entirely satisfactory is that in which the petroleum ether is replaced by Skellysolve "A" or "F". The data obtained by use of this substitution reveal that if the technique is properly executed, there is no significant difference between the results obtained by its use and the Mojonnier procedure. This is illustrated by Figure I which shows the relationship between the Mojonnier method and the modified method in which petroleum ether is replaced by Skellysolve "F". This regression curve reveals practically perfect correlation between the two methods.

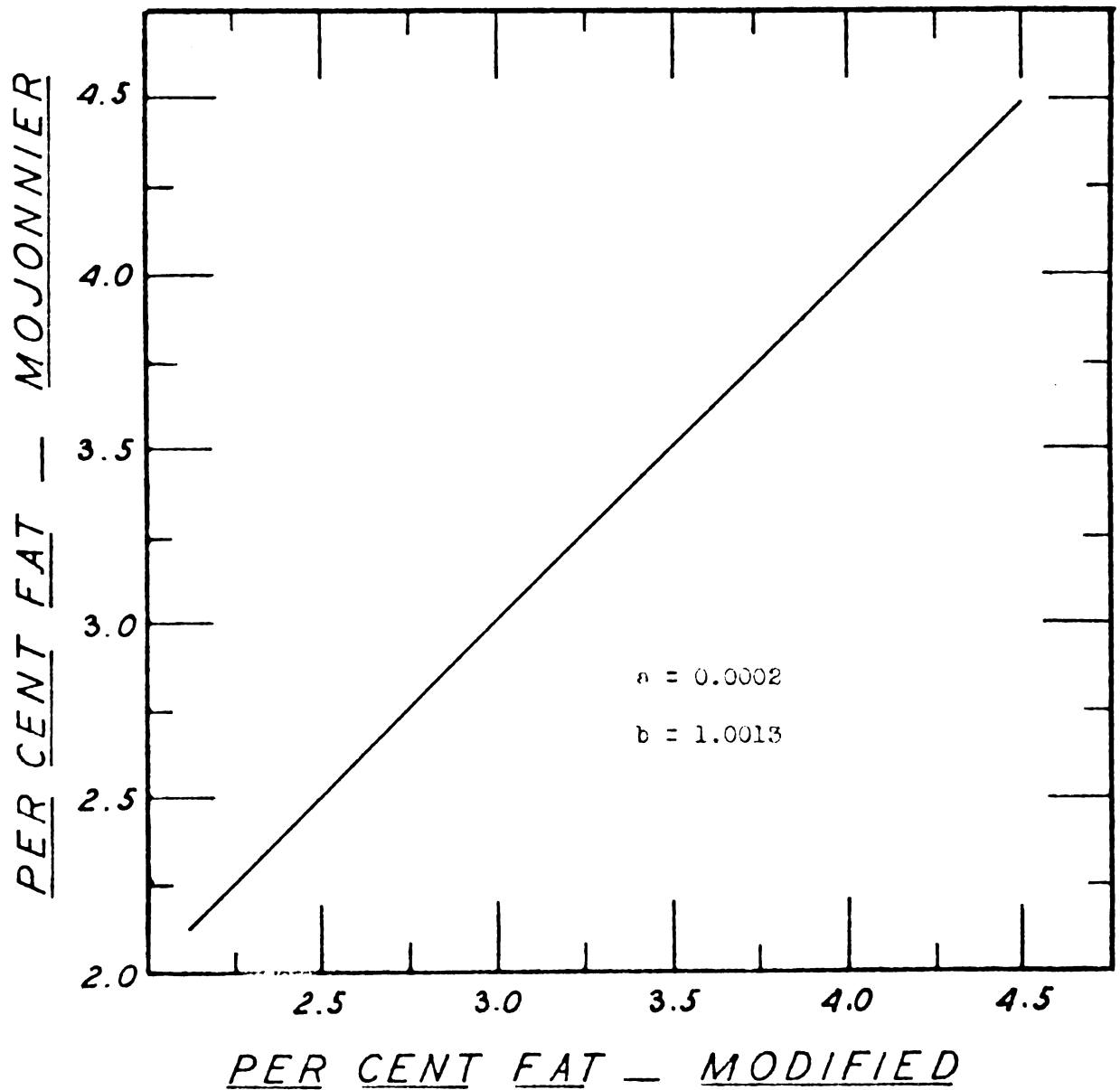


Figure I. Relationship between the Mojonnier Method of Fat Extraction and a Modified Method in which the Petroleum Ether is Replaced by Skellysolve "F"

Of the types of modifications involving the first solvent, ethyl ether, none exhibited the high degree of accuracy demonstrated in the previous substitution modification. Those modifications which utilized reduced quantities of ethyl ether resulted in incomplete extraction to a greater or less degree when compared to the Mojonnier, the efficiency of extraction being directly related to the quantity of ether used. Figures II and III show the regression curves which were established for the reduction modifications using 50 per cent and 25 per cent less ether respectively in the analysis of summer milk. Figure IV shows the relationship between the 50 per cent reduced method and the Mojonnier method in the analysis of winter milk. These graphs reveal that when the 25 per cent reduction method is used in the analysis of summer milk, a difference of 0.0104 per cent can be expected between the modified procedure and the Mojonnier, whereas the 50 per cent reduction method under these conditions results in a difference of 0.0269 per cent. This latter value is increased to 0.0661 per cent under winter conditions. Mojonnier and Troy (48) report that a 50 per cent reduction in ethyl ether resulted in a value which was 0.06 per cent lower than the Mojonnier, a value which is in close agreement with the value herein reported for winter milk.

Substitution for ethyl ether of an equal volume of a mixture of ethyl ether and Skellysolve "A" also yielded inferior results when compared to the standard Mojonnier procedure. Figures V and VI depict the relationship which exists between the Mojonnier method and the 1:1 mixture modification when Skellysolve "F" and Skellysolve "A" respectively are used as second solvents. Figure VII shows the relationship between the Mojonnier method and the 1:1 modification when the comparison is made on winter milk and the regression curve obtained by the comparison of the Mojonnier method is presented in Figure VIII. These graphs reveal that the efficiency of extraction is directly

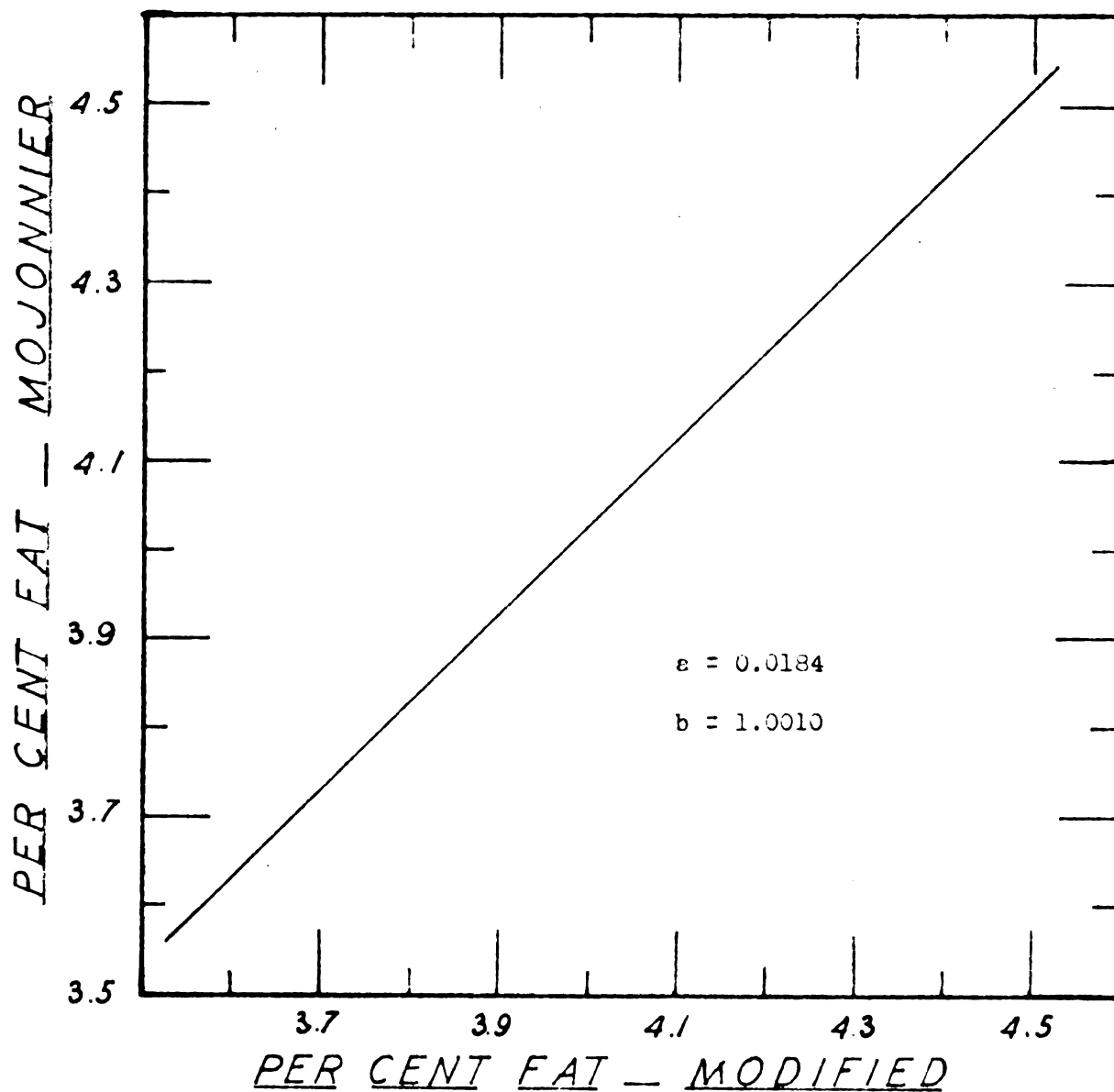


Figure II. Relationship between the Mojonnier Method and a Modified Method which Utilizes 50 per cent of the Standard Quantity of Ethyl Ether

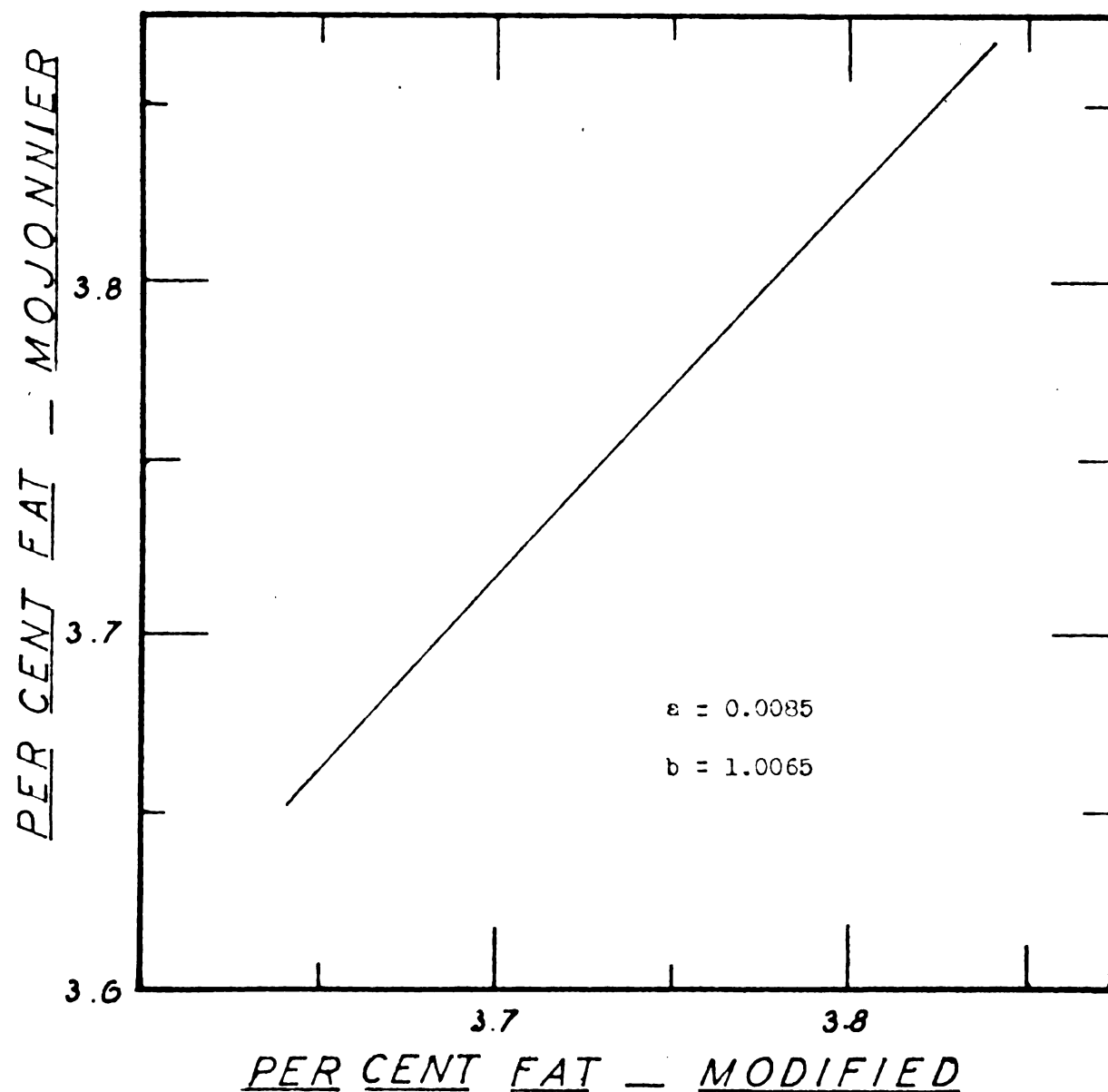


Figure III. Relationship between the Mojonnier Method of Fat Extraction and a Modified Method which Utilizes 75 per cent of the Standard Quantity of Ethyl Ether

