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ANALYSIS

OF

MILK AND MILK PRODUCTS

BY

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THIRD EDITION, REVISED AND ENLARGED
WITH ILLUSTRATIONS

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PREFACE TO THIRD EDITION

This book is intended as a concise manual of the chemical examinations of commercial milk and milk-products. Only processes of practical utility are described. Nothing has been said concerning the food-value of milk or its products. Much text has been used from the second edition of "Select Methods in Food Analysis." I am under obligation to P. Blakiston's Son & Company for permission to use this matter and also for the loan of several cuts.

Since the second edition appeared much change has occurred in some departments of milk analysis, while others have undergone but little modification. The most noticeable changes are in regard to the detection of preservatives. Several recently discovered and useful tests for formaldehyde will be found in the following pages, also convenient tests for abrastol and benzoates. Cochran's method for fat determination will be found to be a useful addition to the facilities for examining condensed milk and infant foods.

Unless otherwise stated all degrees are centigrade and all readings of scale and arc are positive.

I desire to place again on record a protest as to the statements occasionally made concerning the rapid process for determining fat in milk by means of the mixture of amyl alcohol and hydrochloric acid. Much misrepresentation has been current in regard to it. A letter is in my possession which shows that some American chemists have misled foreign chemists in regard to the priority of discovery. The Gerber method

is obviously a mere modification, yet in the bulletin "*Provisional Methods of Food Analysis*," issued by the U. S. Department of Agriculture, the former method is given as if it were original with Gerber, but the authors of that bulletin cannot be unaware of the facts of the case.

PHILADELPHIA, *June*, 1905

CONTENTS

MILK AND MILK PRODUCTS

MILK

Milk, the nutritive secretion of nursing mammals, consists of water, fat, proteids, sugar, and mineral matters. Cow's milk is meant in all cases, unless otherwise stated.

Fat.—This occurs in globules varying from 0.0015 mm. to 0.005 mm. in diameter, in a condition which prevents spontaneous coalescence. It is peculiar among animal fats in containing a notable proportion of acid radicles with a small number of carbon atoms.

Proteids.—The nature of the proteids of milk has been much discussed, but it is now generally conceded that there are at least three forms, casein, albumin, and globulin, the casein being present in by far the greatest amount, and the globulin as traces only.

CASEIN.—Casein is, probably in part, in combination with phosphates. It is precipitated by many substances, among which are acids, rennet, and magnesium sulfate, but not by heat. Acids precipitate it by breaking up the combination with phosphates. The action of rennet is complex and probably partly hydrolytic, splitting the casein into several proteids, some of which are precipitated in the curd. Films of proteid matter occur abundantly in milk, for which reason it is distinctly opaque, even when nearly all the fat has been removed by centrifugal action.

The *albumin* of milk appears to be a distinct form, and is called lactalbumin. It is not precipitated by dilute acids, but is coagulated by heating to 70°—75°. The proportion in cow's milk is usually from 0.35 to 0.50 per cent., but colostrum may contain much larger proportions.

Globulin is present only in minute amounts in normal milk,

but colostrum may contain as much as 8 per cent. It is coagulated on heating.

Lactose.—This is a sugar peculiar to milk.

Citric acid is a normal constituent of the milk of various animals. In human milk, the quantity is about 0.5 gram to the liter; in cow's milk, from 1 to 1.5 grams. It is not dependent on the citric acid present in the food.

Wender states that the following enzyms exist in normal milk:

Milk trypsin or galactase. This is a proteolytic enzym. It dissolves casein and is rendered inactive by exposure to a temperature of 76°.

Milk-catalase. This can decompose hydrogen dioxid and similar compounds. It is rendered inactive by exposure to a temperature of 80°.

Milk-peroxydase, an anaerobic oxydase, that is, a body that has the power to decompose peroxids and carry the oxygen over to other substances. This is the substance which produces the reaction when milk, hydrogen dioxid and tincture of guaiacum are mixed, by which a deep blue is obtained. This enzym is rendered inactive by exposure to a temperature of 83°.

Minute amounts of *nitrogenous bases* occur in milk.

Mineral Matter.—The ash of milk contains calcium, magnesium, iron, potassium, and sodium as chlorids, carbonates, sulfates, and phosphates. It does not exactly represent the salts present in milk.

Richmond has determined the ratio of the ash to the solids not fat of 135 samples of milk. This was found to range from 7.8 to 9.4 per cent., but more usually from 7.8 to 8.5 (average 8.2) per cent. Many ashes were alkaline to turmeric, litmus, and phenolphthalein, the maximum alkalinity being 0.025 per cent. calculated as sodium carbonate.

The following table gives the approximate composition of some milks. Analyses of the milks of less important animals have been published, but the figures are of uncertain value, be-

cause it is not sure that the samples were of average character or the methods of analysis accurate:

	Human.	Cow.	Mare.	Goat.	Ass.	Gamoose.
Fat,	3.5	4.0	1.1	4.3	1.6	5.6
Sugar,	6.8	4.8	6.6	4.0	6.1	5.4
Proteids,	1.5	3.5	1.9	4.6	2.2	3.8
Ash,	0.2	0.7	0.3	0.6	0.5	1.0
	12.0	13.0	9.3	13.5	10.4	15.8

Normal milk is an opaque white or yellowish-white fluid, with an odor recalling that of the animal, and a faint sweet taste. The opacity is due largely but not entirely to the fat globules. The reaction of freshly drawn milk to litmus is usually alkaline, but is sometimes amphoteric; that is, it turns the red paper blue and the blue paper red. The specific gravity varies between 1.027 and 1.035. It usually undergoes a gradual augmentation (sometimes termed Recknagel's phenomenon) for a considerable time after the sample has been drawn. The increase may amount to two units (water being 1000). The specific gravity becomes stationary in about 5 hours if the milk be maintained at a temperature below 15°, but at a higher temperature it may require 24 hours to acquire constancy. The change is not entirely dependent on the escape of gases.

Unless collected with special care and under conditions of extreme cleanliness, milk always contains many bacteria and animal matter of an offensive character, such as epithelium, blood and pus cells, particles of feces, and soil.

At ordinary temperature milk soon undergoes decomposition, by which the milk sugar is converted principally into lactic acid, and the proteids partly decomposed and partly coagulated. The liquid becomes sour and the fat is inclosed in the coagulated casein. In the initial stages of decomposition the proteids frequently undergo transformations into substances which are the cause of the violent poisonous effects occasionally produced by ice-cream and other articles of food into the preparation of which milk enters.

Boiling produces coagulation of the albumin, some caramelization of the sugar, and develops a greater facility of coalescence on the part of the fat globules. Enzyms are rendered inert and most microbes are killed.

When milk is allowed to stand, some of the fat rises gradually and forms a rich layer, constituting cream. The proportion of cream depends on several conditions. The amount formed in a given time cannot be taken as a measure of the richness of the milk. Water added to milk causes a more rapid separation of the cream. Centrifugal action separates nearly all of the fat. The following figures, given by D'Hout as averages, show this effect:

	Whole Milk.	Separated Milk.	Cream.
Specific gravity,	1032	1034	1015
Total solids,	14.10	9.6	26.98
Sugar,	4.70	5.05	3.32
Casein,	3.50	3.62	2.02
Ash,	0.79	0.78	0.58
Fat,	5.05	0.20	21.95

Buttermilk is the residue after removal of the butter by churning. Vieth gives the following analyses:

Total Solids.	Fat.	Solids not Fat.	Ash.
9.03	0.63	8.40	0.70
8.02	0.65	7.37	1.29
10.70	0.54	10.16	0.82

Whey or *Milk-serum* is the liquid freed from curd after precipitation by rennet or acids. In most cases it contains a notable amount of proteids, as shown in the following analyses by Cochran:

Milk		Whey.	
Total solids.	Solids not fat.	Total solids.	Proteids removed.
9.27	9.13	6.62	2.51
9.27	9.13	6.1	3.03
14.05	8.35	6.62	2.33
7.71	7.61	5.98	1.63
8.91	8.71	6.50	2.21

The whey of any given milk has practically the same composition, whether taken from the original milk, skimmed milk, or cream.

Average Proportion of Solids in Milk.—The most extensive data on this point are those obtained by Vieth. The total number of samples was 120,540. The averages of the entire series are as follows:

Fat,	4.1 per cent.
Non-fatty solids,	8.8 "
Total solids,	12.9 "

Richmond's results for several years have confirmed these figures.

Seasonal Variations in the Composition of Milk.—The poorest quality usually occurs during the first half of the year, especially in April. A low figure is also frequently noted about July. In autumn the quality rises, being highest in October and November.

Deficient Solids.—The following are some instances of deficiency of solids in milk known to be genuine:

Sp. Gr.	Fat.	S. N. F.	Total Solids.	Analyst.
1029.6	3.38	7.95	11.33	Cochran.
1030.0	3.62	8.31	11.93	Cochran.
1029.3	3.63	8.02	11.65	Cochran.
...	3.99	8.36	12.35	Leffmann and Beam.
...	3.11	8.33	11.44	Monthly averages N. J. State Agricultural Exp. Station.
...	3.05	8.33	11.38	
...	3.23	8.44	11.67	

The following analyses of milk from individual cows were made by Cochran. The samples were taken under precautions which insured their genuineness. The data are all direct determinations. The total solids were obtained by drying in the usual manner, and the fat by the L-B. method. Low milks have been often noted in the vicinity of Philadelphia.

Sp. Gr.	Fat.	S. N. F.	Total Solids.
1026.6	2.35	6.78	9.13
1028.8	2.95	7.56	10.51
1028.8	2.40	7.56	9.96
1033.5	2.90	8.68	11.58

The mixed milk from a herd of any considerable number will rarely, if ever, show a proportion of non-fatty solids less than 8.5 per cent. nor less than 3.5 per cent. of fat. Cochran examined the milk from each cow of a herd of 59, with the following results:

Fat,.....2.60 to 5.40.
Total solids,.....9.86 to 13.78.

The average milk of the entire herd was:

Fat,..... 3.76 per cent.
Total solids,.....12.33 per cent.

The average of nearly 100 determinations at the University of Wisconsin creamery during a protracted drought in 1895 gave but a trifle over 8.5 per cent. solids not fat. The casein was low in this milk, while the sugar was about normal in amount. Similar conditions have been observed by Van Slyke at the New York station.

Richmond states that when the non-fatty solids of genuine whole milk are low, the deficiency is principally in the milk sugar.

Colostrum.—This is the secretion in the early stages of lactation, and differs from ordinary milk. It contains characteristic structures, known as colostrum corpuscles, and usually contains much less fat than fully developed milk, but a larger proportion of proteids. Colostrum coagulates on boiling. Lactose is in small amount.

U. S. Standard.

Milk (whole milk) is the lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before and

five days after calving, and contains not less than 12 per cent. of total solids, not less than 8.5 per cent. of solids not fat, and not less than 3.25 per cent. of milk fat.

Blended milk is milk modified in its composition so as to have a definite and stated percentage of one or more of its constituents.

Skim milk is milk from which a part or all of the cream has been removed and contains not less than 9.25 per cent. of milk solids.

Analytic Processes.

As already noted, the specific gravity of milk rises gradually for some time after it has been drawn, and the determination is to be made only after this action has ceased. This will require about 5 hours after the milk is drawn, if it has been kept below 15°, but at a higher temperature it will be necessary to allow at least 12 hours. For all other determinations the milk must be analyzed as soon as possible. The following figures, published by Bevan, show that a considerable loss in total solids may occur in 24 hours:

	Total Solids.	Loss.
Evaporated immediately,	11.73	
Evaporated after 24 hours,	10.79	0.94
Evaporated after 48 hours,	10.38	1.35
Evaporated after 120 hours,	9.42	2.31

The decomposition is very irregular, and it is not possible to determine, by estimation of the lactic acid or other products, the original composition of the milk. The pipet used for taking a portion for analysis should have a wide opening, that no cream may be retained when the pipet is discharged.

When rigid accuracy is not essential, it will suffice to measure the portions of milk taken for the determinations. Vieth uses a pipet graduated to deliver 5 grams, and finds that, working with whole and skimmed milk, under the ordinary variations of temperature, the error will not exceed 0.1 on the total solids and is less on the fat.

A good plan is to use a 5 c.c. pipet and to wash out that which adheres to the glass with a little water. The specific gravity of the milk being known, the amount taken can be calculated. The milk should be as near 15.5° as possible.

Specific Gravity.—Air-bubbles are held rather tenaciously by milk, and care must be taken in mixing, preparatory to taking the specific gravity, to avoid as far as possible the inclosure of the air, and to allow sufficient time for the escape of any bubbles that may be present. The specific gravity of milk

Find the temperature of the milk in one of the horizontal lines and the specific gravity in the first vertical column. In the same line with this and the temperature the corrected specific gravity is given.

°F.	50	51	52	53	54	55	56	57	58	59	60	61	62
Sp. Gr.													
21	20.2	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2
22	21.2	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2
23	22.2	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2
24	23.2	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2
25	24.1	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2
26	25.1	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2
27	26.1	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.3
28	27.0	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.3
29	28.0	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.3
30	29.0	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3
31	29.9	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.8	30.9	31.0	31.2	31.3
32	30.9	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.2	32.3
33	31.8	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.2	33.3
34	32.7	32.9	33.0	33.1	33.2	33.3	33.5	33.6	33.7	33.9	34.0	34.2	34.3
35	33.6	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.0	35.2	35.3
°C.	10	10.5	11.1	11.6	12.2	12.7	13.3	13.8	14.4	15.0	15.5	16.1	16.6

is understood to be taken at 15.5°; samples should be brought near to this. If at a few degrees above or below, it will suffice to make the determination at once and obtain the correct figure by reference to the annexed table. The specific gravity of normal milk varies between 1.028 and 1.035. The figure alone does not indicate the character of the sample, but taken in conjunction with the figure for fat or for total solids, it is of value as a check on the results furnished by other determinations.

The simplest method of determining specific gravity is by

Find the temperature of the milk in one of the horizontal lines and the specific gravity in the first vertical column. In the same line with this and the temperature the corrected specific gravity is given.

63	64	65	66	67	68	69	70	71	72	73	74	75
21.3	21.4	21.5	21.6	21.7	21.8	22.0	22.1	22.2	22.3	22.4	22.5	22.6
22.3	22.4	22.5	22.6	22.7	22.8	23.0	23.1	23.2	23.3	23.4	23.5	23.7
23.3	23.4	23.5	23.6	23.7	23.8	24.0	24.1	24.2	24.3	24.4	24.6	24.7
24.3	24.4	24.5	24.6	24.7	24.9	25.0	25.1	25.2	25.3	25.5	25.6	25.7
25.3	25.4	25.5	25.6	25.7	25.9	26.0	26.1	26.2	26.4	26.5	26.6	26.8
26.3	26.5	26.6	26.7	26.8	27.0	27.1	27.2	27.3	27.4	27.5	27.7	27.8
27.4	27.5	27.6	27.7	27.8	28.0	28.1	28.2	28.3	28.4	28.6	28.7	28.9
28.4	28.5	28.6	28.7	28.8	29.0	29.1	29.2	29.4	29.5	29.7	29.8	29.9
29.4	29.5	29.6	29.8	29.9	30.1	30.2	30.3	30.4	30.5	30.7	30.9	31.0
30.4	30.5	30.7	30.8	30.9	31.1	31.2	31.3	31.5	31.6	31.8	31.9	32.1
31.4	31.5	31.7	31.8	32.0	32.2	32.2	32.4	32.5	32.6	32.8	33.0	33.1
32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.4	33.6	33.7	33 9	34.0	34.2
33.5	33.6	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.1	35.2
34.5	34.6	34.8	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.0	36.1	36.3
35.5	35.6	35.8	35.9	36.1	36.2	36.4	36.5	36.7	36.8	37.0	37.2	37.3
17.2	17.7	18.3	18.8	19.4	20	20.5	21.1	21.6	22.2	22.7	23.3	23.8

the *lactometer*, a delicate and accurately graduated hydrometer. The instrument must be immersed carefully so as not to wet the stem above the point at which it will rest. Its accuracy should be verified by immersion in distilled water at 15.5° and milks of known specific gravity.

More accurate determinations may be made with a balance. A special form, the Westphal balance, is adapted to the determination of specific gravity only, the weights being so arranged that a simple enumeration of them gives the gravity directly. The cheap forms of this instrument are not satisfactory, but some made by German houses are excellent. The ordinary analytic balance may also be used. A plummet consisting of a thick glass rod or short sealed tube, weighted with mercury, of a bulk of about 10 c.c. is suspended from the hook of the balance by means of fine platinum wire and the weight ascertained. It is then submerged in distilled water and the weight also noted. The water is contained in a narrow upright cylinder resting on a bench or support above the scale pan. The loss of weight of the plummet is, of course, the weight of the bulk of water that it displaces. The specific gravity of any sample can be determined by weighing the plummet immersed in the sample and dividing the loss in weight by the loss in water. The quotient is the specific gravity.

The specific gravity bottle is not convenient for milk on account of the liability of the upper layer of the liquid to be richer in fat than the lower; the overflow, therefore, does not represent the mixture.

Total Solids.—This determination may often be made with sufficient accuracy for practical purposes by evaporating a measured volume (*e. g.*, 3 or 5 c.c.) in a shallow nickel dish from 5 to 8 cm. in diameter. Nickel crucible-covers are suitable. The thin glass (Petri) dishes used for microbe culture are convenient. When greater accuracy is required, and especially when the ash is to be determined, platinum dishes must be

used. Satisfactory results may be secured by the following simple method: A flat platinum dish, 3.5 cm. in diameter, with sides 0.5 cm. high, is provided with a thin flat watch-glass cover that fits rather closely. The total weight of the cover and dish is noted. 2 or 3 c.c. of the sample are run into the dish from the pipet, the watch-glass placed on, and the weight taken as rapidly as possible. The glass prevents appreciable loss from evaporation during an ordinary weighing. The cover is removed, the dish heated on the water-bath or in the water-oven, and weighed from time to time (with cover on it) until the weight is sensibly constant. The percentage of residue can be easily calculated. About three hours may be required to secure constant weight.

The A. O. A. C. method is: Heat at 100° to constant weight, about 3 grams in a tared platinum, aluminum or tin dish of 5 cm. diameter, with or without the addition of 15 to 30 grams of sand. Cool and weigh.

The use of aluminum or tin as substitutes for platinum is inadvisable; much better results will be obtained with nickel, porcelain or glass.

Ash.—The residue from the determination of total solids is heated cautiously over the Bunsen burner, until a white ash is left. The result obtained in this manner is apt to be slightly low from loss of sodium chlorid. This may be avoided by heating the residue sufficiently to char it, extracting the soluble matter with a few cubic centimeters of water, and filtering (using paper extracted with hydrofluoric acid). The filter is added to the residue, the whole ashed, the filtrate then added, and the liquid evaporated carefully to dryness. The ash of normal milk is about 0.7 per cent. and faintly alkaline. A marked degree of alkalinity and effervescence with hydrochloric acid will suggest the addition of a carbonate.

The method of the A. O. A. C. is as follows: In a weighed dish put 20 c.c. of milk from a weighing bottle; add 6 c.c. of

nitric acid, evaporate to dryness, and burn at a low red heat till the ash is free from carbon.

Fat.—Many methods for fat determination have been devised. The following will suffice for all practical work:

Adams' Method.—This consists essentially in spreading the milk over absorbent paper, drying, and extracting the fat in an extraction apparatus; the milk is distributed in an extremely thin layer, and by a selective action of the paper the larger portion of the fat is left on the surface. A paper, manufactured especially for this purpose by Schleicher & Schuell, is obtainable in strips of suitable size. Each of these yields to ether only from 0.001 to 0.002 gram of extract.

Coils made of thick filter-paper, cut into strips 6 by 62 cm., are thoroughly extracted with ether and alcohol, or the weight of the extract corrected by a constant obtained for the paper. From a weighing bottle about 5 grams of the milk are transferred to the coil by means of a pipet, care being taken to keep dry the end of the coil held in the fingers. The coil is placed, dry end down, on a piece of glass and dried for one hour, preferably in an atmosphere of hydrogen; it is then transferred to an extraction apparatus and extracted with absolute ether, petroleum spirit of boiling-point about 45° or, better, carbon tetrachlorid. The extracted fat is dried and weighed.

The above procedure is very satisfactory, but the drying in hydrogen may usually be omitted. After the coil has received at least twenty washings, the flask is detached, the ether removed by distillation, and the fat dried by heating in an air-oven at about 105°, and occasionally blowing air through the flask. After cooling, the flask is wiped with a piece of silk, allowed to stand ten minutes, and weighed.

Richmond states that to perform a rigidly accurate determination attention to the following points is necessary: The ether must be anhydrous (drying over calcium chlorid and distilling is sufficient). Schleicher & Schuell's fat-free papers

should be used, and one should be extracted without any milk on it, as a tare for the others. Four or five hours' extraction is necessary, and the coils should be well dried before extraction is begun.

Thimble-shaped cases made of fat-free paper are now obtainable and are convenient for holding the absorbent material on which the milk is spread. The fine texture prevents undissolved matter escaping. A case may be used repeatedly. Sour milk may be thinned with ammonium hydroxid before taking the portion for analysis.

Babcock Asbestos Method.—This is recommended by the A. O. A. C.: Provide a hollow cylinder of perforated sheet metal 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disk of the same material. The perforations should be about 0.7 mm. in diameter and 0.7 mm. apart. Fill the cylinder loosely with from 1.5 to 2.5 grams of freshly ignited woolly asbestos free from fine or brittle material. Cool in a desiccator and weigh. Introduce a weighed quantity of milk (about 4 grams) and dry at 100°. The cylinder is placed in the extraction tube and extracted with ether in the usual way. The ether is evaporated and the fat weighed. The extracted cylinder may be dried at 100° and the fat checked by the loss in weight. A higher degree of accuracy is secured by performing the drying operation in hydrogen.

For thorough extraction, especially with difficultly soluble materials and volatile solvents, the continuous extraction apparatus devised by Szombathy, but commonly called the Soxhlet tube, is most suitable.

The apparatus, as shown in figure 1, is provided with a globular metal condenser, but any form may be employed. The material may be placed in a fat-free paper thimble and covered with a plug of cotton to prevent loss of fine particles. In place of the cotton plug, a Gooch crucible may be used, as shown in the cut. The top of the thimble should be a short

distance below, and the top of the crucible a short distance above, the bend of the siphon. The thimble should be supported by a section of glass tubing, 1 to 2 cm. long, with rounded edges; the edge on which the thimble rests should be a little uneven to prevent a close joint, which would hinder the siphoning of some of the liquid.

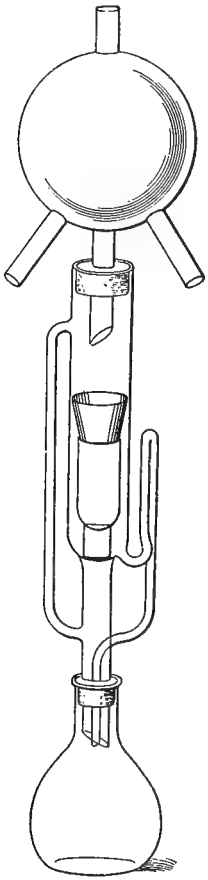

FIG. 1.

Another method is to use a glass tube open at both ends, the material to be extracted being held in position by loose plugs of cotton placed above and below.

Loss of solvent by leakage often occurs. It may be diminished somewhat by soaking the corks in rather strong hot gelatin solution, draining them quickly and then exposing them for some hours to formaldehyd vapor.

The solvents most generally employed are ether and petroleum spirit, but carbon tetrachlorid is well adapted for extraction purposes as it has high solvent power for fats and is not easily inflammable.

When extraction is completed, the carton and materials may be removed from the tube, and, replacing the parts of the apparatus, much of the solvent may be redistilled into the extractor, thus recovering the liquid. Care must be taken not to distil the contents of the flask closely or heat strongly, lest some of the more volatile of the dissolved matters pass into the distillate.

Werner-Schmid Method.—This is suitable for sour milk and for sweetened condensed milk. 10 c.c. of the milk are measured into a long test-tube of 50 c.c. capacity, and 10 c.c. of strong hydrochloric acid added, or the milk may be weighed in a small beaker and washed into the tube with the acid. After mixing, the liquid is boiled 1½ minutes, or the tube may be corked and heated in the water-bath from 5 to 10 minutes, until the liquid turns dark brown. It must not be allowed to turn black. The tube and contents are cooled in water, 30 c.c. of *well-washed* ether added, shaken, and allowed to stand until the line of acid and ether is distinct. The cork is taken out, and a double-tube arrangement, like that of the ordinary wash-bottle, inserted. The stopper of this should be of cork and not of rubber, since it is difficult to slide the glass tube in rubber, and there is a possibility, also, of the ether acting on the rubber and dissolving it. The lower end of the exit-tube is adjusted so as to rest immediately above the junction of the two liquids. The ethereal solution of the fat is then blown out and received in a weighed flask. Two more portions of ether, 10 c.c. each, are shaken with the acid liquid, blown out, and added to the first. The ether is then distilled off and the fat dried and weighed as above.

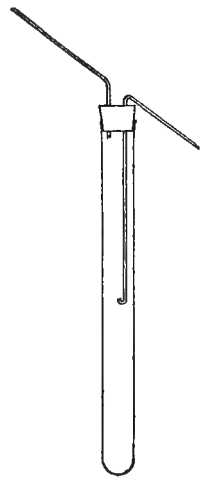

FIG. 2.

Centrifugal Methods.—Among the processes for the rapid determination of fat, those employing centrifugal action have been found most convenient. The following method, devised by Leffmann & Beam in 1889, has proved satisfactory on the score of accuracy, simplicity, and ease of manipulation. This process, which antedates in its successful operation

and public exhibition all the rapid centrifugal methods except the De Laval, is sometimes called the "Beimling" method, but Beimling was merely a patentee of a crude form of centrifugal machine, and had no part in devising the mixture for freeing the fat. The distinctive feature is the use of fusel oil, the effect of which is to produce a greater difference in surface tension between the fat and the liquid in which it is suspended, and thus promote its readier separation. This effect has been found to be heightened by the presence of a small amount of hydrochloric acid.

The test-bottles have a capacity of about 30 c.c. and are provided with a graduated neck, each division of which represents 0.1 per cent. by weight of butter fat.

15 c.c. of the milk are measured into the bottle, 3 c.c. of a mixture of equal parts of amyl alcohol and strong hydrochloric acid added, mixed, the bottle filled nearly to the neck with concentrated sulfuric acid, and the liquids mixed by holding the bottle by the neck and giving it a gyratory motion. The neck is now filled to about the zero point with a mixture of sulfuric acid and water prepared at the time. It is then placed in the centrifugal machine, which is so arranged that when at rest the bottles are in a vertical position. If only one test is to be made, the equilibrium of the machine is maintained by means of a test-bottle, or bottles, filled with a mixture of equal parts of sulfuric acid and water. After rotation for from one to two minutes, the fat will collect in the neck of the bottle and the percentage may be read off. It is convenient to use a pair of dividers in making the reading. The legs of these are placed at the upper and lower limits respectively of the fat, allowance being made for the meniscus; one leg is then placed at the zero point and the reading made with the other. Experience by analysts in various parts of the world has shown that with properly graduated bottles the results are reliable. As a rule, they do not differ more than 0.1 per cent. from

those obtained by the Adams process, and are generally even closer.

For great accuracy, the factor for correcting the reading on each of the bottles should be determined by comparison with the figures obtained by the Adams or other standard process.

Cream is to be diluted to exactly ten times its volume, the specific gravity taken, and the liquid treated as a milk. Since in the graduation of the test-bottles a specific gravity of 1.030 is assumed, the reading must be increased in proportion.

A more accurate result may be obtained by weighing in the test-bottle about 2 c.c. of the cream and diluting to about 15 c.c. The reading obtained is to be multiplied by 15.45 and divided by the weight in grams of cream taken.

The mixture of fusel oil and hydrochloric acid seems to become less satisfactory when long kept. It should be clear and not very dark in color. It is best kept in a bottle provided with a pipet which can be filled to the mark by dipping. Rigid accuracy in the measurement is not needed.

See also *Cochran's method* under "Condensed Milk."

Calculation Methods.—Several investigators have proposed formulæ by which when any two of the data, specific gravity, fat, and total solids, are known, the third can be calculated. These vary according to the method of analysis employed. That of Hehner and Richmond, as corrected by Richmond, was deduced from results by the Adams method of fat extraction, and has been found to be the most satisfactory. It is as follows:

$$T = 0.25\ G + 1.2\ F + 0.14;$$

in which T is the total solids, G the last two figures of the specific gravity (water being 1000), and F the fat. A table based upon this formula is annexed.