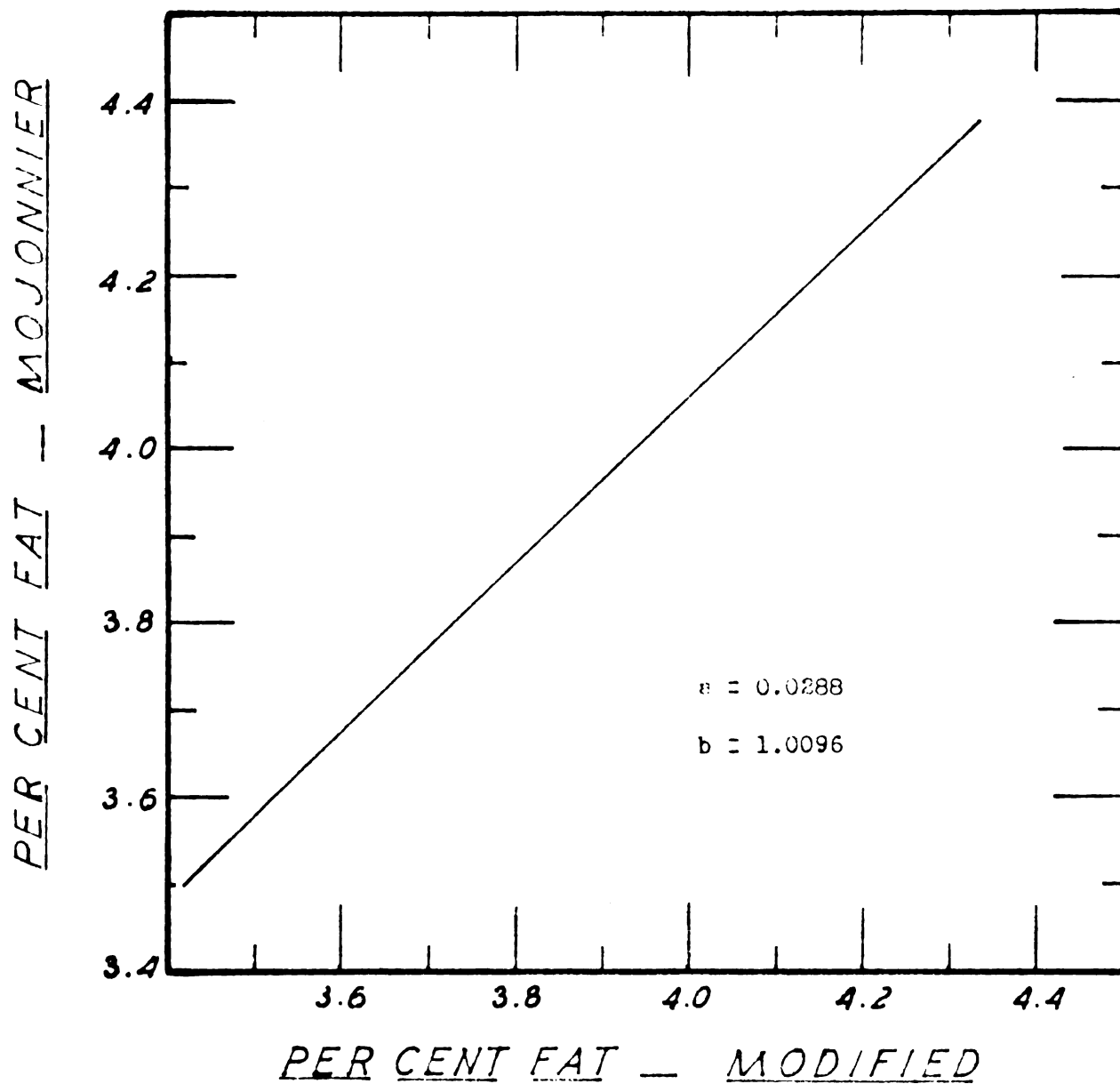


Figure IV. Relationship between the Mojonnier Method of Fat Extraction and a Modified Method which Utilizes 50 per cent of the Standard Amount of Ethyl Ether in the Analysis of Winter Milk

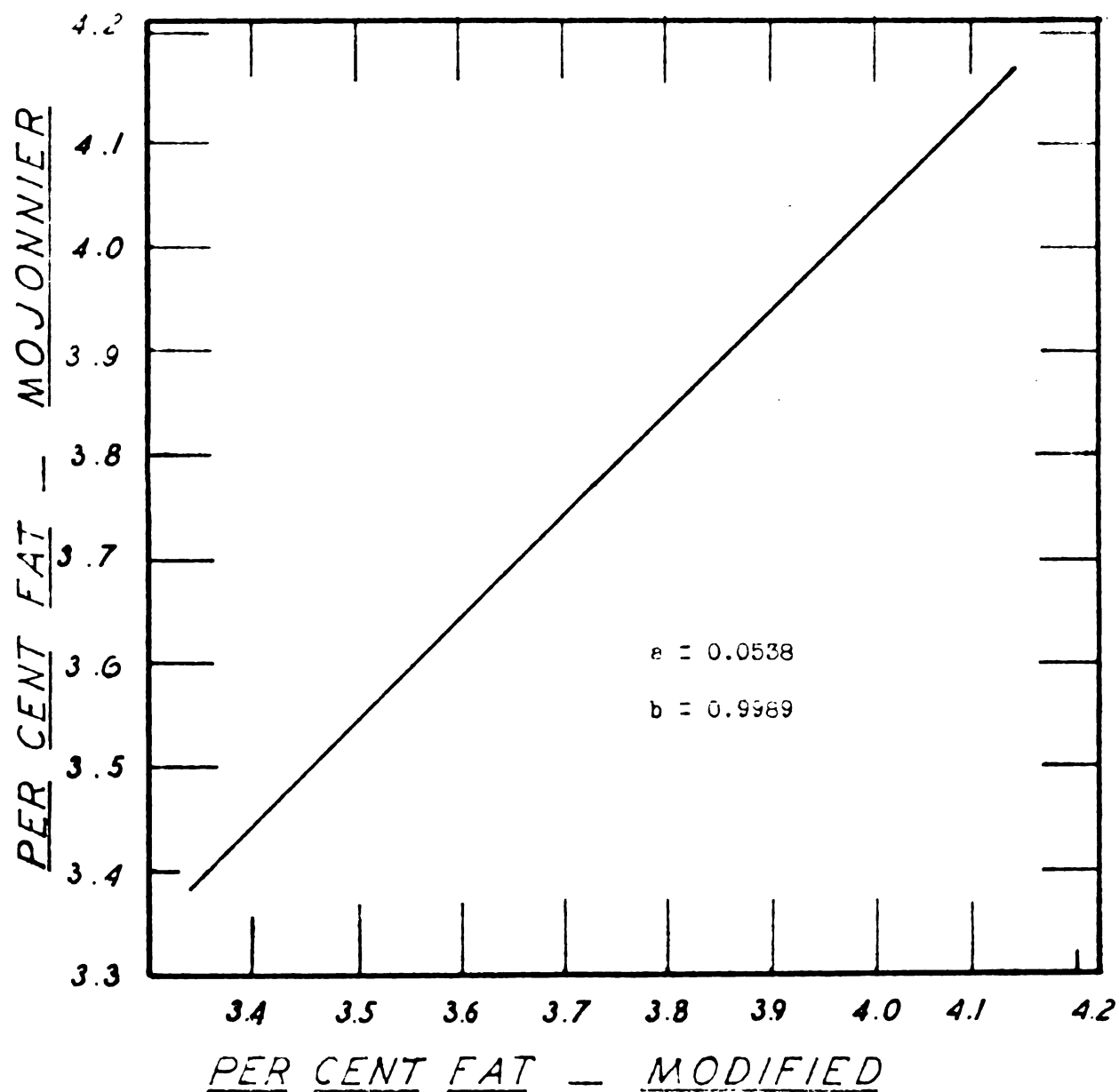


Figure V. The Relationship between the Mojonnier Method and a Modified Method in which a 1:1 Mixture of Ethyl Ether and Skellysolve "A" is Used as First Solvent, Skellysolve "F" being used as Second Solvent

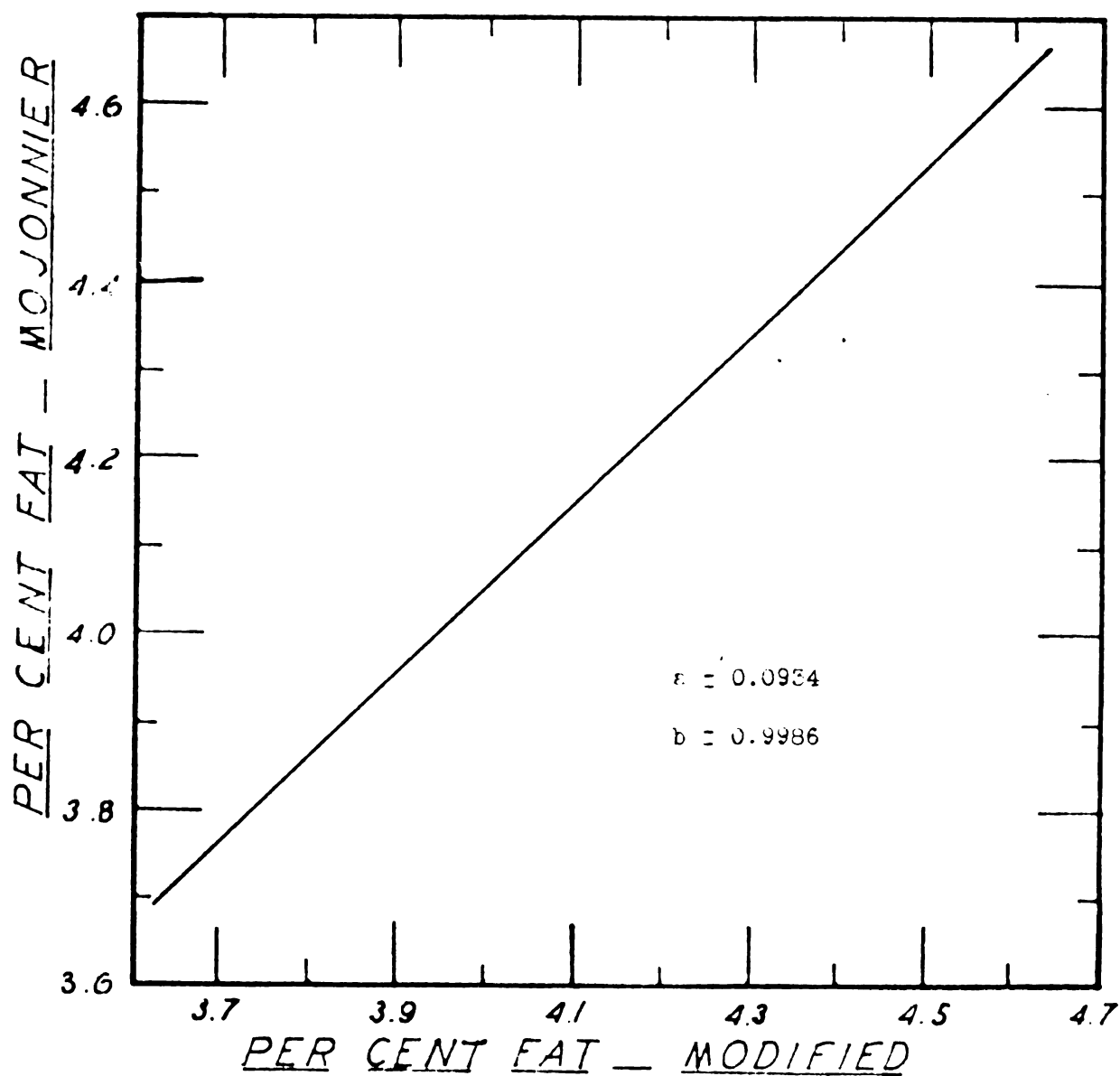


Figure VI. Relationship between the Mojonnier Method of Fat Extraction and a Modified Method in which a 1:1 Mixture of Ethyl Ether and Skellysolve "A" is Used as First Solvent, Skellysolve "A" Being Used as Second Solvent