A formula has been devised by Richmond by which the lac-

TABLE FOR CALCULATION METHOD

Sp. Gr.	Fat.																			
	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9
27.0	10.49	10.61	10.73	10.85	10.97	11.09	11.21	11.33	11.45	11.57	11.69	11.81	11.93	12.05	12.17	12.29	12.41	12.53	12.65	12.77
0.5	10.62	10.74	10.86	10.98	11.10	11.22	11.34	11.46	11.58	11.70	11.82	11.94	12.06	12.18	12.30	12.42	12.54	12.66	12.78	12.90
28.0	10.74	10.86	10.98	11.10	11.22	11.34	11.46	11.58	11.70	11.82	11.94	12.06	12.18	12.30	12.42	12.54	12.66	12.78	12.90	13.02
0.5	10.87	10.99	11.11	11.23	11.35	11.47	11.59	11.71	11.83	11.95	12.07	12.19	12.31	12.43	12.55	12.67	12.79	12.91	13.03	13.15
29.0	10.99	11.11	11.23	11.35	11.47	11.59	11.71	11.83	11.95	12.07	12.19	12.31	12.43	12.55	12.67	12.79	12.91	13.03	13.15	13.27
0.5	11.11	11.23	11.35	11.47	11.59	11.71	11.83	11.95	12.07	12.19	12.32	12.44	12.56	12.68	12.80	12.92	13.04	13.16	13.28	13.40
30.0	10.24	11.36	11.48	11.60	11.72	11.84	11.96	12.08	12.20	12.32	12.44	12.56	12.68	12.80	12.92	13.04	13.16	13.28	13.40	13.52
0.5	11.37	11.49	11.61	11.73	11.85	11.97	12.09	12.21	12.33	12.45	12.57	12.69	12.81	12.93	13.05	13.17	13.29	13.41	13.53	13.65
31.0	11.49	11.61	11.73	11.85	11.97	12.09	12.21	12.33	12.45	12.57	12.69	12.81	12.93	13.05	13.17	13.29	13.41	13.53	13.65	13.77
0.5	11.62	11.73	11.85	11.97	12.09	12.21	12.33	12.45	12.57	12.69	12.82	12.94	13.06	13.18	13.30	13.42	13.54	13.66	13.78	13.90
32.0	11.74	11.86	11.98	12.10	12.22	12.34	12.46	12.58	12.70	12.82	12.94	13.06	13.18	13.30	13.42	13.54	13.66	13.78	13.90	14.02
0.5	11.87	11.99	12.11	12.23	12.35	12.47	12.59	12.71	12.83	12.95	13.07	13.19	13.31	13.43	13.55	13.67	13.79	13.91	14.03	14.15
33.0	11.99	12.11	12.23	12.35	12.47	12.59	12.71	12.83	12.95	13.07	13.19	13.31	13.43	13.55	13.67	13.79	13.91	14.03	14.15	14.27
0.5	12.12	12.24	12.36	12.48	12.60	12.72	12.84	12.96	13.08	13.20	13.32	13.44	13.56	13.68	13.80	13.92	14.04	14.16	14.28	14.40
1034.0	12.24	12.36	12.48	12.60	12.72	12.84	12.96	13.08	13.20	13.32	13.44	13.56	13.68	13.80	13.92	14.04	14.16	14.28	14.40	14.52

tose and proteids may be calculated (approximately), the specific gravity, fat, total solids, and ash being known. Thus:

$$P = 2.8\,T + 2.5\,A - 3.33\,F - 0.7\,\frac{G}{D};$$

in which P is the proteids, T the total solids, A the ash, F the fat, D specific gravity (water at 15.5° being taken as 1), and $G = 1000\,D - 1000$.

The difference between the total solids and the fat, proteids, and ash gives the lactose. In this formula it has been assumed that everything that is not fat, proteids, or ash, is milk-sugar, an assumption which is not strictly correct, and which introduces a small error. Another slight error is introduced by the fact that the ash in milk is not the same as the salts existing in the milk.

Total Proteids.—For practical purposes the total proteids are best estimated by calculation from the total nitrogen obtained by the Kjeldahl-Gunning method. Milk contains, however, a sensible proportion of non-proteid nitrogen. According to Munk, this may range, in cow's milk, from 0.022 to 0.034 per cent., and from 0.014 to 0.026 per cent. in human milk. By these figures, the average proteid nitrogen in cows' milk would be 94 per cent., and in human milk 91 per cent., of the total nitrogen.

KJELDAHL-GUNNING METHOD

Reagents

Potassium Sulfate. A coarsely powdered form free from nitrates and chlorids should be selected.

Sulfuric Acid. This should have a sp. gr. 1.84 and be free from nitrates and ammonium.

Standard Acid. $\frac{N}{2}$ Sulfuric or hydrochloric acid, the strength of which has been accurately determined.

Standard Alkali. $\frac{N}{10}$ Ammonium hydroxid, sodium hydroxid, or barium hydroxid, the strength of which in relation to the standard acid must be accurately determined.

Strong Sodium Hydroxid Solution. 500 grams should be added to 500 c.c. of water, the mixture allowed to stand until the undissolved matter settles, the clear liquor decanted and kept in a stoppered bottle. It will be an advantage to determine approximately the quantity of this solution required to neutralize 20 c.c. of the strong sulfuric acid.

Indicator. Cochineal solution is recommended by the A. O. A. C., but methyl-orange is satisfactory. Phenolphthalein is not well adapted to titration of ammonium compounds.

Digestion Flasks. Pear-shaped round-bottomed flasks of hard, moderately thick, well-annealed glass, about 22 cm. long, maximum diameter of 6 cm., tapering gradually to a long neck, 2 cm. in diameter at the narrowest part, and slightly flared at the mouth.

Distillation Flasks. Jena-glass flasks of about 550 c.c. capacity. A copper flask, such as sometimes used in the manufacture of oxygen, may be substituted.

Combined Digestion and Distillation Flasks. Jena-glass round-bottomed flasks with a bulb 12.5 cm. long and 9 cm. in diameter, the neck cylindrical, 15 cm. long and 3 cm. in diameter, flared slightly at the mouth.

Process. 5 c.c. of the sample are placed in a digestion flask, 10 grams of powdered potassium sulfate and 15 to 25 c.c. (ordinarily about 20 c.c.) of the strong sulfuric acid are added and the digestion conducted as follows: The flask is placed in an inclined position and heated below the boiling-point of the acid for from five to fifteen minutes, or until frothing has ceased. Excessive frothing may be prevented by the addition of a small piece of paraffin. The heat is raised until the acid boils briskly. A small, short-stemmed funnel may be placed in the mouth of the flask to restrict the circulation of air. No further attention is required until the liquid has become clear and colorless, or not deeper than a pale straw.

When Kjeldahl operations are carried out in limited number,

the arrangement used in my laboratory has been found very satisfactory. A double-Y, terra cotta drain-pipe, about 20 centimeters internal diameter, is connected by an elbow directly with the chimney-stack. The digestion flasks are supported as shown in the rough sketch, figure 4 (not drawn exactly to scale). Two flasks can be operated at once. The central opening is convenient for other operations producing fumes. Openings not in use are closed by circles of heavy asbestos.

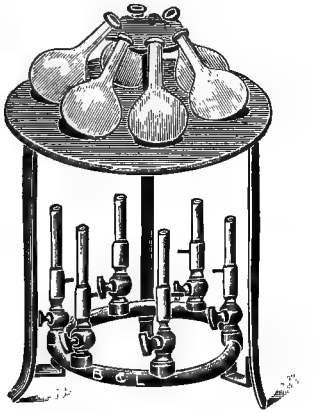

FIG. 3.

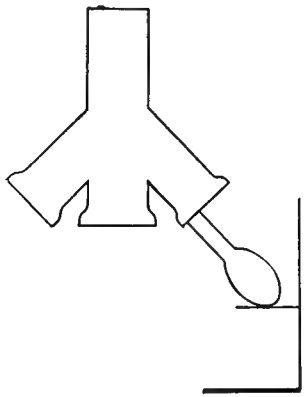

FIG. 4.

The apparatus shown in figure 3 is used when many determinations are made. As corrosive vapors are given off, it must be placed under a hood. The central opening in the ventilating pipe shown in figure 4 will be satisfactory; the mouths of the flasks should be well inside the margin of the pipe.

When the liquid has become colorless or very light straw yellow, it is allowed to cool, diluted with 100 c.c. of water if the smaller form of flask has been used, the liquid transferred to the distilling flask, and the digestion flask rinsed with two portions of water, 50 c.c. each, which are also transferred to the distilling

flask. With the larger form of flask the dilution is made at once by the cautious addition of 200 c.c. of water. Granulated zinc, pumice stone, or 0.5 gram of zinc dust is added. 50 c.c. of the strong sodium hydroxid solution, or sufficient to make the reaction strongly alkaline, should be slowly poured down the side of the flask so as not to mix at once with the acid solution. It is convenient to add to the acid liquid a few drops of phenolphthalein or azolitmin solution, to indicate when the liquid is alkaline, but it must be noted that strong alkaline solutions destroy the former indicator. The flask is shaken so as to mix the alkaline and acid liquids and at once attached to the condensing apparatus. The receiving flask should have been previously charged with a carefully measured volume of the $\frac{N}{2}$ acid (100 c.c. is a convenient amount). The distillation is conducted until about 150 c.c. have passed over. The acid is then titrated with standard alkali and methyl orange, cochineal, or azolitmin, and the amount neutralized by the distilled ammonium hydroxid determined by subtraction. Each c.c. of $\frac{N}{2}$ acid neutralized is equivalent to 0.007 nitrogen. The nitrogen multiplied by 6.38 gives the *total proteids*.

The distillation in this operation requires care, as the amount of ammonium hydroxid is determined by its neutralizing power, hence solution of the alkali of the glass will introduce error. Common glass is not satisfactory. Block-tin is a good material. Moerrs found that Jena-glass tubes resist the action of the ammonium hydroxid.

The most satisfactory condensing arrangement for general laboratory use is a copper tank of good size, through which several condensing tubes pass. Such an arrangement is shown in side-view in figure 5. A detailed view of the construction as applied to Kjeldahl distillations is also shown in figure 5, which is a rough sketch, not drawn to scale. The flask is the standard Jena-glass distilling flask, about 12 cm. diameter, the tank should be high enough to allow of a condensing tube 60

cm. long. The connection of this with the receiving flask is made by means of a bulb tube to allow for occasional drawing-back of the liquid. The cork through which this tube passes into the flask must not fit closely, as opportunity must be given for expansion of the air. The safety tube connecting the distilling flask with the condenser should terminate a little below the water level in the tank. The apparatus should be heated by the low temperature burner or flat evaporating burner. To avoid spurting of the boiling liquid, it is usual to interpose a safety-tube between the distilling flask and the condenser. Many forms have been suggested and are figured in the catalogs of dealers in chemical apparatus. The form shown in figure 5 is somewhat complicated but is satisfactory. The distillation will be hastened if this tube be covered with non-conducting material.

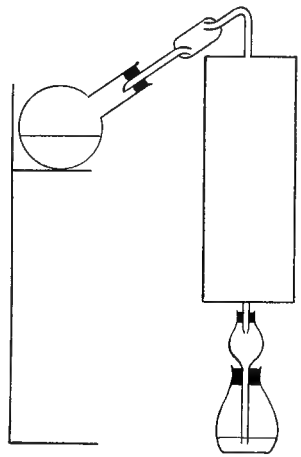

FIG. 5.

Ritthausen Method.—This method depends on precipitation by copper sulfate and sodium hydroxid. It is applicable only to fully developed milks; the proteids of colostrum and whey are only partially precipitated. The reagents are given on page 27.

10 grams of milk are placed in a beaker, diluted with 100 c.c. of distilled water, 5 c.c. of copper sulfate solution added, and thoroughly mixed. The sodium hydroxid solution is then added drop by drop, with constant stirring, until the precipitate settles quickly and the liquid is neutral, or at most very feebly acid. An excess of alkali will prevent the precipitation of some of the proteids.

The reaction should be tested on a drop of the clear liquid, withdrawing it by means of a rod, taking care not to include any solid particles. When the operation is correctly performed, the precipitate, which includes the fat, settles quickly, and carries down all of the copper. It is washed by decantation with about 100 c.c. of water, and collected on a filter (previously dried at 130° and weighed in a weighing bottle). The portions adhering to the sides of the beaker are dislodged with the aid of a rubber-tipped rod. The contents of the filter are washed with water until 250 c.c. are collected, which are mixed and reserved for the determination of the sugar as described below. The water in the precipitate is removed by washing once with strong alcohol, and the fat by six or eight washings with ether. An extraction apparatus may be used for this purpose. The washings being received in a weighed flask, the determination of the fat may be made by evaporating the ether, with the usual precautions.

The residue on the filter, which consists of the proteids in association with copper hydroxid, is washed with absolute alcohol, which renders it more granular, and then dried at 130° in the air-bath. It is weighed in a weighing bottle, transferred to a porcelain crucible, incinerated, and the residue again weighed. The weight of the filter and contents, less that of the filter and residue after ignition, gives the weight of the proteids. The results by this method are slightly high, since copper hydroxid does not become completely converted into copper oxid at 130°.

Richmond & Boseley have modified the process by diluting the milk to 200 c.c., adding a little phenophthalein, and neutralizing any acidity by the cautious addition of dilute sodium hydroxid solution, then adding from 2.0 to 2.5 c.c. of the copper sulfate solution. The precipitate is allowed to settle, washed, and estimated as above.

Casein and Albumin.—The most accurate separation of

casein and albumin is made by Sébelein's method, as follows: 20 c.c. of the sample are mixed with 40 c.c. of a saturated solution of magnesium sulfate and powdered magnesium sulfate stirred in until no more will dissolve. The precipitate of casein and fat, including the trace of globulin, is allowed to settle, filtered, and washed several times with a saturated solution of magnesium sulfate. The filtrate and washings are saved for the determination of albumin. The filter and contents are transferred to a flask and the nitrogen determined by the method described above. The nitrogen so found, multiplied by 6.38, gives the casein.

The filtrate and washings from the determination of casein are mixed, the albumin precipitated by *Almén's tannin reagent*, filtered, and the nitrogen in the precipitate determined as above. The same factor is used.

Almén's reagent is prepared by dissolving 4 grams of tannin in 190 c.c. of 50 per cent. alcohol and adding 8 c.c. of acetic acid of 25 per cent.

In a mixture of milk and whey (prepared with rennet) in about equal parts, Richmond and Boseley found about 0.3 per cent. of albumoses not precipitated by the copper sulfate nor by magnesium sulfate, but precipitable, along with the albumin, by a solution of tannin. The separation may be effected by diluting the filtrate from the magnesium sulfate precipitation, acidifying slightly with acetic acid, and boiling, when the albumin will be coagulated and precipitated. The albumoses may be separated by filtering the solution and precipitating with tannin solution. The precipitated proteids are best estimated by determining the nitrogen in the moist precipitate. The separation of the proteids may be effected, though less accurately, by the use of acetic acid, as recommended by Hoppe-Seyler and Ritthausen.

The following are A. O. A. C. methods:

1. *Provisional Method for the Determination of Casein in*

Cows' Milk.—The determination should be made when the milk is fresh. When it is not practicable to make the determination within 24 hours, add one part of formaldehyde to 2500 parts of milk and keep in a cool place. 10 grams of the sample are diluted with about 90 c.c. of water at between 40° and 42°, 1.5 c.c. of a solution containing 10 per cent. of acetic acid by weight added, allowed to stand for five minutes, washed three times by decantation, pouring the washings through a filter, and the precipitate transferred completely to the filter. If the filtrate is not clear at first, it will generally become so in two or three filtrations, after which the washing can be completed. The nitrogen in the washed precipitate and filter is determined by the Kjeldahl-Gunning method. The nitrogen, multiplied by 6.38, gives the casein.