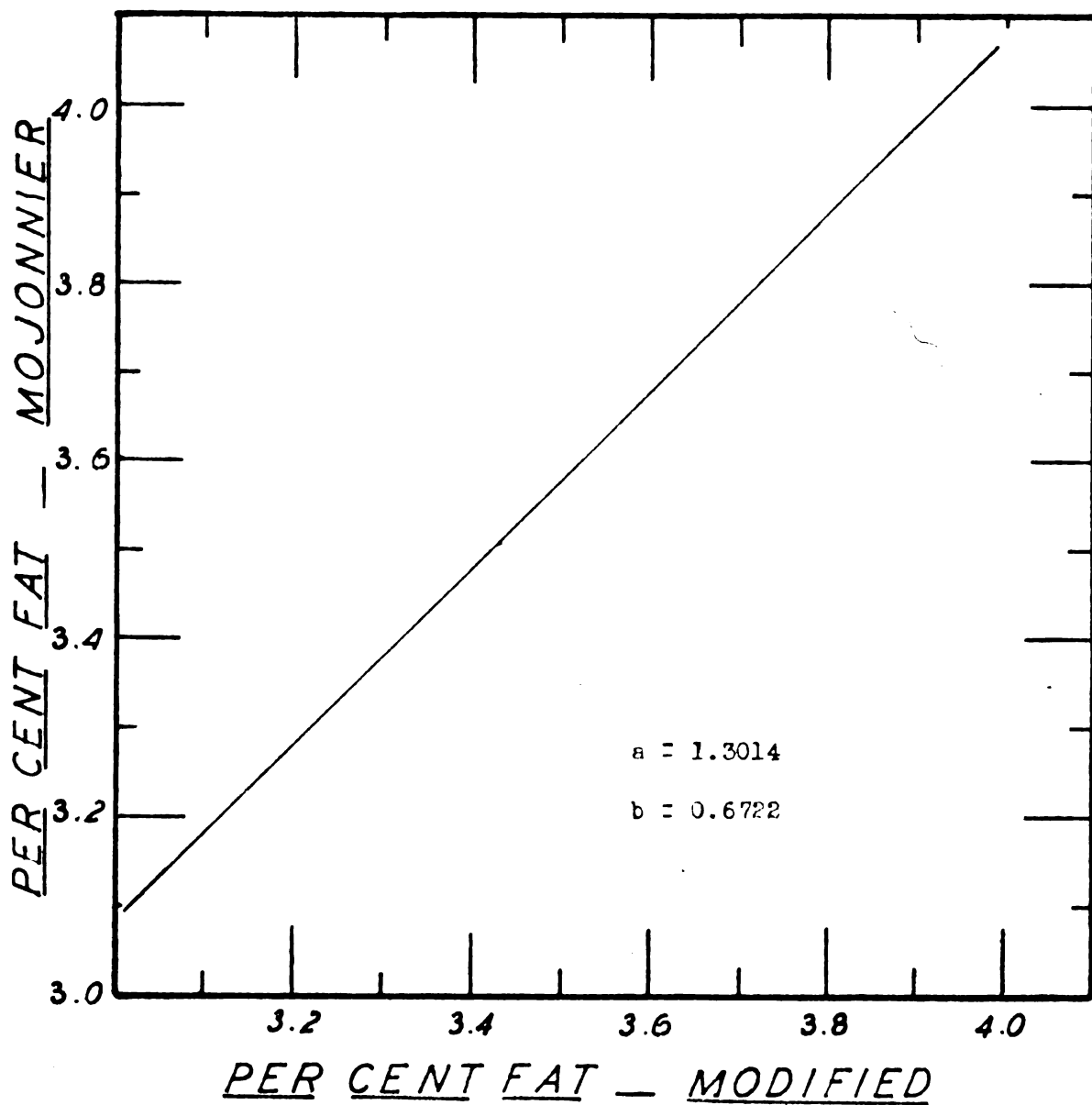


Figure VII. Relationship between the Mojonnier Method and a Modified Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A" in the Analysis of Winter Milk

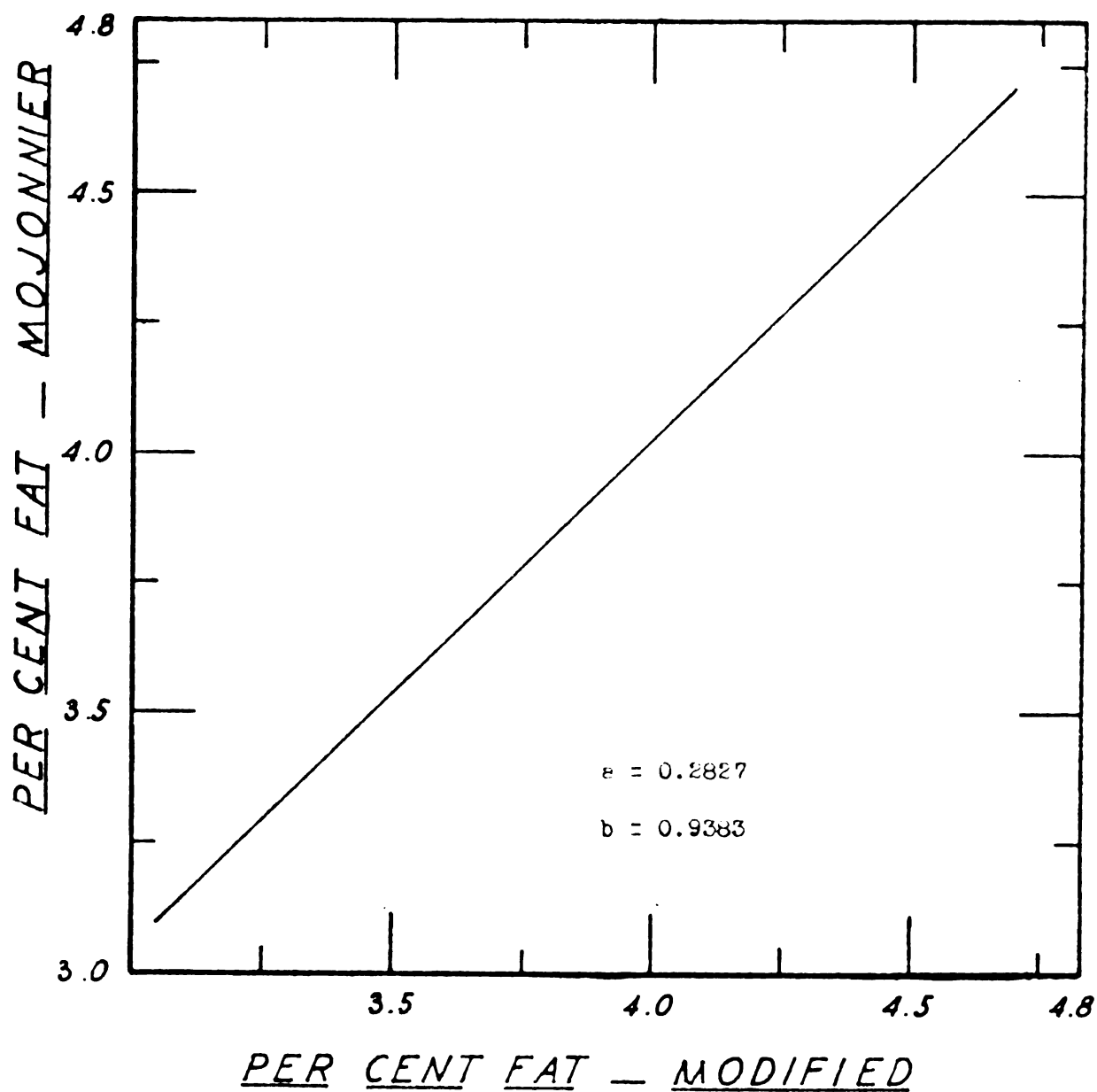


Figure VIII. Relationship between the Mojonnier Method and a Method which Makes Use of a 3:1 Mixture of Ethyl Ether and Skellysolve "A"

related to the proportion of ether in the mixture. In the analysis of summer milk the 1:1 mixture modification averaged 0.0476 ± 0.0046 per cent lower than the Mojonnier and the 3:1 mixture was lower by 0.0245 ± 0.0038 per cent. Although no graph is shown for the 3:2 mixture modification, this technique gave results 0.0298 ± 0.0025 per cent lower than the Mojonnier. In the case of the 1:1 mixture method the difference from the Mojonnier is increased in the winter time to 0.0896 ± 0.0049 per cent. Statistical analyses of all data obtained by use of these mixture modifications show that there is a significant difference between their results and the Mojonnier. However, the difference between Skellysolve "A" and "F" as second solvents in the 1:1 procedure is not significant.

The results obtained by use of the modification which uses only 50 per cent of the normal amount of ethyl ether, without any Skellysolve except as second solvent, indicate that poorer results are obtained by using 40 ml. of the 1:1 mixture than by using 20 ml. of ethyl alone. The decreased efficiency is probably due to the fact that despite the apparent miscibility of the mixture with alcohol, the Skellysolve still exerts an inhibiting action on the penetration ability of the ethyl ether and alcohol.

The results of both the reduced and mixture modifications were noticeably improved by certain innovations in the technique. It was found that in analyzing winter milk, increasing the shaking time from 30 seconds to 60 seconds reduced the average error in both types of modification by about 0.04 per cent. Thus, by creating this extra length of exposure of the fat to the solvent, the inefficiency created by winter conditions was nullified. A similar increase in winter efficiency was obtained by means of heating the sample to 100° F just prior to analysis and using extra amounts of ammonia in the second extraction. Figures IX and X show the respective relationships of these

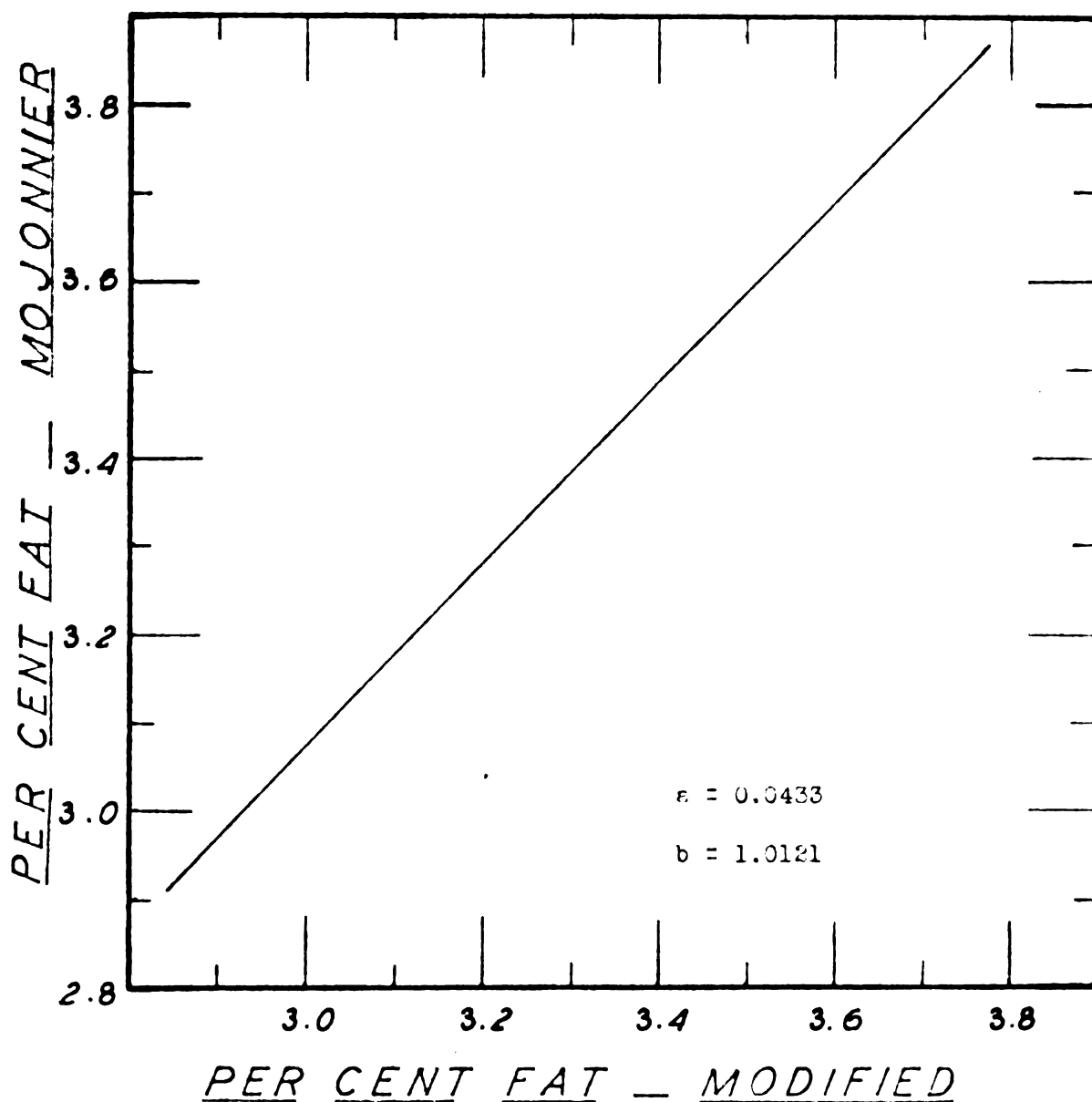


Figure IX. Relationship between the Mojonnier Method and a Method which uses a 1:1 Mixture of Ethyl Ether and Skellysolve "A" when the Shaking Period is Lengthened to Sixty Seconds