In working with milk which has been kept with preservatives, the acetic acid should be added in small portions, a few drops at a time with stirring, and the addition continued until the liquid above the precipitate becomes clear or nearly so.

2. *Provisional Method for the Determination of Albumin in Milk.*—The filtrate obtained in the above operation is neutralized with sodium hydroxid, 0.3 c.c. of the 10 per cent. solution of acetic acid added, and the mixture heated for 15 minutes. The precipitate is collected on a filter, washed, and the nitrogen determined.

The following method has been found satisfactory, avoiding the difficulty of washing the precipitate: 10 c.c. of the milk are mixed with saturated magnesium sulfate solution and the powdered salt added to saturation. The mixture is washed into a graduated measure with a small amount of the saturated solution, made up to 100 c.c. with the same solution, mixed, and allowed to stand until the separation takes place. As much as possible of the clear portion is drawn off with a pipet and passed through a dry filter. An aliquot portion of the filtrate is taken, the albumin precipitated by a solution of

tannin, and the nitrogen in the precipitate determined as above.

The casein is found by subtracting the figure for albumin from that for total proteids.

Lactose.—For this determination, A. O. A. C. employs Soxhlet's method with the following reagents:

Copper sulfate solution.—34.639 grams of pure crystallized copper sulfate are dissolved in water and made up to 500 c.c.

Alkaline tartrate solution.—173 grams of pure sodium potassium tartrate and 50 grams of good sodium hydroxid are dissolved in water and the solution made up to 500 c.c.

Half-normal sodium hydroxid.

25 c.c. of the sample in a 500 c.c. flask are diluted with 400 c.c. of water and 10 c.c. of the copper sulfate solution and 8.8 c.c. $\frac{N}{2}$ sodium hydroxid solution added. The mixture should still have an acid reaction and contain copper in solution. If this is not the case, the experiment must be repeated, using a little less of the alkali. The flask is filled to the mark with water, shaken, and the liquid passed through a dry filter. 50 c.c. of Fehling's solution, obtained by mixing equal parts of the above copper sulfate and alkaline tartrate solutions, are heated to brisk boiling in a 300 c.c. beaker, 100 c.c. of the filtrate obtained as above added, and boiling continued for six minutes; the liquid then promptly filtered, and treated according to methods given below. The amount of lactose is calculated by the table on page 28 from the copper obtained by table. The figures for weights of copper between any two data given in the table may be calculated with sufficient accuracy for practical purposes by allowing 0.0008 gram of lactose for each 0.001 gram of copper.

The precipitated cuprous oxid is usually converted into free copper and weighed as such. Two methods may be employed for reduction: by hydrogen or by electrolysis.

Copper.	Lactose.	Copper.	Lactose.	Copper.	Lactose.
0.100	0.072	0.205	0.151	0.305	0.228
0.105	0.075	0.210	0.154	0.310	0.232
0.110	0.079	0.215	0.158	0.315	0.236
0.115	0.083	0.220	0.162	0.320	0.240
0.120	0.086	0.225	0.165	0.325	0.244
0.125	0.090	0.230	0.169	0.330	0.248
0.130	0.094	0.235	0.173	0.335	0.252
0.135	0.097	0.240	0.177	0.340	0.256
0.140	0.101	0.245	0.181	0.345	0.260
0.145	0.105	0.250	0.185	0.350	0.264
0.150	0.109	0.255	0.189	0.355	0.268
0.155	0.112	0.260	0.192	0.360	0.272
0.160	0.116	0.265	0.196	0.365	0.276
0.165	0.120	0.270	0.200	0.370	0.280
0.170	0.124	0.275	0.204	0.375	0.285
0.175	0.128	0.280	0.208	0.380	0.289
0.180	0.132	0.285	0.212	0.385	0.293
0.185	0.134	0.290	0.216	0.390	0.298
0.190	0.139	0.295	0.221	0.395	0.302
0.195	0.141	0.300	0.224	0.400	0.306
0.200	0.147				

Reduction by Hydrogen.—The cuprous oxid is collected on an asbestos filter. This is arranged most conveniently in a special filtering tube, which is shown in figure 6. The wider part is about 8 cm. long and 1.5 cm. in diameter, the narrower portion about 5 cm. long and 0.5 cm. in caliber. A perforated platinum disk is sealed in just above the point of narrowing. The asbestos is placed on this disk, washed free from loose fibers, dried well, and the tube weighed. The filtering tube is attached to an exhaustion apparatus by passing narrower portion through the cork, and a small funnel is fitted tightly in the top of the tube. The object of this funnel is to prevent the precipitate collecting on the upper part of the tube. The lower end of the funnel should project several centimeters below the bottom of the cork through which it passes.

The filtering apparatus must be arranged prior to the precipitation, so that the cuprous oxid may be filtered without delay. The precipitate is transferred as rapidly as possible to the filter, well washed with hot water, alcohol, and ether successively, dried, and the cuprous oxid reduced by gentle heating in a current of hydrogen. When the reduction is complete, the heat is withdrawn, but the flow of hydrogen is continued until the tube is cold. It is then detached and weighed.

Reduction of Copper by Electrolysis.—The filtration is performed in a Gooch crucible with an asbestos-felt film and the beaker in which the precipitation was made is well washed with hot water, the washings being passed through the filter, but it is not necessary to transfer all the precipitate. When the asbestos film is completely washed, it is transferred with the adhering oxid to the beaker; any oxid remaining in the crucible is washed into the beaker by use of 2 c.c. nitric acid (sp. gr. 1.42), added with a pipet. The crucible is rinsed with a spray of water, the rinsings being collected in the beaker. The liquid is heated until all the copper is in solution, filtered, the filter washed until the filtrate amounts to at least 100 c.c., and electrolyzed.

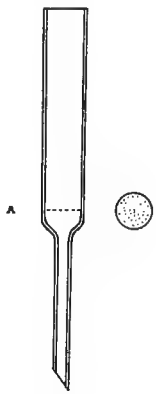

FIG. 6.

Electrolytic apparatus has been constructed in a great variety of forms. When the operation is carried out frequently, it is best to have an electrolytic table. A platinum basin holding not less than 100 c.c. is used. A cylindrical form with flat bottom is convenient. It should rest on a bright copper plate, which is connected with the negative pole of the electrical supply. The positive pole should be also platinum, either a spiral wire, cylinder, or flat foil. Many operators use a funnel-shaped perforated terminal for the negative pole; in which case a glass

beaker or casserole will be a suitable container, the positive terminal being placed within the negative.

Four cells of a gravity battery will suffice for a single decomposition, and will operate two, but more slowly. It is usual to arrange the apparatus so that the operation may be continued during the night. When the electricity is taken from the general supply of the laboratory, it is usually necessary to interpose resistance and to have some means of measuring the current-flow. This is sometimes done with a gas evolution cell and incandescent lamp, but an ammeter and adjustable rheostat are better.

Lactose may be determined by the polarimeter after removal of the fat and proteids, which is best effected, as recommended by Wiley, by a mercuric nitrate solution, prepared by dissolving mercury in twice its weight of nitric acid of 1.42 sp. gr. and adding to the solution five volumes of water. The A. O. A. C. optical method is as follows:

For polarimeters reading to 100 for 26.048 grams sucrose (corresponding to 32.98 grams lactose), measure, in c.c., the amount obtained by dividing double this (*i. e.*, 65.96) by the specific gravity, add 10 c.c. mercuric nitrate solution, make up to 102.6 c.c., shake, filter through a dry filter and examine in a 200 mm. tube. Half the observed reading will be the percentage of lactose. For example, if the specific gravity of the milk is 1.030, the amount taken will be $65.96 \div 1.030 = 64$ c.c.

The allowance for volume of precipitate by making up to 102.6 c.c. is not accurate, except with closely-skimmed milks.

The correction may be made more closely by calculating the actual volume of the precipitate by multiplying the fat-percentage by 1.075 (average specific volume of fat) and the proteid-percentage by 0.8 (average specific volume of coagulated proteids), deducting the sum of these products from 100 c.c. and correcting the observed reading by proportion. For ordinary milk, the volume of the proteids from 65.96 grams may

be taken at 1.68 c.c. Supposing the sample to contain 4.0 per cent. of fat and the polarimetric reading to be 10, the calculation would be thus:

65.96 × 0.04	= 2.63	Amount of fat in milk taken
2.63 × 1.075	= 2.82 c.c.	Volume of fat in precipitate
	1.68 c c.	Est. vol. of proteids in precipitate
	4.50 c.c.	Total volume of precipitate
100 — 4.50	= 95.5 c.c.	Actual volume of liquid.
100 : 95.5 :: 10 : 9.55		9.55 ÷ 2 = 4.75, per cent. lactose

The employment of a factor for correcting for the volume of precipitate may be avoided by Scheibler's method of "double dilution," in which two solutions of different volume are compared. The following is a summary of the method given by Wiley & Ewell: For polarimeters adapted to a normal weight of 26.048 sucrose, 65.82 grams of milk are placed in a 100 c.c. flask, 10 c.c. of the acid mercuric nitrate added, the flask filled to the mark, the contents well mixed, filtered, and a reading taken. A similar quantity of the milk is placed in a 200 c.c. flask and treated in the same way. The true reading is obtained by dividing the product of the two readings by their difference. If the observations are made in a 200 mm. tube the percentage is half the true reading.

The instrument should be accurate, and great care taken in the work, or the results will be less satisfactory than by the method first described, in which an allowance is made for the volume of the precipitate.

Birotation.—When freshly dissolved in cold water, lactose shows a higher rotation than that given above. By standing, or immediately on boiling, the rotatory power falls to the point mentioned. In preparing solutions from the solid, therefore, care must be taken to bring them to the boiling-point previous to making up to a definite volume. This precaution is unnecessary when operating on milk.

Adulterations.—The addition of water to milk is usually detected by the diminution in the amount of solids. The addition of water decreases the specific gravity, while abstraction of fat increases it.

Several observers have found that the whey (milk-serum) obtained by a routine method is of constant composition and that by its specific gravity or refractive index, watering may be detected. Woodman recommends the following method for obtaining a standard whey: 100 c.c. of the sample are mixed with 2 c.c. of dilute acetic acid (sp. gr. 1035, containing 25 per cent. acetic acid), the vessel covered with a watch-glass and heated in the water-bath for 20 minutes, at 70.° It is then placed in ice-water for 10 minutes, and the solution filtered. The specific gravity may be taken under the usual precautions, or, as suggested by Leach, the refractive index may be observed. The routine of precipitation must be closely followed, as the amount of proteids precipitated differs with the method. The total solids and polarimetric reading of the whey might be taken as additional data. The latter figure will be somewhat less than that due to the milk-sugar, as the proteids in solution are levorotatory.

The following are some of the limits recorded, but analysts should make determinations on samples of known composition.

For the Zeiss immersion refractometer, an instrument of special construction, Leach & Lythgoe consider 39 as the lowest permissible reading. This corresponds to 1.3424 on the Abbé refractometer.

From unwatered whole milk, Leach obtained a serum of sp. gr. 1.0287; from unwatered centrifugal skimmed milk, a serum of 1.0296, at 15°.

Vieth has pointed out that in normal milks the ratio sugar: proteids : ash = 13 : 9 : 2 exists, and a determination of these ratios may aid in the attempt to distinguish genuine but abnormal milks from watered milks. In the case of a watered

milk the proportion would remain unchanged, but in abnormal milk it has been found to vary.

Richmond states that the determination of the amount of water that has been added to milk is best calculated from the figures obtained by adding the difference between the specific gravity of the sample and 1000 to the figure representing the percentage of the fat. Thus, if a milk have the specific gravity of 1029.2 and contain 3.27 per cent. of fat, the figure from which the water is calculated is 29.2 + 3.27 = 32.47. The mean figure from unadulterated milks was found to be 36.0, but 34.5 is considered to be a safer limit. Accepting this figure, the percentage of added water in the sample given above will be found by the proportion 34.5 : 23.47 : 100 :: 94.1, *i. e.*, the sample contains 5.9 per cent. of water. Experiments on milks which had been diluted with known proportions of water showed that this method of calculating the added water gave nearer approximations to the truth than by calculating from the figure for non-fatty solids.

It is stated that the watering of milk can be detected by the lowering of the freezing-point. The freezing-point of whole milk ranges from —0.55 to —0.57. Bomstein claims that as little as 5 per cent. added water can be detected by this method. The special apparatus devised for these determinations (known as "cryoscopy") must be used, and the data must be determined by each observer in order to be safely comparable.

For ordinary milk control it will suffice to take the specific gravity by the lactometer (see page 9) and the fat by the Leffmann-Beam method. From the figures thus obtained the total solids can be ascertained from the table or Richmond's slide-rule.

Coloring

Annatto, turmeric, and some coal-tar colors are much used. Caramel is occasionally used, saffron and carotin but rarely. *Annatto* may be detected by rendering the sample slightly alkaline by acid sodium carbonate, immersing a slip of filter-paper,

and allowing it to remain overnight. Annatto will cause a reddish-yellow stain on the paper.

Leys gives the following method for detecting annatto: 50 c.c. of the sample are shaken with 40 c.c. of 95 per cent. alcohol, 50 c.c. of ether, 3 c.c. of water, and 1.5 c.c. of ammonium hydroxid solution (sp. gr. 0.900), and allowed to stand for 20 minutes. The lower layer, which in presence of annatto will have a greenish-yellow tint, is tapped off and gradually treated with half its measure of 10 per cent. solution of sodium sulfate, the separator being inverted without shaking, after each addition. By this treatment the casein separates in flakes which conglomerate and rise to the surface, when the adjacent liquid is tapped off, strained through wire gauze, and placed in four test-tubes. To each of these amyl alcohol is added, and the tubes shaken and immersed in cold water, which is gradually raised to 80°. This causes the emulsion to break up, and the alcohol, holding the annatto in solution, to come to the surface. The alcoholic layer is separated from the lower stratum, evaporated to dryness, and the residue dissolved in warm water containing a little alcohol and ammonium hydroxid. A bundle of white cotton fibers is introduced and the liquid evaporated nearly to dryness on the water-bath. The fiber, which is colored a pale yellow, even with pure milk, is washed and immersed in a solution of citric acid, when it will be immediately colored rose-red if the milk contained annatto. Saffron, turmeric, and the coloring-matter of marigolds do not give a similar reaction.

Coal-tar colors may often be detected by the wool-test (see under "Butter"), but Lythgoe has devised the following method, which is satisfactory: 15 c.c. of the sample are mixed in a porcelain basin with an equal volume of hydrochloric acid (sp. gr. 1.20), and the mass shaken gently so as to break the curd into coarse lumps. If the milk contains an azo-color, the curd will be pink; with normal milk the curd will be white or yellowish. (See next page.)

Salt and cane-sugar are occasionally added to milk that has been diluted with water. The former is detected by the taste, the increased proportion of ash and of chlorin. Cane-sugar may be detected, if in considerable quantity, by the taste. Cotton devised the following test: 10 c.c. of the sample are mixed with 0.5 gram of powdered ammonium molybdate, and 10 c.c. of dilute hydrochloric acid (1 to 10) are added. In a second tube 10 c.c. of milk of known purity or 10 c.c. of a 6 per cent. solution of milk-sugar are similarly treated. The tubes are then placed in the water-bath and the temperature gradually raised to about 80°. If sucrose be present, the milk will assume an intense blue color, while genuine milk or milk-sugar remains unaltered unless the temperature be raised to the boiling-point. According to Cotton, the reaction is well marked in the presence of as little as 1 gram of sucrose to a liter of the milk, and 6 grams and over per liter are usually found in adulterated samples.

The quantitative determination is made by the methods described in connection with condensed milk.

General Method for Colors in Milk.—Leach has devised a general method for detecting colors in milk. 150 c.c. of the sample are coagulated in a porcelain basin, with the addition of acetic acid and heating, and the curd separated from the whey. The curd will often collect in a mass; but if this does not occur, it must be freed from whey by straining through muslin. The curd is macerated for several hours in a closed flask, with occasional shaking, with ether to extract fat. Annatto will also be removed by it. The ether and curd are separated and treated as follows:

The ether is evaporated, the residue mixed with a little weak solution of sodium hydroxid, and passed through a wet filter; and when this has drained, the fat is washed off and the paper dried. An orange tint shows annatto, which may be confirmed by a drop of solution of stannous chlorid, which makes a pink spot.	If the curd be colorless, no foreign coloring-matter is in it; if orange or brown, it should be shaken with strong hydrochloric acid in a test-tube.	
	If the mass turns blue gradually, caramel is probably present. The whey should be examined for caramel (see page 64).	If the mass turns pink at once, an azo-color is indicated.

Thickening Agents.—Several instances of the use of brain matter, dextrin and gelatin have been reported. It is also stated that sugar, starch, and salt have been added. Brain matter is easily detected by microscopic examination; starch by the iodin test; dextrin and sugar by increased polarimetric reading, and the sweet taste of the residue. A solution of calcium hydroxid in sirup has been sold under the name "grossin" for thickening cream. It would easily be detected by the high ash. Thickening agents of pectinous nature are now commercial articles. The most important of these is agar.

Gelatin.—Stokes detects the presence of gelatin in cream or milk as follows: 10 c.c. of the sample, 20 c.c. of cold water, and 10 c.c. of acid mercuric nitrate solution (page 30) are mixed, shaken vigorously, allowed to stand for five minutes, and filtered. If much gelatin be present, it will be impossible to get a clear filtrate. A portion of the filtrate is mixed with an equal bulk of saturated aqueous solution of picric acid. If any gelatin be present, a yellow precipitate will be immediately produced. Picric acid will detect the presence of one part of gelatin in 10,000 parts of water.

Agar (agar-agar) is derived from marine algæ. It forms with water a stiff jelly that does not melt as readily as that from gelatin.

Commercial agar almost always contains diatoms. One characteristic form is *Arachnoidiscus Ehrenbergii.* (See figure 7.) The diatoms may be obtained by oxidizing the organic material with a mixture of nitric and sulfuric acid or nitric and hydrochloric acids. Moist materials should be well dried but not powdered. The diatoms will be found by examining the residue with a power of about 100 diameters.

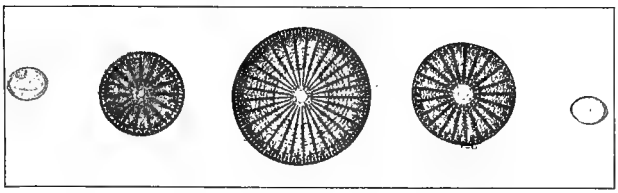

FIG. 7.—*Arachnoidiscus Ehrenbergii.* × 100. The smaller oval diatoms are a species of *Cocconeis.*

PRESERVATIVES

These are largely used, especially in the warmer season, as a substitute for refrigeration. Many of them are sold under proprietary names which give no indication of their composition. Preparations of boric acid and borax were at one time the most frequent in use, but lately *formalin,* a 40 per cent. solution of formaldehyde (methyl aldehyde), has come into favor. Sodium benzoate is now in common use as a preservative of cider, fruit-jellies, and similar articles, and may, therefore, be found in milk. Salicylic acid is not so much employed as in former years. Sodium carbonate is occasionally used to prevent coagulation due to slight souring. Fluorids and abrastol might be used. A mixture of boric acid and borax is more efficient than either alone. The quantity generally used is equivalent to about 0.5 gram of boric acid per liter. Formaldehyde is the most efficient antiseptic. In the proportion of 0.125 gram to the liter, it will keep milk sweet for a week.

Boric acid and borax.—These may be detected by the following test: A few drops of the sample are mixed with a drop of hydrochloric acid and a drop of strong alcoholic solution of turmeric, evaporated to dryness at a gentle heat, and a drop of ammonium hydroxid added to the residue when cold. A dull green stain shows that boric acid is present.

Formaldehyde.—Hehner found that when milk containing this substance is mixed with sulfuric acid containing a trace of ferric salt, a blue color appears. Richmond & Boseley showed that the delicacy of the test is much increased by diluting the milk with an equal bulk of water and adding sulfuric acid of 90 to 94 per cent., so that it forms a layer underneath the milk. Under these conditions, milk, in the absence of formaldehyde, gives a slight greenish tinge at the junction of the two liquids, while a violet ring is formed when formaldehyde is present even in so small a quantity as 1 part in 200,000 of milk. The color is permanent for two or three days. In the absence of formaldehyde, a brownish color is developed after some hours, not at the junction of the two liquids, but lower down in the acid.

One of the most delicate and positive tests for formaldehyde is as follows: To a few c.c. of the suspected liquid, a pinch of phenylhydrazin hydrochlorid is added, the liquid shaken and a drop of a dilute solution of sodium nitroprussid added and then a few drops of sodium hydroxid. Milk containing formaldehyde gives a grayish-green. If the test is applied to the pure solution obtained by distilling the sample, as noted below, a characteristic deep blue is produced. This is a delicate and useful test and should always be applied. It avoids the fallacy noted below in the testing of ice-cream and desserts.

Another test is the addition of a small amount of a solution of 1 per cent. of phloroglucol and about 25 per cent. of sodium hydroxid in water. This produces a rose-red. The test is best applied by running the test solution by means of a pipet under the suspected liquid.

The following test, recently discovered by Bonnet, is due to the vapor of formaldehyde, and avoids the fallacies of some of the older tests. A solution is made by dissolving 0.035 gram pure morphin sulfate in 10 c.c. of sulfuric acid. This solution does not keep well. A convenient amount of the sample is placed in a dish or beaker, a watch-glass containing 1 c.c. of the above solution is floated on it, and the dish covered with a glass plate. The materials are allowed to remain undisturbed at room-temperature for several hours. Formaldehyde is indicated by the development of a color ranging from pink to dark blue. A black discoloration is disregarded. Bonnet found that with 1 part of formaldehyde to 25,000 parts of sample a distinct color appeared in one hour.

Formaldehyde may be obtained pure by distillation of the sample, especially in a current of steam. B. H. Smith found that if 100 c.c. of the sample be mixed with 1 c.c. of dilute sulfuric acid (1 : 3) and distilled, one-third of the formaldehyde present will come over in the first 20 c.c. of distillate. The distillation of milk is troublesome, owing to bumping and frothing, but Smith found that it could be conducted safely by using a flat evaporating burner. It is advisable also to put a few small pieces of pumice-stone in the flask.

In testing ice-cream and similar articles, it must be borne in mind that some flavoring materials, such as vanillin, and lemon and orange extract, being partially aldehydic in nature may simulate formaldehyde. LaWall has obtained reactions of this character from vanillin, and even from a commercial table sugar. The phenylhydrazin and morphin sulfate tests are the least liable to fallacy in this respect.

Determination of Formaldehyde.—B. H. Smith, who also investigated the methods for this purpose, finds that the choice will depend on the strength of the solution. For moderately strong solutions the iodin method of Romijn is satisfactory.

10 c.c. of the solution, which should be diluted so as not to

contain more than 3 per cent. of formaldehyde, are mixed with 25 c.c. $\frac{N}{10}$ iodin solution and sufficient strong sodium hydroxid solution added to make the liquid bright yellow. After standing 10 minutes, hydrochloric acid is slowly added until a marked brown liquid is produced. The iodin is then titrated with thiosulfate in the usual way. The amount of iodin that has been taken up, multiplied by 0.118, will give the amount of formaldehyde. A blank experiment should be made and any necessary correction applied.

For dilute solutions, the potassium cyanid method is best.

30 c.c. of $\frac{N}{10}$ silver nitrate solution are acidulated with 15 drops of nitric acid. 10 c.c. of this solution are mixed with 10 c.c. of normal potassium cyanid solution (6.5 grams in 1000 c.c.), then water to make 50 c.c., the liquid shaken, filtered through a dry filter and 25 c.c. set apart for titration as below (Volhard's method).

Another 10 c.c. of cyanid solution are mixed with a measured amount of the formaldehyde solution (which must not contain more than 0.03 gram of formaldehyde), the mixture added to another 10 c.c. of the acid silver nitrate solution, shaken, made up to 50 c.c., filtered and 25 c.c. of the filtrate taken as before. The two solutions contain excess of silver, but the second contains more, because the formaldehyde converts the cyanid into a compound that does not precipitate silver.

Standard thiocyanate solution is prepared by dissolving 10 grams of potassium thiocyanate (or 8 grams of ammonium thiocyanate) in water to make 1000 c.c. The solution is approximately $\frac{N}{10}$. Its value in silver must be determined thus:

50 c.c. of $\frac{N}{10}$ silver nitrate are mixed with 1 c.c. of nitric acid and 1 c.c. of saturated solution of ammonium ferric sulfate, and thiocyanate solution added until a faint permanent brown is produced.

The titration of the acid filtrates is conducted in the same

manner. To each filtrate is added 1 c.c. of ferric sulfate and then the thiocyanate until the faint permanent brown is obtained. If the thiocyanate is exactly $\frac{N}{10}$, the difference in c.c. required for the two filtrates multiplied by 0.006 will give the amount of formaldehyde in the quantity originally taken. If the thiocyanate is not $\frac{N}{10}$ the result must be reduced to that basis.

Beta naphthol.—Several allied antiseptics of this type may be detected by the following method: 200 grams of the sample are acidified with sulfuric acid and distilled with open steam until 150 c.c. of distillate are obtained. This liquid is shaken with 20 c.c. of chloroform, the latter withdrawn, rendered alkaline with potassium hydroxid, and heated almost to boiling for a few minutes. Color changes as follows:

Salol, ...light red.
Phenol,.....light red, to brown, to colorless.
Beta naphthol,.....deep blue, to green, to brown.

A portion of the distillate may also be tested as follows: 25 c.c. are made faintly alkaline with ammonium hydroxid, then faintly acid with nitric acid and then a drop of strong sodium nitrite solution. Beta naphthol develops a rose red, but the reaction is sometimes uncertain and seems to be affected by light. The so-called hydronaphthol gives the same effect.

Benzoates.—Peter's method: The material is made slightly acid and extracted with chloroform, which is then evaporated spontaneously. The vessel containing the residue is placed in melting ice, 2 c.c. of sulfuric acid added, and stirred until the residue is dissolved. Barium dioxid is dusted into the mass, with constant stirring, until the liquid begins to foam, when 3 c.c. of hydrogen dioxid (3 per cent.) are added drop by drop. The dish is then removed from the cold bath, the contents diluted with water to convenient bulk, and filtered. The acid filtrate is extracted with chloroform. The benzoic

acid will have been converted into salicylic acid by the process and the latter may be detected by dilute solution of ferric chlorid or ammonio-ferric sulfate.

Salicylic acid. This is usually detected by extraction with an immiscible solvent. 25 to 50 c.c. of the sample are rendered feebly acid with a few drops of sulfuric acid and shaken vigorously with about an equal bulk of a mixture of equal parts of ether and petroleum spirit, the liquids are allowed to separate, as much as possible of the solvent is drawn off, filtered, and evaporated at a gentle heat. When salicylic acid has been added as a preservative, distinct needle-like crystals will be usually seen. A few drops of water should be added and then a drop of very dilute ferric chlorid solution. The reaction of salicylic acid is distinct. When a crystalline deposit cannot be obtained, a larger quantity of the sample may be concentrated at a gentle heat and extracted as above.

Some analysts prefer chloroform as the extracting liquid. In this case the shaking should be done in a stoppered separator, that the solvent may be readily drawn. A solution of ammonio-ferric alum is in some respects preferable to ferric chlorid as a testing agent. If 50 c.c. of the sample properly extracted does not give a visible deposit of the acid, it is not likely that it has been added as a preservative.

Saccharin. A suitable amount of the sample (50 or 100 c.c.) is acidified with dilute (25 per cent.) sulfuric acid and extracted with a mixture of equal parts of petroleum spirit boiling below 60° and ether. The solvent is evaporated at a gentle heat. The presence of saccharin in the residue may be detected by the taste. 2 c.c. of a saturated solution of sodium hydroxid are added and the dish heated until the residue dries and then to 210°–215°, and maintained thus for half an hour. The saccharin is converted into salicylic acid, which may be detected in the residue by acidulating it with sulfuric acid and applying the ferric chlorid test. If salicylic acid be present

originally in the sample, the residue from the petroleum spirit and ether solution is dissolved in 50 c.c. of dilute hydrochloric acid, bromin water added in excess, the liquid shaken well, and filtered. Salicylic acid is completely removed as a brominated derivative. The filtrate is made strongly alkaline with sodium hydroxid, evaporated, and fused as described above.

Sodium Carbonate.—The following test is due to Schmidt.

10 c.c. of the milk are mixed with an equal volume of alcohol, and a few drops of a 1 per cent. solution of rosolic acid added. Pure milk shows merely a brownish-yellow color, but in the presence of sodium carbonate a more or less marked rose-red appears. The delicacy of the test is enhanced by making a comparison cylinder with the same amount of milk known to be pure. If the salt is present in considerable amount, it may be detected by the increase in the ash, its marked alkalinity and effervescence with acid.

Abrastol.—1 c.c. of acid mercuric nitrate solution (page 30) is added to 20 c.c. of milk. A yellow tint indicates abrastol. The delicacy of the test may be increased by comparison with an untreated portion of the sample. The absence of other preservatives should be assured.

Fluorids.—50 grams of the sample are made slightly alkaline with ammonium carbonate, heated to boiling, a few centimeters of calcium chlorid solution added, and heating continued for 5 minutes. The precipitate is collected, washed, dried, transferred to a platinum crucible, and ignited. When the mass is cold, a few drops of strong sulfuric acid are added, and the crucible covered with a piece of glass partly protected on the lower side by paraffin. The bottom of the crucible is then heated for an hour at a temperature between 75° and 80°. The glass is etched if fluorids are present.