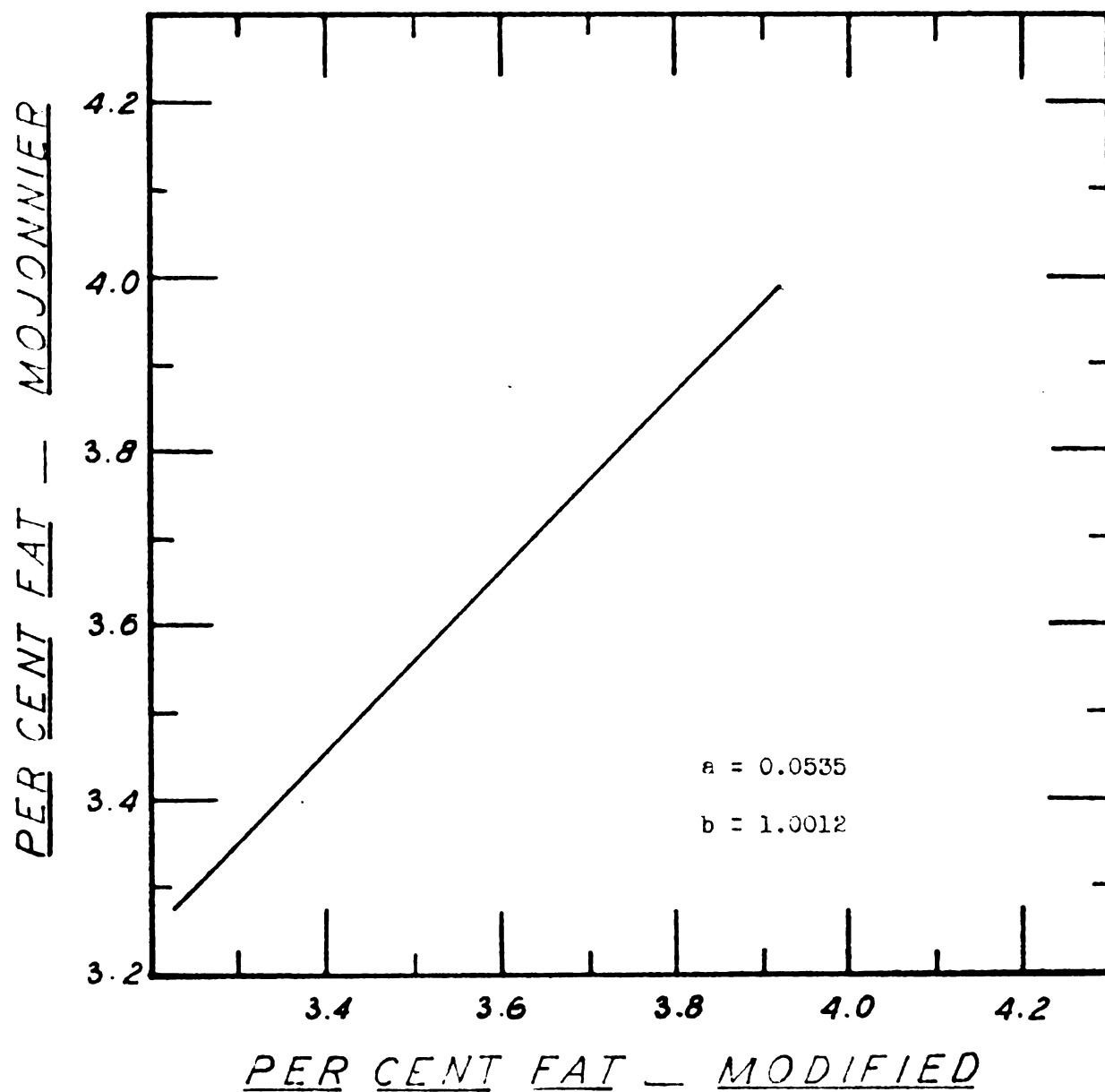


Figure X. Relationship between the Mojonnier Method and a Modified Method which Uses a 1:1 Mixture of Ethyl Ether and Skellysolve "A" with a further Treatment with Heat and Additional Ammonia

variations in technique to the Mojonnier. The action of heat alone tends to cause a slight reduction in the discrepancy, but both effects are needed for most efficient results. The effect of a third extraction with the 1:1 mixture procedure was also studied and resulted in a decrease of 0.0253 per cent in the discrepancy from the Mojonnier method.

As has been stated in the results, certain procedures which gave inferior results likewise exhibited a gelatinous condition and opaque color in the lower layer. This condition was first noticed when Skellysolve "A" was substituted in entirety for ethyl ether, and later in the winter trials using both the mixture and reduced quantity modifications. The most pronounced case of this type was observed when the final sample of milk from the fat feeding trials was analyzed. In general, there seems to be a relationship between the degree of opacity and gelatination and the efficiency of fat extraction. This relationship would be expected if the protein surrounding the fat globules were not completely dissolved, resulting in a flocculent emulsion which prevents complete extraction of the fat. In other trials, the observation was made that this opacity and flocculence was destroyed and a normal appearance created by the use of additional ammonia and heat, indicating a protein effect.

Since this trouble of gelatination was present when winter milk was analyzed by the modified procedures, but was not there during the summer, it was felt that the winter milk had undergone some slight, natural change in protein stability. It is believed that the fat thus trapped in the gelatinous lower layer represents the difference observed between winter and summer efficiency of the modified procedures. This is indicated by the fact that variations in technique which destroyed the opaque color and gelatinous condition gave results which showed deviations from the Mojonnier results which were no greater than the normal summer discrepancy. In connection with the observa-

tions relative to this seasonal influence, studies were made on the effect of low temperature storage of milk and on the effect of changes in ration. The storage of milk had no effect on the method, but the change in ration greatly altered the efficiency of the modified technique. For example, in the fat feeding trials, the final series of analyses conducted on this milk resulted in the greatest inefficiency obtained in any trials by the mixture modification, and the gelatinous condition and opaque color of the lower layer were present at their greatest intensity. If such great changes in fat extraction and in the physical appearance of the samples during analysis by the modified procedures are induced by the change in ration herein made, then surely normal winter practices of feeding may be expected to be a factor in influencing the seasonal changes in certain of these extraction methods.

Another characteristic was noted which is similar to the gelatinous condition and also occurred when poor extraction efficiency was obtained. This difficulty was the presence of a yellowish, fat-like layer at the junction of the two layers in the flask, and was encountered when Skellysolve "A" was substituted entirely for ethyl ether and when mixtures of ethyl ether and Skellysolve "A" were used which contained less than 50 per cent ethyl ether. This condition is also believed to be due to the trapping of fat by undissolved protein, the yellow color being due to a greater proportion of fat in the protein mass. The reason that this condition should exist only when less than 50 per cent ethyl ether is present in the mixture is probably because the solvent is immiscible with alcohol under these conditions.

Immiscibility of the solvent with alcohol has a profound effect on the efficiency of the extraction since the extraction efficiency is due to the complete penetration of the solvent to all particles of fat, any substance which is entirely immiscible with the transporting agent would likely be less

effective than ethyl ether. In the Mojonnier method of fat extraction, the alcohol acts as a transporting agent, carrying the solvent to the aqueous suspension of fat. The ethyl ether though only slightly miscible with water, is readily brought in contact with the fat by the alcohol which is miscible with both water and ethyl ether.

As indicated in the results, Skellysolve itself is completely immiscible with alcohol. However, the miscibility of alcohol and Skellysolve is improved by the addition of ethyl ether, and a 50 per cent mixture of ethyl ether and Skellysolve is completely miscible with alcohol, even when water or milk is included in the system. The greater quantity of ethyl ether required when water or milk is present is probably due to the additional volume through which the alcohol must be distributed. The water or milk serves to partition a certain amount of the alcohol, leaving a smaller amount remaining in the solvent phase. As small quantities of alcohol reduce miscibility with Skellysolve to a greater extent than large quantities, it is logical that greater amounts of ether are required to keep the mixture in a miscible state.

The volume of the lower layer of liquid in the flask is also affected by the miscibility of the reagents. In the process of conducting the standard Mojonnier method, the volume of the lower layer after the addition of ethyl ether is only 48.8 per cent as great as the volume of non-ethereal liquids which have been added. The difference corresponds approximately to the amount of alcohol which has been added and it is likely that it is included in the upper layer at this point. In the case of the substituted procedure, when Skellysolve is added, and the emulsion allowed to settle out, the volume of the lower layer is even greater than the amount of milk, ammonia and alcohol which are thought to compose it, indicating that certain small amounts of Skellysolve are retained in the lower layer. The presence of small amounts

of Skellysolve at this stage of the extraction procedure will cause an increase in the volume of the lower layer, even though the miscibility of the ether-Skellysolve mixture, alcohol, and water is complete. However, these small variations in the volume of the lower layer are of minor importance so long as miscibility is maintained.

Upon the addition of the second solvent, petroleum ether, to the mixture in the standard Mojonnier procedure the volume of the lower layer is still less than the total volume of milk, alcohol and ammonia by approximately the amount of alcohol which has been added in the second extraction. Thus, petroleum ether does not occlude all of the alcohol or other non-etheral substances from the ethereal layer. In the case of the modified procedure, using 100 per cent Skellysolve, little change is effected in the volume of the lower layer by the addition of the second solvent, indicating that the Skellysolve does not permit the inclusion of alcohol or other liquids in the upper layer. Further evidence that some non-etheral substances are present in the ether layer of the Mojonnier samples is indicated by the fact that in all trials, the Mojonnier extract took a considerably longer time to become completely evaporated, and there was some indication that the boiling temperature of the Mojonnier extract was higher than that of the modified procedures.

Further reasons for the inefficiency of the several Skellysolve modifications may be revealed by the data obtained in the analysis of low-fat products. These data show that a great difference exists between (a) the methods using ethyl ether as the first solvent and (b) the methods using mixtures of ethyl ether and Skellysolve "A" as the first solvent.

In the analysis of skimmilk and powdered skimmilk, both the mixture modification and the reduced quantity modification give similar results, indicating they are about equally efficient. However, in the analysis of churned

buttermilk, the 1:1 mixture procedure extracted only 55 per cent as much fat as the Mojonnier method, whereas the 50 per cent reduced quantity procedure was 92.13 per cent efficient.

It may be speculated that these differences in results obtained by the two methods in the analysis of these products may be due to a difference in the composition of the products. Apparently there is some ether-soluble material in the buttermilk which is not extracted by the Skellysolve-ether mixture. It is known that churned buttermilk contains a relatively high proportion of phosphatides, and that skim milk contains a relatively low percentage. It is further known that these substances are rather efficiently extracted by ether. If it were found that the phosphatides are not extracted by the Skellysolve-ether mixtures, then a good portion of the discrepancies found between these mixture methods and the Mojonnier would be explained.

A final important consideration in the comparison of the Mojonnier method with these various modifications is the cost of the several reagents. The present price of ethyl ether is quoted at from \$1.80 to \$2.25 per gallon, and petroleum ether at \$2.50 per gallon. In contrast, Skellysolve "A" and "F" cost \$0.65 per gallon. On this basis, the complete replacement of petroleum ether by Skellysolve results in a saving of 74 per cent of the cost of second solvents. The replacement of ethyl ether by a 1:1 mixture of ethyl ether and Skellysolve "A" results in a saving of 30 to 36 per cent, and use of the 3:1 mixture gives a saving of 12 to 19 per cent. The use of reduced quantity modifications have obvious effects on the cost of reagents. The availability of ethyl ether has been affected somewhat by war production demands, but similar circumstances have not diminished the supply of Skellysolve. Naturally, the economic factor is of no consideration unless the efficiency of the procedure is satisfactory.

SUMMARY AND CONCLUSIONS

This study was conducted for the purpose of developing modifications of the Mojonnier method which employ less expensive and more easily available solvents. The modifications which were attempted are as follows: (a) The complete substitution of Skellysolve "F" and Skellysolve "A" for the petroleum ether of the Mojonnier method. (Skellysolve "F" is essentially a mixture of hexanes with a boiling range of 39° - 48° C., and Skellysolve "A" is essentially normal pentane with a reported boiling point of 33° - 33.5° C.) (b) The use of various mixture of ethyl ether and Skellysolve "A" in place of the ethyl ether of the Mojonnier method. (c) The use of reduced amounts of ethyl ether used alone as first solvent. The graphic relationships between these modifications and the Mojonnier control procedure are presented in Figures I - X of the discussion.

The most successful modification is the one in which the petroleum ether is replaced by Skellysolve "A" or "F". This substitution gave results which were practically identical to those obtained with the Mojonnier procedure.

Mixtures of Skellysolve "A" and ethyl ether were substituted for ethyl ether as first solvent in proportions of 1:1, 3:2, and 3:1 parts of ethyl ether and Skellysolve "A" respectively. The extraction efficiency was found to be directly related to the proportion of ethyl ether in the mixture, but all of the modifications gave values which were lower than those obtained by the Mojonnier method. In the analysis of summer milk the procedures involving the 1:1, 3:2 and 3:1 mixtures resulted in the following respective discrepancies from the Mojonnier method: 0.0476 ± 0.0037 per cent, 0.0298 ± 0.0025 per

cent and 0.0245 ± 0.0038 per cent. Analysis of milk produced during the winter time gave even lower recovery of the fat, the difference between the 1:1 mixture and the Mojonnier procedure amounting to 0.0896 ± 0.0049 per cent.

The finer dispersion of fat caused by homogenization, and the presence of greater amounts of fat resulted in greater differences between these modifications and the Mojonnier control method. With the 1:1 mixture the following products resulted in the accompanying average differences: homogenized milk, 0.0713 ± 0.0036 per cent; evaporated milk, 0.2783 per cent; ice cream, 0.1857 ± 0.0307 per cent. The 3:2 mixture gave the following average differences: homogenized milk, 0.0452 ± 0.0141 per cent; evaporated milk, 0.0615 ± 0.0375 per cent; ice cream, 0.1289 ± 0.0496 per cent. The 3:1 mixture resulted in the following average discrepancies: homogenized milk, 0.0165 ± 0.0037 per cent; evaporated milk, 0.0461 per cent; ice cream, 0.0641 per cent.

The third modification utilized smaller quantities of ethyl ether than are used in the standard Mojonnier procedure. Reduction in the quantity of ethyl ether by 50 and 25 per cent gave summer extraction values which were respectively 0.0249 ± 0.0037 and 0.0104 per cent lower than the Mojonnier method. Winter conditions affected these methods to the extent that the 50 per cent reduced method resulted in a difference of 0.0661 per cent. Homogenized milk and high-fat products gave greater discrepancies which for the 50 per cent reduced method are as follows: homogenized milk, 0.0259 ± 0.0019 per cent; evaporated milk, 0.1009 per cent; and ice cream, 0.1662 per cent. In the case of the 25 per cent reduction these differences are: homogenized milk, 0.0164 ± 0.0026 per cent; evaporated milk, 0.0353 per cent; and ice cream, 0.0698 per cent.

Both the reduced and mixture modifications are less efficient in the analysis of winter milk. This is believed to be caused by a change in the con-

stituents of the milk, particularly protein, resulting in a condition whereby the solvent extracts the fat with more difficulty.

The seasonal effect was nullified in both the reduced and mixture techniques by either of two expedients: (a) Lengthening of the shaking period from 30 seconds to 60 seconds and (b) Application of heat at 100° F. just prior to analysis, together with the use of additional ammonia in the second extraction. Three extractions were also found to reduce the error in the 1:1 mixture by 0.0253 per cent.

In the analysis of milk which had been preserved with corrosive sublimate or milk which had been subjected to low temperature storage, neither the mixture nor the reduced procedure was affected to any greater extent than the Mojonnier method. However, in the analysis of milk from cows on fat-feeding experiments, the 1:1 mixture procedure extracted 0.9664 per cent less fat than the Mojonnier, indicating that feeding conditions have a pronounced deleterious effect on the ability of the ethyl ether-Skellysolve mixture to extract fat.

In the substitution of a solvent in fat extraction procedures, the efficiency of the solvent is doubtless affected by its miscibility with alcohol. Skellysolve "A", not being miscible with alcohol, will not be efficiently dispersed through the milk, thus resulting in incomplete extraction. The addition of ethyl ether to Skellysolve "A" increases its miscibility, and a mixture of 1:1 proportions of ethyl ether and Skellysolve "A" is completely miscible with alcohol. The results indicate that the efficiency of this miscible mixture is greatly improved over the efficiency of the Skellysolve "A" alone.

The miscibility of the reagents not only affects the extraction efficiency of the method, it plays a role in governing the volume of the lower, non-ethereal layer of liquid. When Skellysolve is one of the reagents, the

upper layer contains no aqueous or alcoholic liquid and the result is that the volume of the lower layer is slightly greater than normal. However, in the standard Mojonnier procedure, the volume of the lower layer is decidedly lower than normal, indicating that it does not include all of the non-ethereal solutions which are present. Therefore, certain of these aqueous or alcoholic substances are included in the ethereal layer.

Analysis of skimmilk and buttermilk by the method involving substitution of a 1:1 Skellysolve-ether mixture for ethyl ether and by the method using 50 per cent of the normal quantity of ethyl ether revealed wide differences in the extracting ability of these methods. When compared to the Mojonnier in the analysis of churned buttermilk, the 50 per cent reduced method was found to be 97.5 per cent efficient whereas the 1:1 mixture modification was found to be only 54.6 per cent efficient. In a similar comparison to the Mojonnier method conducted on skimmilk, the modification using the 1:1 mixture of ethyl ether and Skellysolve "A" extracted 92.13 per cent of the fat.

REFERENCES

1. Adorjan, Iosef Apparatus for Fat Determination
1906 Landw. Vers. - Wes. 9, 1063-66. Chem. Absts. 1, 1151.
2. Ardenghi, A. O. Review of the Current Standard Tests for Fat Determination. Rev. facultad. cienc. quim. 13, 131-34. Chem. Absts. 33, 5076.
3. Bartlett, J. M. A Method for the Determination of Fat in Sweetened Condensed Milk. U. S. Bur. Chem. Bul. 122, 152.
4. Bieda French Patent. Chem. Absts. 3, 256.
1906
5. Biesterfeld, C. H. and Evanson, O. L. Estimation of Fat in Condensed Milk and Milk Powder. Jour. Ind. Eng. Chem. 9, 1111-13.
1917
6. Bigelow, W. D. and Fitzgerald, F. F. A Review of Methods for the Analysis of Evaporated Milk. Natl. Canners Assn. Res. Bul. 5, 23.
1915
7. Brodrick-Pittard, N. A. Weitere Mittheilung zur Methodik der fettbestimmung im Käse. Z. Nahr. Genussm. 32, 354-8.
1916
8. Broeman, F. C. Determining Fat in Sweetened Condensed Milk.
1913 Jour. Ind. Eng. Chem. 4, 672.
9. Cobleigh, W. M. A Modified Babcock Test Applied to Homogenized Cream.
1916 Am. Food Jour. 11, 457.
10. Cochrane, C. B. Estimation of Fat in Infant and Invalid Foods.
1905 Jour. Am. Chem. Soc. 27, 906-7.
11. Consortium für elektrochemische Industrie New Fat Solvents and Extraction Media. Chem. Ztg. 31, 1095-96. Chem. Absts. 2, 717.
1907
12. Croll, W. L. An Investigation to Determine the Accuracy of a Modified Meigs Method for the Quantitative Determination of Fat in Milk. Biochem. Bul. 2, 509-18.
1913
13. Dyer, B. Minutes of a Meeting of the Society of Public Analysts.
1899 Analyst 24, 174-5.
14. Eichloff, R. Studien über die Ademssche Fettbestimmungsmethode.
1907 Milchw. Zentbl. 4, 120-24.
15. _____ and Grimmer. Abgeändertes Verfahren zur Bestimmung des Fettgehalts nach Rose-Gottlieb in Milch und Molkerelprodukten. Milchw. Zentbl. 6, 114-121.
1909

16. Fleming, R. S. Notes on the Composition and Analysis of Dessi-
1912 cated Milk and Cream. Jour. Ind. Eng. Chem. 4,
543-4.
17. Gerber's Co. German Patent. Chem. Absts. 2, 1641.
1906
18. Gottlieb, E. Estimation of Fat in Milk. Landw. Vers.-Stat. 40,
1906 1-27. Abst. Jour. Chem. Soc. 62, 549-550.
19. Greisheim Elektron Chemical Works Studies on the use of Carbon
1905 Tetrachloride as a Solvent. Chem. Ztg. 31, 326-328.
Chem. Absts. 1, 1778.
20. Grossfeld, J. A New Method for the Determination of Fat in Cheese.
1923 Pharm. Weekblsd. 60, 1069-1078. Chem. Absts. 17, 3897.
21. Gudeman, E. A New Method for Fat Analysis in Evaporated Milk or
1909 Other Concentrated Dairy Product. Jour. Soc. Chem.
Ind. 28, 738-40.
22. Hackman, C. The Estimation of Fat in Cheese. Milch. Forsch. 3,
1926 43-54. Chem. Absts. 20, 2374.
23. Hak, S. and Gregg, H. Über die Refraktometrische Methode der Fett-
1902 bestimmung in Milch nach Prof. Dr. Woolney.
Milch. Ztg. 31, 433.
24. _____ and Klykksen Über Fettbestimmung in kondensierter Milch.
1907 Z. Nahr.-Genussm. 13, 338-45.
25. Harding, E. and Parkin, G. A Rapid, Accurate Gravimetric Method for
1914 the Analysis of Evaporated Milk and Powdered Milk.
Jour. Ind. Eng. Chem. 6, 131.
26. _____ A Rapid, Accurate Gravimetric Method for
1914 the Analysis of Ice Cream. Jour. Ind. Eng. Chem. 5,
843-5.
27. Harris, C. P. The Use of O-dichlorobenzine in the Determination of
Fat by Change in Specific Gravity. Jour. Ind. Chem.,
Eng. Anal. Ed. 2 #1, 410-13. Chem. Absts. 24, 5519.
28. Hesse, A. A Simple Method for the Determination of Fat in Butter.
1904 Z. Untersuch. Nahr. Genussm. VIII, 673. Abst. in
Analyst 30, 86.
29. Horsley, J. A Method for the Estimation of Fat in Milk. Chemical
1874 News, May 1874, 224. Abst. in Am. Jour. Pharm. 46,
479.
30. Hortvet, J. Report of the Referee. Assoc. Off. Agr. Chem. Jour.
1915 1, 186.

31. Hortvet, J. Report of the Referee. Assoc. Off. Agr. Chem. Jour.
1917 2, 238.
32. Report of the Referee. Assoc. Off. Agr. Chem. Jour.
1920 4, 201.
33. Report of the Referee. Assoc. Off. Agr. Chem. Jour.
1926 9, 235.
34. Jaffe, E. A Study of the Use of Ethylene Glycoll as a Fat Sol-
1932 vent. Ann. Chim. applicata. 22, 426-433. Chem.
Absts. 26, 6167.
35. Johnson, J. J. The Use of Isopropyl Ether in a Modified Mojonnier
1937 Fat Test. Jour. Dairy Sci. 20, 563-6.
36. Keister, J. T. The Determination of Fat in Malted Milk. Jour.
1922 Assoc. Off. Agr. Chem. 5, 176.
37. Koenig, F. Application of the Gum Tragacanth Method to the
1913 Analysis of Butter. Apoth. Ztg. 29, 223. Chem.
Absts. 8, 2008.
38. Kohmen, E. F. A Rapid Test for the Determination of Fat in Butter.
1919 Jour. Ind. Eng. Chem. 11, 38.
39. Kropat, K. Application of the Gum Tragacanth Method to the
1913 Analysis of Cheese, Cream, and Butter. Arch. Pharm.
252, 76-82. Chem. Absts. 8, 2434.
40. Kuhn, F. Milch. Ztg. 27, 772. Cited by Fisher and Walts,
1898 Conn. Agr. Exp. Sta. Bul. 131.
41. Kurz, O. A Micro-Analysis for Fat. Microchemie 1, 78-86.
1923 Chem. Absts. 18, 1718.
42. Lempestrasse British Patents. Chem. Absts. 1, 921.
1906
43. Leithe, W. A Pycnometer Method for the Determination of Fat in
1934 Dairy Products. Z. Untersuch. Lebensm. 67, 441-446.
Chem. Absts. 28, 3490.
44. McLellen, B. G. A Comparison of Methods of Fat Extraction on Dried
1908 Milk. Analyst 33, 353.
45. Mendel, H. The Use of Benzene in Barthel's Method of Fat Extrac-
1917 tion. Svensk Kem. Tidskrift 29, 181-3. Chem. Absts.
12, 1402.
46. Meigs, A. V. A Method for Fat Determination in Dairy Products.
1882 Philadelphia Medical Times 12, 660.

47. Mitchell, L. C. and Alfend, S. A Lend-Gooch Method of Analysis of
1926 Butter. Jour. Assoc. Off. Agr. Chem. 9, 209-20.
48. Mojonnier, T. and Troy, H. C. The Technical Control of Dairy Products.
1925 2nd ed. Pub. by Mojonnier Bros. Co., Chicago, Ill.
936 pp. illus.
49. Notbohm, E. F. and Anigerhausen, J. Nachprüfung der "Neusal-Methode
1910 von Dr. Wendler" zur Fettbestimmung in Milch.
Z. Nahr. Genussm. 20, 495-8.
50. Olson, G. A. A Comparison of Ether Extraction Methods in the
1906 Analysis of Cheese. Proc. Assoc. Off. Agr. Chem. 23.
51. Oven, A. V. A Review of Various Old and New Methods of Fat Ex-
1915 traction. Milch. Zentbl. 43, 285-95. Chem. Absts. 9,
1080.
52. Patrick, G. E. Review of the Current Methods for Fat Determination
1913 in Dairy Products. U. S. Dept. of Agr. Bur. Chem.
Cir. 66.
53. Popp, M. Observations on Rose's Method of Fat Determination.
1904 Milch. Ztg. 33. Cited by Fisher and Walts, Conn.
Agr. Exp. Sta. Bul. 131, 1925.
54. Popp, M. and Siegfeld Zeit. für Untersuchung Nahr. Genussm. 7, 772.
1904 Cited by Fisher and Walts, Conn. Agr. Expt. Sta.
Bul. 131.
55. Rammstedt, O. Comparison of Ether Extraction and Carbon Tetra-
1909 chloride Extraction. Chem. Ztg. 33, 93-4. Chem.
Absts. 3, 1097.
56. _____ Comparison of Rapid Methods and Gravimetric Methods
1912 of Fat Analysis. Z. Angew. Chem. 25, 754-8.
Chem. Absts. 6, 1938.
57. Richmond, G. and Musgrave, W. A Special Extraction Method for Horlick's
1908 Malted Milk. Phillipine Jour. Sci. 3, 87-90.
58. Richmond, H. D. Modification of the Rose-Gottlieb Method for the
1908 Analysis of Dried Milk. Analyst 33, 389.
59. _____ Dairy Chemistry. 3rd ed. revised. Pub. by Charles
1920 Griffin & Co. Ltd. London. 490 pp. illus.
60. _____ and Rosier, C. H. The Estimation of Fat in Milk Using
1899 Petroleum Ether as a Solvent. Analyst 24, 172-175.
61. Rieter, E. A New Apparatus for the Rose-Gottlieb Test. Ann. Chim.
1909 Applicatu. 14, 54-57. Chem. Absts. 3, 1222.

62. Rijnveld, L.
1911 Comparison of Bonnema, Dekker and Soxhlet Method of Fat Extraction. Pharm. Weekblad. 48, 1204. Chem. Absts. 6, 517.
63. Rohrig,
1905 Apparatus for the Determination of Fat in Dairy Products. Amer. Zeit. Nahr. Genussn. 9, 531-33. Cited by Fisher and Walts, Conn. Agr. Expt. Sta. Bul. 131.
64. Roop, J. H.
1918 The Use of an Acid Medium in the Rose-Gottlieb Test. Chem. Analyst 26, 21.
65. Rose, B.
1888 The Determination of Fat in Milk. Z. Angewandte. Chem. 1, 100-107. Abst. in Jour. Chem. Soc. 54, 1135.
66. Rupp, E. and Müller, A.
1912 Über ein beschleunigtes Verfahren der Gottlieb-Rose'schen Fettbestimmung in Milch. Z. Nahr. Genussn. 23, 338-9.
67. Rusting, N.
1898 The Use of Tragacanth in Ether Extraction Methods. Nederl. Tydschr. Pharm. 10, 163-70. Abst. In Chem. Centr. 2, 393.
68. Scheringa, K.
1931 Observations on the Weibull-Smetham Method of Fat Extraction. Chem. Weekblad. 28, 654. Chem. Absts. 26, 1038.
69. Schreib, H.
1888 Comparison of the Rose-Gottlieb and Adams Methods of Fat Extraction. Jour. Chem. Soc. 54, 1135.
70. Sichler, A.
1903 German Patent. Chem. Absts. 2, 347.
71. _____
1904 German Patent. Chem. Absts. 1, 2435.
72. Skinner, W. W.
1940 Methods of Analysis of the Assoc. Off. Agr. Chem. 5th ed. Pub. by Assoc. Off. Agr. Chem., Washington, D. C.
73. Supplee, G. C. and Bellis, B.
1922 Fat Analysis of Milk Powders. Jour. Dairy Sci. 5, 39-50.
74. Sutton, R. W.
1925 A Modification of the Werner-Schmid Test for Condensed Milks. Analyst 50, 17-18.
75. Szilasi, J.
1914 Analyses of Human Milk. Chem. Zeit. 14, 1202-3. Abst. in Jour. Chem. Soc. 62, 1892.
76. Taufel, K. and Standigl, L.
1930 Comparison of Solvents Used in the Soxhlet Method. Allgem. Öl. Fettzg. 27, 127-29. Chem. Absts. 24, 4176.