Preservation of Milk-samples.—Formaldehyde is now generally used; 0.05 per cent. will keep milk for a month and larger proportions for an indefinite period.

Bevan has, however, noted the fact that the total solids of milk containing formaldehyde are always higher, and that the increase is much greater than can be accounted for, even assuming that all the formaldehyde remains in the residue.

The rate at which formaldehyde disappears from milk has been investigated by Hehner, who found that at the end of a week none could be detected in a sample to which had been added 1 part in 100,000; after two weeks none could be detected in a sample of 1 part in 50,000; after three weeks only a trace could be detected with 1 part in 25,000.

Detection of Boiled Milk.—Dupouy proposed the following method: A few drops of a solution of 1-4-diamidobenzene in water are added to 5 c.c. of the sample, and then a few drops of hydrogen dioxid solution. Raw milk gives a blue color; milk that has been heated to over 79° gives no color. The solution of diamidobenzene must be freshly prepared. Rosier has found that 1-3-diamidobenzene will serve, and that if the blue milk be shaken with amyl alcohol, the blue color passes into the latter and is more stable. These tests are applicable for distinguishing between pasteurized and sterilized milks, as the reactivity of milk is lost between 75° and 80°.

Faber has shown that raw milk may be distinguished from boiled milk or milk that has been heated above 75° by the fact that such treatment coagulates or alters the albumin so that if the liquid be saturated with magnesium sulfate, the albumin is separated along with the albumin casein.

Richmond & Boseley recommend the following methods to distinguish new milk from milk which has been sterilized:

(*a*) 100 c.c. of the sample are allowed to stand in a graduated cylinder for six hours at 15.5° and the percentage of cream noted. If less than 2.5 per cent. of cream has risen for each 1 per cent. of fat in the milk, the milk may be considered suspicious; if the cream falls decidedly below 2 per cent. for each 1 per cent. of fat, it is probable that sterilized milk is present.

(*b*) The albumin is determined by means of magnesium sulfate. If less than 0.35 per cent. is found, sterilized milk may be considered to be present.

(*c*) The milk-sugar is determined by the polarimeter, and also gravimetrically, in duplicate. If the difference between the two estimations be more than 0.2 per cent., it will be corroborative evidence of the presence of sterilized milk. It is doubtful whether a proportion of sterilized milk much below 30 per cent. can be detected.

The following figures, by Stewart, show the percentage of soluble albumin found in milk raised to various temperatures:

Time of Heating.	Soluble Albumin in Fresh Milk.	Soluble Albumin in Heated Milk.
10 minutes at 60°	0.423	0.418
30 " " 60°	0.435	0.427
10 " " 65°	0.395	0.362
30 " " 65°	0.395	0.333
10 " " 70°	0.422	0.269
30 " " 70°	0.421	0.253
10 " " 75°	0.380	0.07
30 " " 75°	0.380	0.05
10 " " 80°	0.375	none.
30 " " 80°	0.375	none.

CONDENSED MILK

The form of condensed milk called "evaporated cream" consists merely of whole milk concentrated to about two-fifths of its bulk, but most condensed milks contain a considerable amount of cane-sugar. These samples represent, usually, whole milk concentrated to about one-third or two-sevenths of its original volume. A small amount of invert-sugar may be present. Portions of the lactose may crystallize from condensed milk, and when solutions are prepared for analysis, abnormal polarimetric reading will result unless the liquid stands for some hours or is heated for a short time to 100°. The most common defect in condensed milks is deficiency in fat,

due to preparation from closely-skimmed milks. Preservatives (other than cane-sugar) and coloring-matters are rarely used, nor is it likely that foreign fats will be present.

ANALYSES OF COMMERCIAL CONDENSED MILKS

Total Solids.	Fat.	Proteids.	Lactose.	Sucrose.	Ash.	Analyst.
36.7	10.5	9.7	14.2	none	2.1	Pearmain and Moor
31.2	9.6	9.2	10.9	none	1.5	F. J. Aschman
28.1	8.8	8.5	9.8	none	1.8	F. J. Aschman
78.4	9.3	9.1	13.4	40.4	2.0	F. J. Aschman
74.2	9.0	9.3	10.2	43.7	1.9	F. J. Aschman
70.9	1.4	11.4	14.6	41.9	1.6	Pearmain and Moor

The sucrose in the last sample was determined by difference.

The analysis of unsweetened condensed milks is conducted as with ordinary milk, the sample having been previously diluted with several times its weight of water heated to boiling, cooled, and made up to a definite volume. The fat may be readily estimated by the L-B. process.

The full analysis of sweetened condensed milk is difficult, and many of the published figures are erroneous. The cane-sugar interferes with the extraction of the fat by solvents. The same difficulty occurs in the analysis of some prepared infant-foods, such as mixtures of milk with malt and glucose.

For the general operations, a portion of the well-mixed contents of a freshly opened can should be accurately weighed, diluted with a known amount of water, and well mixed, from which mass the portions for analysis may be taken and the results calculated to the original sample. 50 grams mixed with 150 c.c. of water will be a convenient quantity. For the polarimetric determination of lactose, a special procedure will be necessary; but for determination of solids, ash, total proteids, and total reducing sugars, the examination may be made as with ordinary milk upon this diluted sample.

Fat.—The Adams method is not satisfactory under ordinary conditions, owing to the sucrose. Geisler substituted petro-

leum spirit or a mixture of this with anhydrous ether, extracting for five hours. Bryant has obtained better results with carbon tetrachlorid, which is, moreover, safer.

Some analysts have advised the extraction of the fat from the precipitate obtained with copper sulfate (see page 23). This is collected on fat-free filter paper (hardened paper will answer), washed and dried. The folded filter is placed on a fat-free thimble and extracted with carbon tetrachlorid for several hours.

The Werner-Schmid method may be employed, but the fat is apt to be contaminated with caramel. It should be dissolved in anhydrous ether, by which the caramel will be left adhering to the glass; and after washing this with a little more ether, it should be dried and weighed and the fat determined by difference.

The estimation of fat by centrifugal method is seriously impeded by the carbonization of the sucrose, and various methods have been proposed for overcoming this difficulty. Leach devised the following method, which he finds to be more trustworthy than ordinary extractions with solvents. Leach applied the process to a centrifugal method not identical with the one described on page 16, but this is not important:

25 c.c. of diluted material are measured into the test-bottle, water added sufficient to fill it to the beginning of the stem, and then 4 c.c. of the copper sulfate solution used for sugar determination, the mixture allowed to stand for a few minutes, then shaken well, and the precipitate settled by whirling the bottle in the machine. The supernatant liquid is drawn off. The precipitate is washed twice with water by the same method, settling the precipitate in each case by the use of the centrifuge, taking care that the mass is well stirred with the water before each whirling. After the second washing, about 15 c.c. of water are put in, the precipitate stirred up, the amyl alcohol mixture added, then the sulfuric acid, as directed on page 16,

the mixture whirled, and the fat measured. The percentage of fat will be that based on the 25 c.c. used, and the amount in the original sample may be calculated from the dilution.

Cochran's method.—This is based on the solution of the curd by the DeLaval method and solution of the fat in ether. It may be applied by means of the L-B. bottles and centrifuge, or with a special flask (which does not require a centrifuge) devised by Cochran. If L-B. bottles are used, the reading must be multiplied by 3, since only 5 c.c. of the sample are taken. The process is especially adapted to sweetened condensed milk and cereal foods containing fat. The fat of normal cereals can be accurately determined by it. The curd is dissolved by a mixture of equal parts sulfuric acid and 80 per cent. acetic acid. This mixture may be made beforehand or the acids may be added in succession to the material in the bottle or flask. With the flask, all materials must be added through the side-tube.

Ordinary milk is taken undiluted, but condensed milk is diluted. Sweetened condensed milk is diluted with 3 times its weight of water; unsweetened condensed with an equal weight of water.

5 c.c. of the prepared sample are introduced into the flask by means of the side-tube, 5 c.c. of the acid mixture added slowly with shaking, taking care that the liquid does not get into the graduated tube. If the liquid becomes dark brown and free from lumps of undissolved curd, the flask is allowed to cool and 4 c.c. of ether added (common ether will answer). If the mixture produced by the acid is lumpy, the flask is set in tepid water, heated gradually (not above 80°) and shaken gently until all flocculent matter is dissolved. Care must be taken not to continue this heating until masses of caramel are formed, as this will prevent correct results being obtained.

When the flocculent matter has disappeared (the liquid will in any case show some turbidity from the emulsion of fat), the flask is cooled and ether added as noted above. The flask is

well shaken to cause the ether to take up all the fat, taking care not to bring the liquid up into the graduated tube. When the fat is dissolved, the flask is placed in water at about 40°, kept still and the temperature raised slowly until all ether is vaporized, then rapidly until the boiling-point is reached, and this continued until the solution ceases to bubble, and the fat forms a clear layer on the surface of the dark but clear acid solution. The flask should not be shaken while evaporating the ether. Water heated to nearly boiling is now run cautiously into the side-tube until the flask is three-quarters full. If any fat is in the side-tube, it may be removed by blowing gently into it. If the liquid is producing but few bubbles, more hot water should be run in until all the fat is within the limits of the graduation. If the bubbling is still violent when the tube is only three-quarters full, the lower half of the flask should be cooled by immersion in cold water, when the bubbling will nearly cease, and the fat may then be raised into the neck by adding more hot water. The flask may stand for a minute, if necessary to allow the fat column to unite, but it should be measured as soon as possible. The graduation is percentage of fat by weight, based on 5 c.c. of milk (say 5.16 grams). If the sample has been diluted, the reading must be increased by the factor of dilution.

The process is easy of accurate operation and is especially adapted to materials that do not yield fat to common extraction methods. The special point is to avoid prolonged or excessive heating with the acid liquid, as this will produce lumps of partly carbonized matter. If these form, the operation must be discontinued and the flask cleaned promptly. This lumpy material should be distinguished from a brown flocculent matter which rests between the acid and ether layer at the early part of the operation, but which disappears later.

For the examination of malted cereals, 1.72 grams are taken and introduced by the side-tube, taking care that no more material adheres than can be washed into the flask by not more

than 5 c.c. of water. The mass is mixed thoroughly by shaking, 3 c.c. of the acid mixture are introduced and the process is carried out as described, taking especial care not to overheat. The volume of fat multiplied by 3 gives percentage.

Most malted cereals are easily treated by the method, but some contain insoluble cellular matter. With care, this will not interfere. Sometimes previous treatment with diluted sulfuric acid will render the material more tractable.

The flasks should be cleaned promptly.

Sugars.—If regard is to be given to the presence of invert-sugar, a special method must be followed. The processes first given consider lactose and sucrose only.

Lactose.—The heating employed in the manufacture of condensed milk may reduce the rotatory power of the sugar sufficiently to cause error in the polarimetric method. The reducing power with alkaline copper solutions is not seriously affected.

Sucrose.—This determination may be made by difference; that is, subtracting the sum of the other ingredients from the total solids. This will serve for ordinary inspection purposes, since the amount present is almost always large, generally more than the total of milk-solids, and an error even of several per cent. does not affect the judgment as to the wholesomeness of the sample. Exact work requires, however, that the cane-sugar be determined directly, and several processes have been devised for the purpose. Sucrose exerts but little action on Fehling's solution, but invert-sugar acts powerfully, and some processes depend on determining the reducing power before and after inversion. Since the polarimetric reading is also markedly changed by the inversion, the difference in polarization may be employed. Processes of fermentation may be so conducted as to remove the sucrose (also any form of glucose) while the lactose is unaffected. This method is chiefly valuable for recognizing invert-sugar or either of its constituents.

When inversion methods are used, they must be such as to

secure prompt inversion of the sucrose without affecting the lactose. Experiment shows that citric acid and invertase are the most suitable agents. Stokes & Bodmer have worked out the citric acid method substantially as follows:

25 c.c. of the diluted sample are coagulated by addition of 1 per cent. of citric acid, without heating, and made up to 200 c.c. plus the volume of the precipitated fat and proteids (see p. 31). The liquid portion, which now measures 200 c.c., is passed through a dry filter. The reducing power with alkaline copper solutions is determined at once upon 50 c.c. of this filtrate. To another 50 c.c., 1 per cent. of citric acid is added, the solution boiled at least 30 minutes, and the reducing power also determined. The increase over that of the first solution is due to the invert-sugar formed by the action of the citric acid on the sucrose. It is necessary to bear in mind that the reducing equivalents of lactose and invert-sugar are not the same. Volumetric method may be employed.

The following method is based on the difference in polarimetric reading before and after action of invertase. 75 c.c. of the diluted milk are placed in a 100 c.c. flask, diluted to about 80 c.c., heated to boiling, to correct birotation, cooled, and 10 c.c. of acid mercuric nitrate solution added. The mixture is made up to 100 c.c., well shaken, filtered through a dry filter, and the polarimetric reading taken at once. It will be the sum of the effect of the two sugars. The volume of the sugar-containing liquid is calculated by allowing for the precipitated proteids and fat, as described on page 31.

50 c.c. of the filtrate are placed in a flask marked at 55 c.c., a piece of litmus paper dropped in, and the excess of nitric acid cautiously neutralized by sodium hydroxid solution. The liquid is then faintly acidified by a single drop of acetic acid (it must not be alkaline), a few drops of an alcoholic solution of thymol are added, and then 2 c.c. of a solution of invertase, prepared by grinding half a cake of ordinary compressed yeast

with 10 c.c. of water and filtering. The flask is corked and allowed to remain at a temperature of 35° to 40° for 24 hours. The cane-sugar will be inverted, while the milk-sugar will be unaffected. The flask is filled to the mark (55 c.c.) with washed aluminum hydroxid and water, mixed, filtered, and the polarimetric reading taken. The amount of cane-sugar can be determined from the difference in the two readings by the formula

$$S = \frac{a \pm b}{143 - \frac{t}{2}}$$

in which S is the percentage of cane sugar; *a*, the reading before, *b*, the reading after inversion; t, the temperature.

Bigelow and McElroy propose the following routine method for the determination of the sugars, including invert-sugars, in condensed milk. The solutions used are:

Acid Mercuric Iodid.—Mercuric chlorid, 1.35 grams; potassium iodid, 3.32 grams; glacial acetic acid, 2.c.c.; water, 64 c.c.

Alumina-cream.—A cold saturated solution of alum is divided into two unequal portions, a slight excess of ammonium hydroxid is added to the larger portion and the remainder added until a faintly acid reaction to litmus is obtained.