77. Thomsen
1905 Fat Extraction from Milk. Tidskrift for Landoeconomi
1905 #2, 101. Abst. in Analyst 30, 128.
78. Timpe
1907 Dehydration of Ether. Chem. Ztg. 31, 1107. Chem.
Absts. 2, 715
79. Van Lennep, D. R. P. and Ruys, J. Use of Trichlorethylene in Extrac-
1912 tion. Chem. Weekblad. 9, 654. Chem. Absts. 6, 3477.
80. Vizern and Guillot Comparison of Various Solvents. Chemie and industrie.
1932 Special No. 580, 1-March. Chem. Absts. 26, 3687.
81. Vollrath, F.
1905 Observations on the Use of Carbon Tetrachloride as a
Solvent. Chem. Ztg. 31, 398. Chem. Absts. 1, 1778.
82. de Waal, D. C.
1922 A New Method for Fat Analysis. Tijdschr. Vergelijk.
Geneskunde 8, 138. Chem. Absts. 17, 441.
83. Weibull, M.
1907 Eine Beobachtung bei der Gottlieb'schen Methode der
Fettbestimmung. Z. Nahr. Genussm. 17, 442-5.
84. Wendler, O.
1909 U. S. Patent. Chem. Absts. 4, 2528.
85. Windisch, Karl
1908 Ueber das Sincidver Fahren und das Salverfahren zur
Fettbestimmung in der Milch. Milch. Zentbl. 8, 344-52.
86. Woll, F. W.
1906 Report to the Assoc. Off. Agr. Chem. Proc. Asso. Off.
Agr. Chem. 23.
87. Woosnam, R. W.
1897 A Modified Schmid Process. Analyst 22, 91.

A P P E N D I X

Table I. Effect of Replacing Petroleum Ether with Skellysolve "F" when a Slow Shaking Procedure Is Used

Product	Trial No.	Fat Obtained		Difference between Methods percent	Difference between Triplicates			
		Mojonnier percent	Modified percent		Mojonnier		Modified SKF	
					Maximum percent	Average percent	Maximum percent	Average percent
Milk	1	4.1052	4.0709	0.0343	0.0142	0.0094	0.0166	0.0110
	2	3.8294	3.7999	0.0295	0.0038	0.0025	0.0191	0.0127
	3	3.3459*	3.8086*	0.0373*	0.0532*	0.0384*	0.0270	0.0146
	4	5.5316	5.4862	0.0454	0.0280	0.0186	0.0457	0.0304
	5	3.5212	3.5129	0.0083	0.0161	0.0161	0.0157	0.0097
	6	4.4425	4.4043	0.0382	0.0189	0.0126	0.0093	0.0062
	7	3.6383	3.6165	0.0218	0.0183	0.0122	0.0183	0.0122
	8	3.5699*	3.5388*	0.0311*	0.0398*	0.0264*	0.0190	0.0126
	9	5.3737	5.3391	0.0346	0.0247	0.0247	0.0179	0.0109
	10	3.8291	3.3277	0.0014	0.0313	0.0208	0.0226	0.0150
	11	3.2542	3.2259	0.0283	0.0070	0.0046	0.0251	0.0167
	12	3.8980*	3.8605*	0.0375*	0.0348*	0.0232*	0.0175	0.0116
	13	3.4434	3.4077	0.0357	0.0238	0.0158	0.0084	0.0056
	14	5.5720	5.5336	0.0384	0.0208	0.0138	0.0471	0.0314
	15	5.6550	5.6457	0.0093	0.0200	0.0133	0.0308	0.0205
	16	4.3273*	4.2783*	0.0490*	0.0381*	0.0280*	0.0141	0.0094
	17	2.5710*	2.5458*	0.0252*	0.0687*	0.0454*	0.0154	0.0102
	18	4.4746	4.4557	0.0189	0.0145	0.0130	0.0288	0.0156
	19	4.4702*	4.4475*	0.0227*	0.0343*	0.0226*	0.0138	0.0092
	20	3.7040	3.6781	0.0259	0.0065	0.0043	0.0312	0.0218

*Not included in averages.

Table II. Effect of Replacing Petroleum Ether with Skellysolve "F" when a Vigorous Shaking Procedure Is Used

Product	Trial No.	Fat Obtained			Difference between Methods percent	Difference between Duplicates	
		Mojonnier percent	Modified percent	SKF		Mojonnier percent	Modified SKF percent
Milk	21	3.4927	3.9388		0.0039	0.0107	0.0065
	22	4.0904	4.0955		0.0051	0.0154	0.0110
	23	2.9748	2.9710		0.0038	0.0002	0.0032
	24	2.9813	2.9775		0.0038	0.0003	0.0027
	25	2.4156	2.4144		0.0012	0.0050	0.0045
	26	3.3841	3.3775		0.0066	0.0151	0.0153
	27	4.1977	4.1851		0.0126	0.0125	0.0165
	28	3.9953	3.9909		0.0044	0.0201	0.0118
	29	3.4867*	3.5012*		-0.0145*	0.0370*	0.0204
	30	3.7953	3.7803		0.0150	0.0058	0.0098
	31	4.9136	4.9200		-0.0014	0.0194	0.0105
	32	4.2985	4.2896		0.0089	0.0038	0.0203
	33	4.2233	4.2067		0.0166	0.0127	0.0050
	34	3.4957	3.5068		-0.0111	0.0038	0.0035
	35	1.0637	1.0623		0.0014	0.0010	0.0232
Homo-genized Milk	36	3.3844	3.3758		0.0086	-	-
	37	3.8765	3.9886		-0.0121	0.0105	0.0075
	38	3.7816	3.7687		0.0129	0.0138	0.0073
	39	3.7668	3.7644		0.0024	0.0121	0.0094
	40	3.3829	3.3642		0.0187	0.0111	0.0057
	41	3.7255	3.7200		0.0055	0.0098	0.0048
	42	4.5581	4.5535		0.0046	0.0072	0.0169
	43	4.5607	4.5501		0.0106	0.0145	0.0245

Table II. Continued.

Product	Trial No.	Fat Obtained			Difference between Duplicates	
		Mojonnier percent	Modified percent	SKF percent	Mojonnier percent	Modified percent
	44	3.9770	3.8743		0.0027	0.0037
Homo-genized Milk	45	3.8923	3.8843		0.0080	0.0045
	46	3.7059	3.7118		0.0059	0.0018
	47	3.7865	3.7736		0.0129	0.0056
	48	3.9909	3.9902		0.0007	0.0096
	49	4.0324	4.0274		0.0050	0.0013
	50	4.5121	4.5095		0.0026	0.0045
Ice Cream Mix	51	10.4080*	10.2292*	0.1798*	0.0125	0.2670*
	52	10.4467*	10.3607*	0.0860*	0.0299	0.1472*
	53	10.2014*	10.0395*	0.1619*	0.0238	0.1269*
	54	10.9576	10.9248	0.0328	0.0209	0.0314
	55	11.0834	11.0544	0.0290	0.0069	0.0037
	56	0.6503	0.6373	0.0130	0.0046	0.0124
	57	10.4772	10.5279	-0.0507	0.0124	0.0208
	58	10.5534	10.5189	0.0345	0.0353	0.0241
	59	10.5777	10.5910	-0.0133	-	-
	60	10.5835	10.6071	-0.0236	0.0316	0.0097
	61	10.7220	10.6567	0.0653	0.0240	0.0691
	62	10.7479	10.7079	0.0400	0.0085	0.0234
Evapo-rated Milk	63	7.9326	7.9060	0.0266	0.0089	0.0181
	64	7.8833	7.8924	-0.0091	0.0510	0.0044
	65	7.9784	7.9377	-0.0593	0.0170	0.0004
	66	7.8764	7.8747	0.0017	0.0430	0.0118

*Not included in averages.

Table III. Effect of Substituting Skellysolve "A" Entirely for Ethyl Ether when Petroleum Ether Is Used as the Second Solvent

Product	Trial No.	Fat Obtained		Difference between Methods	Difference between Triplicates			
		Mojonnier per cent	Modified SKA percent		Mojonnier		Modified SKA	
					Maximum	Average	Maximum	Average
				percent	percent	percent	percent	percent
Milk	1	3.6556	3.3070	0.3486	0.0086	0.0086	0.0650	0.0433
	2	3.4911	3.2520	0.2391	0.0104	0.0104	0.0638	0.0425
	3	5.2919	4.9188	0.3731	0.0036	0.0036	0.2929	0.1986
	4	5.2567	5.0523	0.2044	0.0069	0.0046	0.0234	0.0156
	5	2.2328	2.1532	0.0796	0.0067	0.0044	0.0322	0.0181
	6	4.5530	4.4117	0.1413	0.0303	0.0202	0.0424	0.0282
	7	4.5472	4.2777	0.2695	0.0115	0.0076	0.0006	0.0006
	8	3.9432	3.6006	0.3426	0.0023	0.0015	0.0713	0.0475
	9	3.9187	3.6083	0.3104	0.0234	0.0156	0.0364	0.0243
	10	4.0106	3.4665	0.5441	0.0177	0.0118	0.2945	0.1963
	11	3.9970	3.5280	0.3690	0.0108	0.0072	0.1235	0.0823
	12	4.0659	3.7779	0.2880	0.0352	0.0234	0.2385	0.1590
	13	4.0999	3.6966	0.3933	0.0248	0.0165	0.0309	0.0462
	14	4.3903	4.0509	0.3294	0.0370	0.0243	0.1538	0.1538
	15	4.0315	3.8715	0.1600	0.0142	0.0094	0.0490	0.0327
	16	3.9317	3.8622	0.1195	0.0138	0.0092	0.0087	0.0087
	17	4.0227	3.6854	0.3373	0.0029	0.0019	0.1326	0.0384
	18	4.0966	4.0241	0.0625	0.0143	0.0095	0.0193	0.0132
	19	4.0250	3.9488	0.0762	0.0013	0.0013	0.0353	0.0235
	20	4.0181	3.9099	0.1082	0.0188	0.0125	0.0282	0.0423
	21	3.1437	3.0478	0.0959	0.0362	0.0241	0.0520	0.0350
	22	3.9127	3.7867	0.1260	0.0397	0.0265	0.0416	0.0277

Table III. Continued.

Product	Trial No.	Fat Obtained		Difference between Methods	Difference between Triplicates			
		Mojonnier percent	Modified SKA percent		Mojonnier		Modified SKA	
				percent	Maximum percent	Average percent	Maximum percent	Average percent
Milk	23	5.2875	5.2479	0.0396	0.0233	0.0155	0.0395	0.0263
	24	3.4747	3.3105	0.1642	0.0126	0.0084	0.0166	0.0111
	25	4.1090	3.8703	0.2387	0.0217	0.0144	0.1144	0.0763
	26	4.1655	4.0904	0.0851	0.0193	0.0128	0.0707	0.0471
	27	4.0739	3.9454	0.1285	0.0243	0.0162	0.0223	0.0145
	28	3.9078	3.8894	0.0184	0.0010	0.0010	0.0532	0.0354
	29	4.1646	4.0872	0.0773	0.0127	0.0085	0.0427	0.0288
	30	3.7715	3.6844	0.0871	0.0300	0.0200	0.0202	0.0134
	31	4.1457	4.0923	0.0534	0.0146	0.0097	0.1316	0.0874
	32	13.8208	0.6084	13.2124	-	0.00114	0.1548	0.1032
Mix	33	11.6327	0.4798	11.1529	-	0.0048	0.1164	0.0776
	34	11.8101	0.9054	10.9047	-	0.0212	0.1388	0.0925

Table IV. Comparison of the Skellysolve "A" Substitution Method and a Mojonnier Method Modified by
Reducing the Quantity of Alcohol Used

Product Sample No.	Fat Obtained				Difference in Extraction				Difference between Duplicates			
	Mojonnier		Modified SKA		Mojonnier		Modified SKA		Mojonnier		Modified SKA	
	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent
Milk	1	4.1014	4.1063	4.0367	0.0049	0.0647	-	0.0036	0.0088			
	2	3.3990	3.8637	3.7655	-0.0353	0.1335	0.0296	0.0137	0.2590			
	3	4.1474	4.1245	4.0594	0.0229	0.0880	0.0065	0.0481	0.0328			
	4	4.1424	4.1424	4.0706	0.0000	0.0718	-	0.0103	0.0085			
	5	4.1201	4.1319	3.4413	0.0118	0.6788	0.0048	0.0380	0.1312			

Table V. The Use of a Proportion of Skellysolve "A" and Ethyl Ether when Skellysolve "F" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	50% Mixture percent		Mojonnier percent	50% Mixture percent
Milk	1	4.0530	3.9895	0.0635	0.0252	0.0305
	2	4.0073	3.9644	0.0429	0.0059	0.0159
	3	4.0029	3.9501	0.0528	0.0178	0.0160
	4	3.5428	3.4645	0.0783	0.0034	0.0050
	5	3.7079	3.5463	0.0616	0.0164	0.0246
	6	3.6156	3.5990	0.0166	0.0162	0.0111
	7	3.9627	3.9364	0.0263	0.0036	0.0039
	8	3.9337*	3.8900*	0.0437*	0.0580*	0.0228
	9	3.8923	3.8365	0.0558	0.0037	0.0043
	10	3.7832	3.7483	0.0349	0.0043	0.0207
	11	3.5383	3.4995	0.0388	0.0012	0.0060
	12	3.4135	3.3804	0.0331	0.0197	0.0028
	13	5.8161	5.7750	0.0411	0.0077	0.0293
	14	3.9748	3.8972	0.0776	0.0018	0.0321
	15	3.7696	3.7350	0.0346	0.0158	0.0260
	16	3.9160*	3.9250*	0.0090*	0.0480*	0.0041
	17	3.7204	3.6840	0.0364	0.0127	0.0025
	18	3.7489	3.7266	0.0223	0.0289	0.0200
	19	3.5939	3.5435	0.0504	0.0027	0.0517
	20	3.9207	3.8267	0.0940	0.0024	0.0456
	21	4.6261	4.5563	0.0698	0.0011	0.0422
	22	3.7525*	3.7439*	0.0086*	0.0523*	0.0253
	23	3.6983	3.6595	0.0388	0.0057	0.0221

Table V. Continued.