The entire contents of the can are transferred to a porcelain dish and thoroughly mixed. A number of portions of about 25 grams are weighed carefully in 100 c.c. flasks. Water is added to two of the portions, and the solutions boiled. The flasks are then cooled, clarified by means of a small amount of the acid mercuric iodid and alumina-cream, made up to mark, filtered, and the polarimetric reading noted. Other portions of the milk are heated in the water-bath to 55°; one-half of a cake of compressed yeast is added to each flask and the temperature maintained at 55° for five hours. Acid mercuric iodid and alumina-cream are then added, the solution cooled to room temperature, made up to mark, mixed, filtered, and polarized. The amount of cane-sugar is determined by formula given above. Correction

for the volume of precipitated solids may be made by the double-dilution method. The total reducing sugar is estimated in one of the portions by one of the reducing methods, and if the sum of it and the amount of cane-sugar obtained by inversion is equal to that obtained by the direct reading of both sugars before inversion, no invert-sugar is present. If the amount of reducing sugar seems to be too great, the milk-sugar must be re-determined as follows: 250 grams of the condensed milk are dissolved in water, the solution boiled, cooled to 80°, a solution of about 4 grams of glacial phosphoric acid added, the mixture kept at 80° for a few minutes, then cooled to room temperature, made up to mark, shaken, and filtered. It may be assumed that the volume of the precipitate is equal to that obtained by mercuric iodid solution. Enough sodium hydroxid is then added to not quite neutralize the free acid, and sufficient water to make up for the volume of the solids precipitated by the phosphoric acid. The mixture is then filtered and the filtrate is measured in portions of 100 c.c. into 200 c.c. flasks. A solution containing 20 milligrams of potassium fluorid and half a cake of compressed yeast is added to each flask, and the mixture allowed to stand for 10 days at a temperature between 25° and 30°. The invert-sugar and cane-sugar are fermented and removed by the yeast in the presence of a fluorid, while milk-sugar is unaffected. The flasks are filled to the mark and the milk-sugar determined either by reducing or by the polariscope. The amount of copper solution reduced by the lactose and invert-sugar, less the equivalent of lactose remaining after fermentation, is due to invert-sugar.

BUTTER

Butter is a mixture of fat, water, and curd. The water contains milk-sugar and the salts of the milk. Common salt is usually present, being added after the churning. Artificial coloring is frequently used.

Butter-fat is distinguished from other animal fats in that it contains a notable proportion of acid radicles with a small number of carbon atoms. Thus, about 91 per cent. consists of palmitin and olein and the remainder of butyrin and caproin, along with small amounts of caprylin, caprin, myristin, and some others. According to the experiments of Hehner & Mitchell, stearin is present only in very small quantity. The exact arrangement of the constituents is unknown.

The composition of commercial butter usually varies within the following limits:

Fat,	78	per cent. to	94	per cent.
Curd,	1	" "	3	"
Water,	5	" "	14	"
Salt,	0	" "	7	"

Butter containing over 40 per cent. of water is sometimes sold. Such samples are pale and spongy, lose weight, and become rancid rapidly.

The official methods of the A. O. A. C. for the analysis of butter are as follows:

Preparation of the Sample.—If large quantities of butter are to be sampled, a butter trier or sampler may be used. The portions thus drawn, about 500 grams, are to be perfectly melted in a closed vessel at as low a temperature as possible, and when melted the whole is to be shaken violently for some minutes until the mass is homogeneous and sufficiently solidified to prevent the separation of the water and fat. A portion is then poured into the vessel from which it is to be weighed for analysis, and should nearly or quite fill it. This sample should be kept in a cold place until analyzed.

Water.—From 1.5 to 2.5 grams are dried to constant weight at the temperature of boiling water, in a dish with flat bottom, having a surface of at least 20 sq. cm. The use of clean dry sand or asbestos with the butter is admissible, and is necessary if a dish with round bottom be employed.

Fat.—The dry butter from the water determination is dissolved in the dish with absolute ether or with petroleum spirit (sp. gr. 0.680). The contents of the dish are then transferred to a weighed Gooch crucible with the aid of a wash-bottle filled with the solvent, and are washed until free from fat. The crucible and contents are heated at the temperature of boiling water till the weight is constant.

The fat may also be determined by drying the butter on asbestos or sand, and extracting by anhydrous alcohol-free ether. After evaporation of the ether the extract is heated to constant weight at the temperature of boiling water and weighed.

Casein, Ash, and Chlorin.—The crucible containing the residue from the fat determination is covered and heated, gently at first, gradually raising the temperature to just below redness. The cover is removed and the heat continued until the material is white. The loss in weight represents casein, and the residue mineral matter. In this mineral matter dissolved in water slightly acidulated with nitric acid, chlorin may be determined gravimetrically with silver nitrate, or, after neutralization with calcium carbonate, volumetrically, using potassium chromate as indicator.

Salt.—About 10 grams are weighed in a beaker in portions of about 1 gram at a time taken from different parts of the sample. Hot water (about 20 c.c.) is now added to the beaker, and after the butter has melted, the mass is poured into the bulb of a separating funnel, which is then closed and shaken for a few moments. After standing until the fat has all collected, the water is allowed to run into an Erlenmeyer flask, with care not to let fat globules pass. Hot water is again added to the beaker, and the extraction is repeated from ten to fifteen times, using each time from 10 to 20 c.c. of water. The resulting washings contain all but a mere trace of the salt originally present in the butter. The chlorin is determined

volumetrically in the filtrate by means of standard silver nitrate and potassium chromate indicator and calculated to sodium chlorid.

Adulteration with Foreign Fats.—The chief adulteration of butter consists in the substitution of foreign fats, especially the product known as oleomargarin.

When fats are saponified and the soap treated with acid, the individual fatty acids are obtained. It is upon the recognition of the peculiar acid radicles existing in butter that the most satisfactory method of distinguishing it from other fats is based. Since the relative proportion of these radicles differs in different samples, the quantitative estimation cannot be made with accuracy; but when the foreign fats are substituted to the extent of 20 per cent. or more, the adulteration can be detected with certainty and an approximate quantitative determination made.

The detection of adulteration of butter-fat by other fats is generally carried out by the determination of the volatile acid, but some other confirmatory processes are occasionally employed.

Volatile Acids.—This method was first suggested by Hehner & Angell, but was systematized by Reichert, and hence is generally called the Reichert process. In this form it is carried out by saponifying 2.5 grams of the fat, adding excess of sulfuric acid, distilling a definite portion of the liquid, and titrating the distillate with $\frac{N}{10}$ alkali. The number of cubic centimeters of this solution required to overcome the acidity of the distillate is called the *Reichert number*. E. Meissl suggested the use of 5 grams, and the number so obtained is called the *Reichert-Meissl number*. Alcoholic solution of potassium hydroxid was originally used for saponification, but the solution devised by Leffmann & Beam, namely, sodium hydroxid in glycerol, is more satisfactory. The reagents and operation are as follows:

Glycerol-soda.—100 grams of pure sodium hydroxid are dissolved in 100 c.c. of distilled water and allowed to stand until clear. 20 c.c. of this solution are mixed with 180 c.c. of pure concentrated glycerol. The mixture can be conveniently kept in a capped bottle holding a 10 c.c. pipet, with a wide outlet.

Sulfuric Acid.—20 c.c. of pure concentrated sulfuric acid, made up with distilled water to 100 c.c.

Sodium Hydroxid.—An approximately decinormal, accurately standardized, solution of sodium hydroxid.

Indicator.—Solution of phenolphthalein or methyl-orange.

A 300 c.c. flask is washed thoroughly, rinsed with alcohol and then with ether, and thoroughly dried by heating in the water-oven. After cooling, it is allowed to stand for about 15 minutes and weighed. (In ordinary operation this preparation of the flask may be omitted.) A pipet, graduated to 5.75 c.c., is heated to about 60° and filled to the mark with the well-mixed fat, which is then run into the flask. After standing for about 15 minutes the flask and contents are weighed. 20 c.c. of the glycerol-soda are added and the flask heated over the Bunsen burner. The mixture may foam somewhat; this may be controlled, and the operation hastened by shaking the flask. When all the water has been driven off, the liquid will cease to boil, and if the heat and agitation be continued for a few moments, complete saponification will be effected, the mass becoming clear. The whole operation, exclusive of weighing the fat, requires about five minutes. The flask is withdrawn from the heat and the soap dissolved in 135 c.c. of water. The first portions of water should be added drop by drop, and the flask shaken between each addition in order to avoid foaming. When the soap is dissolved, 5 c.c. of the dilute sulfuric acid are added, a piece of pumice dropped in (this must not be omitted), and the liquid distilled until 110 c.c. have been collected. The condensing tube should be of glass, and the distillation con-

ducted at such a rate that the above amount of distillate is collected in 30 minutes.

The distillate is usually clear; if not, it should be thoroughly mixed, filtered through a dry filter, and 100 c.c. of the filtrate taken. A little of the indicator is added to the distillate, and the standard alkali run in from a buret until neutralization is attained. If only 100 c.c. of the distillate have been used for the titration, the number of cubic centimeters of alkali should be increased by one-tenth.

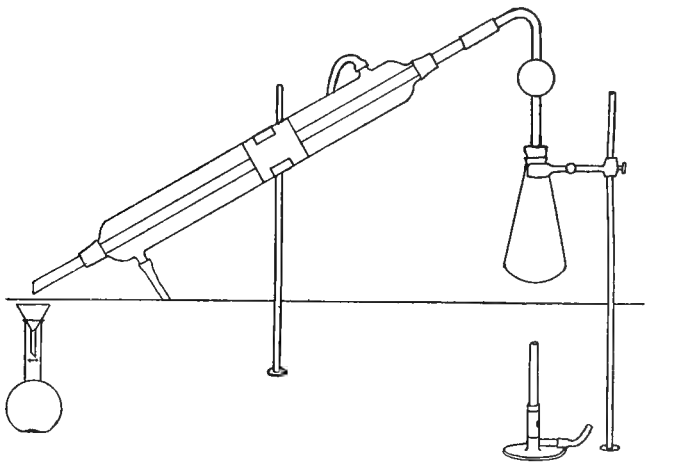

FIG. 8.

The distilling apparatus shown in figure 8 is that recommended by the A. O. A. C. (and since adopted in Great Britain), and the directions for preparing the flask are also from the same source, but when it is intended merely to distinguish butter from oleomargarin, it will be sufficient to saponify 3 c.c. of the clarified fat, dilute, acidify, distil 100 c.c. in the ordinary manner and titrate as directed. "Straight oleos," that is, samples containing inappreciable amounts of butter, will give a distillate requiring only a few c.c. of alkali.

Butter (5 grams) yields a distillate requiring from 24 to 34 c.c. of decinormal alkali. Several instances have been published in which genuine butter has given a figure as low as 22.5 c.c., but such results are uncommon. The materials employed in the preparation of oleomargarin yield a distillate requiring less than 1 c.c. of alkali. Commercial oleomargarin is usually churned with milk in order to secure a butter flavor, and, thus acquiring a small amount of butter-fat, yields distillates capable of neutralizing from 1 to 2 c.c. of alkali.

If coconut oil has been used in the preparation of the oleomargarin, the figure will be higher, but there will still be no difficulty in distinguishing pure butter.

The determination of the Reichert number will usually give sufficient information as to the nature of a butter sample. In doubtful cases it may be of advantage to apply other tests as corroborative evidence.

Saponification Value.—In the absence of coconut oil, the saponification value will give valuable indications as to the purity of a butter sample. It is possible to make oleomargarin, by the addition of coconut oil, which would have the same saponification value as pure butter.

Specific Gravity.—According to Skalweit, the greatest differences between the specific gravity of butter and its adulterants are found at a temperature of 35°, but the determination is more conveniently made at the temperature of boiling water. The Sprengel tube or Westphal balance may be employed for the purpose.

Index of Refraction.—This datum differs notably in different oils, but it is not of much value in detecting adulteration unless considerable of the adulterant be present. Several instruments have been devised for making refraction determination; the familiar ones are the refractometer of Abbé and the butyrorefractometer of Zeiss.

The butyrorefractometer has been strongly recommended for

the examination of butter. It is equally adapted for the general examination of fats and oils, and may be used for the determination of the index of refraction as well. As these instruments are made by only one firm and are furnished with directions for use, further description will not be required.

Milk test.—The following test was proposed by Waterhouse: 50 c.c. of fresh whole milk are placed in a 100 c.c. beaker, heated nearly to boiling and a lump of the sample (5 to 10 grams) stirred in, preferably with a wooden rod, until the fat is melted. The beaker is placed in cold water and the stirring continued until the temperature falls to the solidifying point of the fat. Butter fat will be granular and not easily collected into a lump, but oleomargarin will collect readily.

Refractometric Examination.—This is most satisfactorily made by the oleorefractometer or the butyrorefractometer. Jean prepares the sample for examination in the former as follows: 30 grams of butter are melted in a porcelain dish at a temperature not exceeding 50°, stirred well with a pinch or two of gypsum, and allowed to settle out at the same temperature. The supernatant fat is decanted through a hot-water funnel plugged with cotton and poured while warm into the prism of the apparatus, stirred with the thermometer until the fat has cooled to 45°, and the deviation observed. Ether must not be used for the solvent, as minute traces of it seriously influence the result.

Heating test.—Oleomargarin and butter exhibit characteristic differences on heating, which may be utilized for rapidly sorting a collection of samples. When butter is heated in a small tin dish directly over a gas flame, it melts quietly, foams, and may run over the dish. Oleomargarin, under the same conditions, sputters noisily as soon as heated and foams but little. Even mixtures of butter and other fats show this sputtering action to a considerable extent. The effect depends upon the condition in which the admixed water exists,

and the test is not applicable to butter which has been melted and reworked (renovated or process butter).

Saponification test.—An alcoholic solution of sodium hydroxid, boiled up with butter, and then emptied into cold water, gives a distinct odor of pineapples, while oleomargarin gives only the alcoholic odor.

Renovated Butter.—So-called "process" or "renovated" butter, made by rendering old or inferior samples, purifying the fat, coloring, salting, and molding it, is now a familiar commercial article. Process butter when heated in a dish sputters with but little foaming, as does oleomargarin; but yields with alcoholic soda the pineapple odor, as does butter. The fat of process butter gives refractometric data and Reichert-Meissl number similar to those of ordinary dairy butter, but is said to give a different figure with Valenta's test. If, therefore, a sample sputters in the pan, but gives the other reactions for butter, as just noted, it may be assumed to be process butter. Hess & Doolittle state that the curd of process butter has characteristic qualities, and propose the following method for detecting it:

50 grams of the sample are melted in a beaker at about 50°. Ordinary butter yields a clear fat almost as soon as melted, while with process butter the fat may remain turbid for a long while. When the curd has largely settled, as much of the fat is poured off as possible, and the remaining mixture is thrown on a wet filter, by which the water will drain away, carrying the soluble proteids and salt. A few drops of acetic acid are added to the filtrate and the mixture is boiled. The filtrate from ordinary butter gives a slight milkiness, but that from process butter gives a flocculent precipitate. Quantitative examination is made by dissolving 50 grams of the sample in ether; if it is ordinary butter, the curd is so finely divided that it remains suspended for some time. As much as possible of the solution is decanted and the mass transferred

to a separator, the casein, water, and salt removed, and the remainder washed three times, at least, with ether to remove the fat. The curd is collected on a filter, washed with water, and the nitrogen determined by treating the precipitate with the filter by the Kjeldahl-Gunning method. The filtrate from the curd is made slightly acid with acetic acid, boiled, the precipitated proteids collected on a filter, and the total nitrogen determined. The factor 6.38 may be used in each case for converting the nitrogen into proteids.