Product Tested	Trial No.	Fat Obtained		Difference between Methods percent	Difference between Duplicates	
		Mojonnier percent	50% Mixture percent		Mojonnier percent	50% Mixture percent
Milk	24	4.1680	4.1156	0.0524	0.0125	0.0587
	25	3.7087	3.6427	0.0660	0.0211	0.0629
	26	3.7274	3.7553	0.0321	0.0076	0.0147
	27	3.8607	3.8129	0.0478	0.0088	0.0088
	28	3.3508	3.3219	0.0289	0.0030	0.0049
	29	3.7435	3.6919	0.0516	0.0018	0.0408
	30	3.7983	3.7452	0.0531	0.0093	0.0318
	31	3.3977	3.3604	0.0373	-	0.0243
	32	3.7674	3.7102	0.0572	0.0066	0.0032
	33	4.1500	4.0890	0.0610	0.0037	0.0103
	34	4.0454	4.0104	0.0350	0.0092	0.0505
	35	3.7137	3.6377	0.0760	0.0014	0.0312
	36	3.4263	3.4347	0.0516	0.0024	0.0454
	37	3.7737	3.7089	0.0648	0.0054	0.0119
	38	3.9620	3.9122	0.0498	0.0147	0.0732
Homogen- ized Milk	39	3.8763	3.4476	0.4287	0.0082	0.2475
	40	4.8409	4.5985	0.2424	0.0077	0.1363
	41	4.2625	4.1669	0.0956	0.0037	0.0484
	42	4.1921	4.0646	0.1275	0.0096	0.0076
	43	4.0855	4.0158	0.0697	0.0038	0.0187
	44	4.0886	4.0286	0.0600	0.0176	0.0035
	45	3.9683	3.8398	0.1285	-	0.0195
	46	4.0177	3.9045	0.1132	0.0048	0.0177

Table V. Continued.

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	50% Mixture percent		Mojonnier percent	50% Mixture percent
Homogenized Milk	47	4.1448	4.0351	0.1097	0.0045	0.0558
	48	4.2107	4.0888	0.1215	0.0095	0.0092
Evaporated Milk	49	7.8839	7.8642	0.0297	0.0346	0.0058
	50	7.9378	7.8544	0.0834	0.0238	0.0180
	51	8.2958	8.1957	0.1001	0.0100	0.0037
	52	8.3688*	8.2126*	0.1562*	0.0358*	0.0182
	53	8.2679	8.1333	0.1346	-	0.0473
	54	8.3244	8.1897	0.1347	-	0.0147
Ice Cream Mix	55	12.9125	12.2866	0.6259	0.0241	-
	56	9.9319	9.6986	0.2333	0.0307	-
	57	11.4014	11.2179	0.1835	0.0370	0.0555
	58	11.6112	11.2785	0.3327	0.0302	0.0310
	59	12.2395	11.8559	0.3836	0.0068	-
	60	10.1296	9.9882	0.1414	0.0290	0.0165

* Not included in averages.

Table VI. Effect upon Fat Recovery when Three Extractions of a 1:1 Mixture of Skellysolve "A" and Ethyl Ether Are Used with Skellysolve "F" as Second Solvent

Product Trial Tested No.	Fat Obtained			Difference between Methods			Difference between Duplicates		
	Mojonnier			50/50 Mixture			Mojonnier		
	Method	Regular	3rd	Procedure	Extraction	Regular	3rd	Regular	3rd
								Procedure	Extraction
Milk									
1	3.5364	3.4997	3.5221	0.03670	0.0143	0.0174	0.0043	0.0014	
2	3.7903	3.5949	3.7545	0.0954	0.0358	0.0026	0.0089	0.0223	
3	5.4324*	5.3604*	5.3855*	0.0720	0.0519	0.0388*	-	-	
4	4.1728	4.1231	4.1425	0.0497	0.0303	0.0055	0.0517	0.0539	
5	3.3623	3.3702	3.3757	0.0079	0.0134	0.0202	0.0223	0.0233	
6	4.0797	4.0153	4.0407	0.0644	0.0390	0.0157	0.0474	0.0483	
7	4.4216	4.3657	4.3981	0.0559	0.0235	0.0086	0.0295	0.0124	
8	3.9072	3.8109	3.8711	0.0963	0.0361	0.0098	0.0269	0.0558	
9	3.9592	3.9863	3.9171	0.0729	0.0492	0.0064	0.0833	0.0814	

* Not included in averages.

Table VII. Use of a 2:3 Proportion of Skellysolve "A" and Ethyl Ether when Skellysolve "F" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	40/60 Mixture percent		Mojonnier percent	40/60 Mixture percent
Milk	1	3.4181	3.3628	0.0553	0.0136	0.0172
	2	3.4070	3.3813	0.0257	0.0078	-
	3	3.5017	3.4813	0.0204	0.0154	0.0024
	4	4.1251	4.1010	0.0241	0.0139	0.0089
	5	3.4533	3.4027	0.0506	0.0197	0.0121
	6	5.0084	4.9725	0.0359	0.0144	0.0119
	7	3.8200	3.7764	0.0436	0.0094	0.0077
	8	3.9047	3.7940	0.0107	0.0228	0.0108
	9	1.7224	1.7171	0.0053	0.0067	0.0131
	10	3.2284	3.1962	0.0322	0.0093	0.0321
	11	4.3395	4.3283	0.0112	0.0070	0.0094
	12	4.1610	4.1187	0.0423	0.0154	0.0478
	13	4.0957*	4.0339*	0.0618*	0.0312*	0.0132
	14	3.9775	3.9537	0.0238	0.0004	0.0147
	15	3.5332	3.4972	0.0360	0.0134	0.0226
Homogen-ized Milk	16	3.9562	3.8991	0.0571	0.0087	0.0113
	17	3.6098	3.5724	0.0374	0.0127	0.0322
	18	3.9363	3.9121	0.0242	0.0128	0.0150
	19	3.9930	3.9422	0.0508	0.0075	0.0156
	20	3.9097	3.8638	0.0459	0.0068	0.0349
	21	3.6218	3.5657	0.0561	0.0005	0.0320

Table VII. Continued.

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	40/60 Mixture percent		Mojonnier percent	40/60 Mixture percent
Evapo- ated Milk	22	7.9292	7.8715	0.0557	0.0523	0.0433
	23	7.8992	7.8897	0.0095	0.0053	0.0193
	24	7.9929	7.9141	0.0788	0.0330	-
	25	7.9981	7.9088	0.0893	0.0100	0.0079
	26	8.0247	7.9503	0.0744	0.0051	0.0551
	27	8.0772*	8.0122*	0.0650*	0.0705*	0.0329
Ice Cream Mix	28	11.6374	11.3287	0.3087	0.0237	0.0243
	29	11.4239	11.3452	0.0787	0.0449	0.0930
	30	10.1281	10.0741	0.0540	0.0055	0.0240
	31	11.1384	11.0058	0.1326	0.0318	0.0844
	32	12.9728	12.8695	0.1033	0.0320	0.0163
	33	9.9552	9.8593	0.0959	0.0222	0.0283

* Not included in averages.

Table VIII. The Use of a 1:3 Proportion of Skellysolve "A" and Ethyl Ether when Skellysolve "F" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	25/75 Mixture percent		Mojonnier percent	25/75 Mixture percent
Milk	1	3.4375	3.4020	0.0355	0.0043	0.0141
	2	4.4562	4.4357	0.0205	0.0161	0.0026
	3	5.4054	5.3784	0.0370	0.0044	0.0200
	4	3.1861	3.1510	0.0351	0.0172	0.0020
	5	4.0648	4.0391	0.0257	0.0092	0.0197
	6	3.7009	3.6820	0.0189	0.0067	0.0069
	7	4.6107	4.5838	0.0269	0.0054	0.0150
	8	4.6096	4.5920	0.0176	0.0134	0.0100
	9	3.0447	3.0270	0.0177	0.0032	0.0077
	10	4.5501	4.5295	0.0206	0.0068	0.0098
Homogen-ized Milk	11	4.7853	4.7609	0.0244	0.0089	0.0293
Milk	12	4.7801	4.7788	0.0013	0.0032	0.0065
	13	4.1633	4.1404	0.0229	0.0100	0.0218
	14	4.0976	4.0830	0.0146	0.0068	0.0150
	15	4.7842	4.7623	0.0219	0.0088	0.0355
	16	4.8590	4.8564	0.0026	0.0217	0.0043
	17	4.8768	4.8570	0.0198	0.0091	0.0022
	18	4.5271	4.5025	0.0246	0.0009	0.0316
	19	10.1628	10.1079	0.0549	0.0109	0.0907
Ice Cream Mix	20	10.0223	9.9314	0.0909	0.0141	0.0349
	21	11.0174	10.9709	0.0465	0.0236	0.0027
	22	7.9042	7.8377	0.0665	0.0356	0.0510
Evapo-rated Milk	23	7.9576	7.9180	0.0396	0.0159	0.0069
	24	7.9331	7.9008	0.0323	0.0165	0.0175

Table IX. The Use of a 1:1 Proportion of Skellysolve "A" and Ethyl Ether when Skellysolve "A" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	50/50 Mixture percent		Mojonnier percent	50/50 Mixture percent
Milk	1	2.3552	2.2846	0.0706	0.0038	0.0042
	2	3.9295	3.8913	0.0382	0.0049	0.0095
	3	5.4471	5.4091	0.0380	0.0001	0.0143
	4	4.2105	4.1996	0.0109	0.0204	-
	5	4.7314	4.6772	0.0542	0.0034	0.0329
	6	3.7564	3.7308	0.0256	0.0054	0.0313
	7	3.3326	3.2756	0.0570	0.0049	0.0120
	8	3.3376	3.2751	0.0625	0.0152	0.0191
	9	4.3569	4.3205	0.0364	0.0135	0.0270
	10	3.6689*	3.6622*	0.0067*	0.0337*	0.0106
Homogen-ized Milk	11	3.8735	3.7397	0.0338	0.0060	0.0611
Milk	12	3.8468	3.7381	0.0587	0.0051	0.0622
	13	3.8572	3.7793	0.0779	0.0044	0.0435
	14	3.8513	3.7920	0.0593	0.0122	0.0140
	15	3.8420	3.7306	0.0614	0.0119	0.0195
	16	3.8551	3.7665	0.0386	0.0050	0.0166
	17	3.8436	3.7737	0.0639	0.0045	0.0330
	18	3.8513	3.7338	0.0675	0.0126	0.0116
	19	3.8414	3.7712	0.0702	0.0021	0.0141
	20	3.7370	3.7104	0.0266	0.0203	0.0065
Ice Cream Mix	21	10.5592	10.4763	0.0329	0.0295	0.0098
	22	11.4071	11.2427	0.1644	0.0093	0.0765

Table IX. Continued.

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier	50/50 Mixture		Mojonnier	50/50 Mixture
		Percent	percent	percent	percent	percent
Ice Cream Mix	23	11.4912	11.2585	0.1327	0.0136	-
	24	10.4172	10.4005	0.0167	0.0257	0.1240
	25	11.1567	10.8980	0.2587	0.0091	0.0368
	26	10.6666	10.5455	0.1211	0.0356	0.0341
	27	10.7184	10.5310	0.1874	0.0522	0.0254
	28	12.3937	12.2030	0.1907	0.0136	-
	29	11.4711	11.0544	0.4167	0.0229	0.0065
Evapo-rated Milk	30	8.0659	7.8111	0.2548	0.0224	-
	31	7.9310	7.6313	0.2997	0.0089	0.2911
	32	8.0445	7.7376	0.3069	0.0381	0.1062
	33	7.9228	7.7378	0.1850	0.0050	0.0946
	34	7.9234	7.5784	0.3450	0.0234	0.0330
Butter-milk	35	0.5700	0.2322	0.2978	0.0039	0.0245
	36	0.6059	0.3220	0.2839	0.0090	0.0058
Skim-milk	37	0.1725	0.1654	0.0071	0.0046	0.0056
	38	0.0636	0.0478	0.0258	0.0012	0.0038
	39	0.1873	0.1712	0.0161	0.0004	0.0098

* Not included in averages.

Table X. The Use of a 2:3 Proportion of Skellysolve "A" and Ethyl Ether when Skellysolve "A" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	40/60 Mixture percent		Mojonnier percent	40/60 Mixture percent
Composite Milk Samples	1	3.3945	3.3382	0.0563	0.0141	0.0345
	2	3.4925	3.4396	0.0529	0.0005	0.0051
	3	4.9728	4.8041	0.0687	0.0165	0.0371
	4	3.3523	3.2818	0.0705	0.0058	0.0291

Table XI. The Effect of Using 50 percent of the Standard Quantity of Ethyl Ether when Petroleum Ether Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	Modified percent		Mojonnier percent	Modified percent
Milk	1	3.3885	3.3419	0.0466	0.0018	0.0252
	2	3.6131	3.5695	0.0436	0.0035	0.0363
	3	3.9442	3.8154	0.0288	0.0077	0.0032
	4	3.1951	3.1600	0.0351	0.0046	0.0218
	5	3.7357	3.6982	0.0375	0.0212	0.0465
	6	3.7109	3.6924	0.0185	0.0041	0.0233
	7	3.5461	3.6034	0.0427	0.0041	0.0610
	8	3.3375	3.3141	0.0234	0.0008	0.0079
	9	3.3450	3.3191	0.0259	0.0132	0.0118
Homogen-ized Milk	10	5.0136	4.9644	0.0492	0.0148	0.0130
Milk	11	3.8595	3.8188	0.0407	0.0043	0.0445
	12	3.9678	3.9800	0.0878	0.0105	0.0480
	13	3.9467	3.8217	0.0250	0.0032	0.0109
	14	3.8349	3.8182	0.0167	0.0070	0.0346
	15	3.8060	3.7769	0.0291	0.0069	0.0202
	16	3.8116	3.7926	0.0190	0.0173	0.0295
	17	3.8068	3.7778	0.0290	0.0107	0.0174
	18	3.4716	3.4401	0.0315	0.0025	0.0161
Evapo-rated Milk	19	7.8909	7.8231	0.0678	0.0419	0.0132
	20	7.9088	7.8529	0.0559	0.0194	0.0319
	21	7.8730	7.8322	0.0408	0.0043	0.0099
Ice Cream Mix	22	10.9978	10.9263	0.0715	0.0165	0.0653
	23	10.5079	10.4828	0.0251	0.0426	0.0079
	24	10.6556	10.6460	0.0096	0.0416	0.0363
Butter Milk	25	0.6518	0.6315	0.0203	0.0099	0.0004

Table XII. The Effect of Using 75 percent of the Standard Quantity of Ethyl Ether when Petroleum Ether Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	Modified percent		Mojonnier percent	Modified percent
Milk	1	3.9102	3.3835	0.0267	0.0107	0.0391
	2	3.5409	3.5354	0.0055	0.0028	0.0071
	3	3.8536	3.8380	0.0156	0.0091	-
	4	3.8236	3.8290	0.0054	0.0010	0.0103
Ice Cream Mix	5	10.5470	10.5297	0.0173	0.0110	0.0251
	6	10.4758	10.4762	0.0004	0.0227	-
	7	9.6708	9.6167	0.0541	0.0225	0.0168

Table XIII. The Effect of Using 50 per cent of the Standard Quantity of Ethyl Ether when Skellysolve "A" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	Modified percent		Mojonnier percent	Modified percent
Milk	1	4.2025	4.1819	0.0206	0.0168	0.0058
	2	3.9932	3.9772	0.0160	0.0099	0.0071
	3	3.7237	3.7132	0.0105	0.0049	0.0030
	4	3.7106	3.6866	0.0240	0.0227	0.0054
	5	8.5030	8.4739	0.0291	0.0099	0.0050
	6	1.9246	1.8942	0.0304	0.0139	0.0247
	7	3.6712	3.6769	0.0057	-	0.0039
	8	3.9946	3.9643	0.0303	0.0090	-
Homogenized Milk	9	3.8336	3.8132	0.0204	0.0186	0.0280
	10	4.4970	4.4637	0.0333	0.0141	0.0177
	11	3.9615	3.8370	0.0245	0.0006	0.0211
	12	4.4918	4.4647	0.0271	0.0114	0.0213
	13	4.2957	4.2769	0.0188	0.0255	0.0218
	14	5.1840	5.1640	0.0200	0.0272	-
	15	4.2130	4.1824	0.0306	0.0009	0.0118
	16	4.1719	4.1400	0.0319	0.0178	0.0369
Evapo-rated Milk	17	7.9136	7.8890	0.0246	0.0012	0.0210
	18	7.8934	7.8480	0.0454	0.0468	0.0783
	19	8.1695	7.9368	0.2327	0.0170	0.0003
Ice Cream Mix	20	10.5924	10.4749	0.1175	0.0128	0.0425
	21	10.1332	9.9684	0.1648	0.0347	0.0323
	22	10.9042	10.6880	0.2162	0.0178	0.0348

Table XIV. The Effect of Using 75 percent of the Standard Quantity of Ethyl Ether when Skellysolve "A" Is Used as the Second Solvent

Product Tested	Trial No.	Fat Obtained		Difference between Methods	Difference between Duplicates	
		Mojonnier percent	Modified percent		Mojonnier percent	Modified percent
Milk	1	3.7283	3.7005	0.0278	-	-
	2	3.6534	3.6483	0.0051	0.0045	0.0002
	3	6.2704	6.2738	0.0034	0.0122	0.0114
	4	3.7218	3.7105	0.0113	0.0055	0.0096
Homogenized Milk	5	3.8610	3.8362	0.0248	0.0283	0.0039
	6	3.8508	3.8376	0.0132	0.0258	0.0247
	7	3.8609	3.8513	0.0096	0.0189	0.0050
	8	3.8631	3.8436	0.0195	0.0044	0.0002
	9	3.8640	3.8565	0.0075	0.0098	0.0018
	10	3.8736	3.8550	0.0186	0.0129	0.0112
	11	3.8572	3.8533	0.0039	0.0177	0.0144
	12	3.8670	3.8234	0.0436	0.0052	0.0304
	13	3.8619	3.8498	0.0121	0.0076	0.0039
	14	3.7698	3.7584	0.0114	0.0064	0.0023
Evapo-rated Milk	15	7.9832	7.9655	0.0177	0.0119	0.0154
	16	7.9367	7.8876	0.0491	0.0058	0.0092
	17	7.9192	7.8803	0.0389	0.0024	0.0059
Ice Cream Mix	18	10.0206	9.9338	0.0868	0.0066	0.0500
	19	10.6556	10.6205	0.0351	0.0416	0.0853
	20	10.5079	10.4204	0.0875	0.0426	0.0112

Table XV. Comparison of the Mojonnier Method with the Modified Methods which Used 1:1 Mixture of Ethyl Ether and Skellysolve "A" and 50 per cent less than the Standard Amount of Ethyl Ether in the Analysis of Winter Milk

Trial No.	Fet Obtained		Difference between Methods percent	Difference between Duplicates	
	Mojonnier percent	1:1 Mixture percent		Mojonnier percent	1:1 Mixture percent
1	3.9947	3.8953	0.0994	0.0099	0.0162
2	3.3862	3.7874	0.0988	0.0039	0.0156
3	4.3816	4.3003	0.0813	0.0030	0.0005
4	3.9929	3.8407	0.1522	0.0181	0.0175
5	4.0018	3.8665	0.1353	0.0052	0.0205
6	3.7663	3.5360	0.1303	0.0018	0.0265
7	3.5762	3.4634	0.1128	-	0.0241
8	3.3334	3.7435	0.0899	0.0028	0.0188
9	4.0045	3.8508	0.1437	0.0085	0.0363
10	3.0848	2.9805	0.1043	0.0221	-
11	4.9493	4.8244	0.1249	0.0024	0.0229
12	4.1541	4.0782	0.0759	0.0033	-
13	4.1979	4.0886	0.1093	0.0073	0.0156
14	3.8604	3.7922	0.0682	0.0185	0.0112
15	3.9905	3.9207	0.0698	0.0052	0.0287
16	4.0509	3.9858	0.0651	0.0026	0.0041
17	3.6855	3.6169	0.0686	0.0183	0.0360
18	3.9027	3.8396	0.0631	0.0082	0.0204
19	2.5316	2.4560	0.0756	0.0027	0.0045
20	3.3035	3.2333	0.0702	0.0013	0.0036
21	2.8356	2.7545	0.0811	0.0055	0.0082
22	3.6838	3.5710	0.1128	0.0062	0.0009
23	3.5595	3.5043	0.0552	0.0002	0.0351
24	3.8869	3.8316	0.0553	0.0029	0.0303
25	3.7385	3.6633	0.0752	0.0189	0.0393
26	3.7556	3.6741	0.0815	0.0159	0.0192
27	3.4030	3.3523	0.0507	0.0000	0.0122
28	3.3806	3.3315	0.0491	0.0026	0.0090
29	3.9283	3.8634	0.0649	0.0161	0.0275
30	3.3932	3.7888	0.1044	0.0227	0.0062
31	3.9206	3.8004	0.1202	0.0030	-
32	3.8718	3.7924	0.0794	0.0195	0.0370
33	3.9561	3.8674	0.0887	0.0065	0.0069

Table XV. Continued

Trial No.	Fat Obtained		Difference between Methods percent	Difference between Duplicates	
	Mojonnier percent	Reduced 50% percent		Mojonnier percent	Reduced 50% percent
1	4.7596	4.7081	0.0515	0.0052	0.0042
2	2.5623	2.5318	0.0305	0.0051	0.0219
3	3.6503	3.6088	0.0415	0.0040	0.0205
4	4.0072	3.9684	0.0388	0.0118	0.0104
5	3.7509	3.7110	0.0399	0.0054	0.0462
6	3.3553	3.3293	0.0260	0.0025	0.0064
7	3.8086	3.6879	0.1207	0.0098	0.0328
8	3.3988	3.7973	0.1015	0.0064	0.0291
9	3.7549	3.6477	0.1072	0.0135	0.0068
10	3.5948	3.4869	0.1179	0.0047	0.0136
11	4.9167	4.9124	0.1043	0.0038	0.0281
12	4.0344	3.9761	0.0583	0.0136	0.0140
13	3.5938	3.5508	0.0430	0.0172	0.0095
14	3.3943	3.3248	0.0695	0.0095	0.0111
15	1.9704	1.9281	0.0423	0.0312	0.0272
16	5.6465	5.5926	0.0539	0.0016	-
17	5.0725	4.9798	0.1073	0.0065	0.0030
18	3.5826	3.4980	0.0846	0.0117	-
19	3.4995	3.4189	0.0806	0.0208	0.0028
20	3.6391	3.5302	0.1089	0.0129	0.0092
21	5.5798	5.4596	0.1202	0.0094	0.0396
22	3.9947	3.9869	0.0078	0.0099	0.0070
23	3.9862	3.8528	0.0334	0.0039	0.0100
24	4.3816	4.3358	0.0458	0.0030	-
25	3.3929	3.9502	0.0427	0.0181	0.0056

Table XVI. Comparison of the Mojonnier Method with a Modified Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A" and a Method Employing the Same Mixture with a 60 Second Shaking Period

Product No.	Trial	Fat Obtained			Deviation from Mojonnier	
		Mojonnier percent	Modified percent	Modified with 60 sec. shaking percent	Modified percent	Modified with 60 sec. shaking percent
Milk	1	3.6855	3.6169	3.6530	0.0686	0.0325
	2	3.9027	3.8396	3.8590	0.0631	0.0437
	3	2.5316	2.4560	2.4886	0.0756	0.0430
	4	3.3035	3.2333	3.2449	0.0702	0.0586
	5	2.8356	2.7545	2.8255	0.0811	0.0101
	6	3.6838	3.5710	3.5666	0.1128	0.0172
	7	3.5595	3.5043	3.5339	0.0552	0.0556
	8	3.9869	3.8316	3.8456	0.0553	0.0413
	9	4.0018	3.8665	3.9480	0.1353	0.0538
	10	3.7663	3.6360	3.7197	0.1303	0.0466
	11	3.8334	3.7435	3.7562	0.0899	0.0772

Table XVII. Comparison of the Mojonnier Method with a Modified Method which Employs Reduced Quantities of Ethyl Ether and a Method Using the Same Modification with a 60 Second Shaking Period

Product No.	Trial	Fat Obtained			Deviation from Mojonnier	
		Mojonnier	Modified	Modified with 60 sec. Shaking	Modified	Modified with 60 sec. Shaking
		percent	percent	percent	percent	percent
Milk	1	4.7596	4.7081	4.7145	0.0515	0.0451
	2	2.5623	2.5318	2.5365	0.0305	0.0258
	3	3.6503	3.6088	3.6201	0.0415	0.0302
	4	4.0072	3.9684	3.9697	0.0388	0.0375
	5	3.7509	3.7110	3.7297	0.0399	0.0212
	6	3.8553	3.8293	3.8504	0.0260	0.0049

Table XVIII. Comparison of the Mojonnier Method with the Modified Method which Utilizes a 1:1 Mixture of Ethyl Ether and Skellysolve "A" in the Analysis of Milk which Has Been Treated to 100° F. for a Few Minutes Immediately before Testing

Trial No.	Fat Obtained			Deviation from Mojonnier	
	Mojonnier percent	Regular Modified percent	Modified plus Heat percent	Regular Modified percent	Modified plus Heat percent
1	3.7385	3.6633	3.6656	0.0752	0.0729
2	3.7556	3.6741	3.6992	0.0815	0.0564
3	3.4030	3.3523	3.3523	0.0507	0.0507
4	3.3806	3.3315	3.2804	0.0491	0.1002
5	3.9283	3.8634	3.8968	0.0649	0.0315
6	3.8932	3.7888	3.8238	0.1044	0.0694
7	3.9206	3.8004	3.8699	0.1202	0.0507
8	3.8718	3.7924	3.8345	0.0794	0.0372

Table XIX. Comparison of the Mojonnier Method with Two Modified Methods which Use Reduced Quantities of Ethyl Ether and a 1:1 Mixture of Ethyl Ether and Skellysolve "A" in the Analysis of Low-Fat Products

Product	Trial No.	Fat Obtained			Deviation from Mojonnier	
		Mojonnier percent	Mixture percent	Reduced 50% percent	Mixture percent	Reduced 50% percent
Churned Buttermilk	1	0.8517		0.6315		0.0202
	2	0.6281		0.6088		0.0193
	3	0.6237		0.6175		0.0062
	4	0.6281	0.3866		0.2415	
	5	0.5700	0.2821		0.2879	
	6	0.6060	0.3220		0.2840	
Skimmilk	7	0.1725	0.1654		0.0071	
	8	0.0634	0.0478		0.0156	
	9	0.1873	0.1712		0.0161	
Powdered Buttermilk	10	5.898	4.777	5.518	1.121	0.380
	11	5.925	4.904	5.503	1.021	0.422
Powdered Skimmilk	12	0.770	0.524	0.0450	0.246	0.320
	13	0.7713	0.4695	0.5266	0.3018	0.2447

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