A distinction between ordinary and process butter may often be made by microscopic examination under polarized light with crossed nicols (*i. e.*, dark field), when the process butter appears mottled, owing to the presence of crystals.

Butter Colors.—Butter and butter substitutes are usually artificially colored. Preparations of turmeric and annatto or azo-colors allied to methyl-orange are used. The latter forms may be detected by the test devised by Geisler. A small amount of the sample, or, better, the fat filtered from it, is mixed on a porcelain plate with a little fullers' earth. Azo-colors give promptly a red mass, while if they are not present, the mixture becomes only yellow or light brown. All samples of fullers' earth are not equally active, and tests should be made with different samples by using fat known to contain the azo-compound until a good specimen of the earth is secured.

For the detection of very minute quantities of the color, the sample may be dissolved in light petroleum, and the fullers' earth added to the solution, when the pink color will appear as a distinct ring or zone at the edge of the deposited layer of the reagent.

Low has proposed the following test for the yellow azo-color: A few cubic centimeters of the filtered fat are mixed in a large test-tube with an equal volume of a mixture of one part strong sulfuric acid and four parts glacial acetic acid. The contents of the tube are then heated almost to boiling and thoroughly

mixed by violently agitating the bottom of the tube. When now allowed to stand and separate, the lower layer of mixed acids will be strongly colored wine-red if the azo-color be present. Pure butter-fat imparts no color to the acids, or, at most, only a faint brownish tinge.

For turmeric and annatto mixtures, Martin's test will usually be satisfactory: 2 c.c. carbon disulfid are mixed with 15 c.c. of alcohol, by adding small portions of the disulfid to the alcohol and shaking gently; 5 grams of the butter-fat are added to this mixture in a test-tube and shaken. The disulfid falls to the bottom of the tube, carrying with it the fatty matter, while any artificial coloring-matter remains in the alcohol. The separation takes place in from one to three minutes. If the amount of the coloring-matter is small, more of the fat may be used. If the alcoholic solution be evaporated to dryness and the residue treated with concentrated sulfuric acid, annatto will be indicated by the production of a greenish-blue color. With many samples of oleomargarin, a pink tint will be produced, which indicates an azo-color.

Palm oil is sometimes used as a coloring agent in butter-substitutes. Crampton & Simons have found that two tests devised for detection of rosin-oil can be satisfactorily adapted to detection of palm oil. Success depends on several points. The sample must be kept in a cool dark place until used, filtered at a temperature not above 70°, the heating as brief as possible, and promptly tested. The reagents must be pure and colorless.

Cochran finds that annatto will simulate palm oil in these tests, and hence the absence of the former must be assured (see above) before inferring the presence of the latter.

Halphen method. 100 c.c. of the filtered fat are dissolved in 300 c.c. petroleum spirit and shaken out with 50 c.c. of potassium hydroxid solution (0.5 per cent. of hydroxid). The water is drawn off, made distinctly acid with hydrochloric acid, and shaken out with 10 c.c. of carbon tetrachlorid. This solution

is drawn off, and part of it tested by adding to it 2 c.c. of a mixture of 1 part crystallized phenol in 2 parts carbon tetrachlorid. To this add 5 drops of hydrobromic acid (sp. gr. 1.19). The test is best performed in a porcelain basin and the contents mixed by agitating gently. Palm oil gives almost immediately a bluish-green liquid.

Liebermann-Storch method. 10 c.c. of the filtered fat are shaken with an equal volume of acetic anhydrid, one drop of sulfuric acid (sp. gr. 1.53) is added and the mixture shaken for a few seconds. If palm oil be present, the heavier layer separating will be blue with a tint of green.

Egg-yolk has been proposed as a color for oleomargarin, and although its use is unlikely, the possibility of it should be borne in mind. To detect it, about 10 grams of the filtered fat should be shaken with warm alcohol, the liquid drawn off as closely as possible and evaporated to dryness. The coloring matter of egg-yolk is soluble in alcohol, but insoluble in water. It may be distinguished from turmeric by moistening it with a few drops of a mixture of boric and hydrochloric acids, and drying at a gentle heat. Turmeric becomes brown; egg-color is not affected. Egg-yolk contains considerable lecithin, a phosphoric acid derivative. Pure fats contain no phosphorus compound. If, therefore, a few grams of the fat, carefully freed from water or curd, are charred and the mass extracted by boiling with nitric acid, the filtered solution should not give an appreciable precipitate with ammonium molybdate.

Vegetable colors may be detected by boiling up the filtered fat with water, drawing off the watery liquid, adding a few drops of hydrochloric acid and heating the mixture with a piece of clean, undyed wool. True butter colors will not dye wool under these circumstances.

Caramel may be detected by shaking the watery solution with fuller's earth and filtering. The filtrate is notably paler if

caramel was present. Fuller's earth varies in efficiency, and each sample should be tested on known solutions.

Preservatives.—The preservatives used in milk may be found in limited amount in butter, but a mixture of boric acid and borax is often added as a substitute for salt.

Glucose is sometimes used as a preservative, especially in butter intended for export to tropical countries. Crampton found as much as 10 per cent. in a sample of highly colored butter intended for exportation to Guadeloupe. For the detection of glucose the phenylhydrazin test might be used. For determination Crampton used the following method: 10 grams of the sample were washed with successive portions of convenient bulk, the solution made up to 250 c.c., and an aliquot portion determined, as given for lactose on page 27. The solution may also be clarified by alumina-cream or acid mercuric nitrate and examined in the polarimeter.

Geisler found *paraffin* in oleomargarin; he uses the specific gravity of the rendered fat as a sorting test, making special examination only of samples that show below 0.9018 at $\frac{37.8°}{37.8°}$. Microscopic examination under polarized light will often show amorphous masses of paraffin mixed with the crystals of fat. To isolate the paraffin, Geisler saponifies 2.5 grams of the fat with 20 c.c. of alcohol and 1 gram of potassium hydroxid, and dilutes the liquid with an equal bulk of water. By alternately heating and cooling the liquid much of the unsaponifiable matter may be collected. Most fats contain unsaponifiable matter, and hence the material must be identified as paraffin.

CHEESE

Cheese is the curd of milk which has been separated from it, pressed, and undergone some fermentation. The precipitation is produced either by allowing the milk to become sour

—when the lactic acid is the agent—or by rennet. The first-named method is mainly applied to the manufacture of so-called Dutch or sour-milk cheese, green Swiss cheese, and cottage cheese. More commonly cheese is obtained by means of rennet derived from the fourth stomach of the calf. The action is due to an enzym which acts directly on the proteids and does not produce its effect through the intervention of acids. The curd (cheese) undergoes, by keeping, various decompositions, some essentially putrefactive, and due to the action of microbes. The decomposition of the cheese is termed "ripening."

In the sour milk cheeses, ripening is restricted intentionally, since there is liability to an irregular and miscellaneous bacterial growth by which the fermentations may be carried too far, undesirable and even harmful products being formed. Such cheeses are intended for prompt use.

Cheese contains no casein, if by this term is meant the proteid as it exists in milk, or when precipitated from milk by acids. When milk is coagulated by rennet, only a part of the proteids enter into the curd; true casein contains about 15.7 per cent. of nitrogen, but the proteid matter of cheese contains about 14.3 per cent. Under the process of ripening this is further decomposed, amido- and ammonium compounds, peptones and albumoses being formed.

The following figures, obtained by Van Slyke, will serve to give some idea of the extent to which the curd is changed in ripening. The figures represent average percentage on the total nitrogen. The cheese was an American cheddar:

	Green Cheese.	After Five Months.
Soluble nitrogen compounds,	4.23	35.52
" amido "	none	11.66
" ammonium "	none	2.92

Van Slyke's experiments seem also to indicate that the cheese ripened more rapidly when the curd was precipitated by a

larger quantity of rennet and, especially, that cheese rich in fat ripened more rapidly than skim-milk cheese.

In addition to the fat and nitrogenous compounds just mentioned, cheese may contain a small amount of milk-sugar and of lactic and other organic acids. There is present also a certain proportion of mineral matter, alkaline and earthy phosphates, along with any salt that has been added. Traces of nitrates have been found.

Skimmed milk is not infrequently used for the production of cheese. Partially-skimmed milk is used in the preparation of certain Dutch cheeses. Foreign fats, such as are used in the manufacture of oleomargarin, are sometimes incorporated, the article being known as "filled cheese."

The ash of cheese consists largely of calcium phosphate and salt. Mariani & Tasselli have estimated the total ash, chlorin, calcium, and phosphoric acid in 15 samples of cheese. The amounts of salts (calculated from the chlorin) depend on the mode of salting. The proportion of phosphoric oxid was always greater than that necessary to form tricalcium phosphate, ranging from 1.07 and 1.08 equivalents of phosphoric anhydrid to calcium oxid in cheese made from sour milk to 1.56 to 1 in Gorgonzola, 1.67 to 1 in skim-milk cheese, and 1.75 to 1 in Edam cheese. The largest quantities of calcium and phosphoric oxid were found in sheep's-milk cheese and in cheese made from sour milk, whence it follows that acidity does not prevent the precipitation of calcium phosphate in the curds. The excess of phosphoric oxid obtained was attributed to acid phosphates.

The salt in cheese usually ranges between 1 and 4 per cent.

Analytic Methods.—The analytic points usually determined in regard to cheese are water, fat, casein, ash, the presence of fats other than butter-fat, and coloring-matters.

In addition to this, especially in comparing the qualities of

genuine cheeses, the proportion of proteic, amidic, and ammoniacal nitrogen is of value.

Care should be taken to select for analysis a sample which represents the average composition of the entire cheese.

The following methods for the determination of water, fat, ash, total nitrogen, and acidity have been adopted by the A. O. A. C.:

Sampling.—When the cheese can be cut, a narrow wedge-shaped segment, reaching from the outer edge to the center of the cheese, is taken. This is to be cut into strips and passed through a sausage-grinding machine three times. When the cheese cannot be cut, samples are taken by a cheese trier. If only one plug can be obtained, this should be perpendicular to the surface, at a point one-third of the distance from the edge to the center of the cheese. The plug should reach entirely through, or only half-way through, the cheese. When possible, draw three plugs—one from the center, one from a point near the outer edge, and one from a point half-way between the other two. For inspection purposes, the rind may be rejected; but for investigations requiring the absolute amount of fat in the cheese, the rind is included in the sample. It is preferable to grind the plugs in a sausage machine, but when this is not done, they should be cut very fine and carefully mixed.

Water.—Between 2 and 5 grams of the sample should be placed in a weighed platinum or porcelain dish which contains a small amount of material, such as freshly ignited asbestos or sand, to absorb the fat that may run out. This is then heated in a water-oven for 10 hours and weighed; the loss in weight is considered as water. If preferred, the dish may be placed in a desiccator over concentrated sulfuric acid and dried to constant weight, but this may require many days. The acid should be renewed when the cheese has become nearly dry.

Fat.—The extraction-tube described on page 14 is prepared as follows: Cover the perforations in the bottom of the tube

with asbestos, and on this place a mixture containing equal parts of anhydrous copper sulfate and pure dry sand to the depth of about 5 cm., packing loosely, and cover the upper surface with a film of asbestos. On this are placed from 2 to 5 grams of the sample, the mass extracted for 5 hours with anhydrous ether, then removed and ground to fine powder with pure sand in a mortar. The mixture is placed in the extraction tube, the mortar washed free from all matters with ether, the washings being added to the tube, and the extraction is continued for 10 hours. The fat so obtained is dried at 100° to constant weight.

Here, as in most extractions, carbon tetrachlorid can be substituted for ether, but the results obtained are not necessarily equivalent.

Total Nitrogen.—This is determined by the Kjeldahl-Gunning method, using 2 grams of the sample. The percentage, multiplied by 6.38, gives the nitrogen compounds.

Ash.—The dry residue from the water determination may be taken for the ash. If the cheese be rich, the asbestos will be saturated therewith. This mass may be ignited carefully, and the fat allowed to burn off, the asbestos acting as a wick. No extra heating should be applied during the operation, as there is danger of spurting. When the flame has died out, the burning may be completed in a muffle at low redness. When desired, the salt may be determined in the ash by titration with silver nitrate and potassium chromate.

Provisional Method for the Determination of the Acidity in Cheese.—Water at a temperature of 40° is added to 10 grams of finely divided cheese until the volume equals 105 c.c., agitated vigorously, and filtered. Portions of 25 c.c. of the filtrate corresponding to 2.5 grams of the cheese are titrated with decinormal solution of sodium hydroxid, using phenol-phthalein as indicator. The amount of acid is expressed as lactic acid.

The above processes may be advantageously modified in

some respects. The determination of water may be made by the extraction of the cheese with alcohol and ether and drying of the alcohol-ether extract and fat-free solids separately. Blyth recommends this method as more accurate and less tedious than the direct drying. In the determination of ash it will be better to extract the charred mass with water and proceed as described in the determination of the ash of milk.

The fat extracted by ether may be examined for other than butter-fat by the distillation method in the usual way. When the composition of the fat is alone desired, it may often be extracted by simple methods. Pearmain & Moor recommend that 50 grams be chopped fine and tied up in a muslin bag, which is placed in a water-bath. When the water is heated, the fat will generally run out clear. If not clear, it can be filtered through paper.

Henzold suggests the following: 300 grams of the powdered cheese are agitated in a wide-neck flask with 700 c.c. of 5 per cent. solution of potassium hydroxid previously warmed to 20°. In about 10 minutes the cheese dissolves, the fat floats, and by cautious shaking may be collected in lumps. The liquid is diluted, the fat removed, washed in very cold water, kneaded as dry as possible, melted, and filtered. It is claimed that the fat is not altered in composition by the process.

The fat of cheese may be estimated by the centrifugal method, as follows:

About 3 grams of the mixed cheese in small fragments are weighed and transferred to the bottle, the last portions being washed in with the acid of water. A few drops of ammonium hydroxid are added, and sufficient water to make the liquid about 15 c.c. The liquid is warmed with occasional shaking until the cheese is well disintegrated, and then treated as a sample of milk. The percentage of fat is found by multiplying the percentage reading by 15.45 and dividing by the number of grams of cheese taken for analysis.

Chattaway, Pearmain & Moor use the following modification: 2 grams of the cheese are placed in a small dish and heated on the water-bath with 30 c.c. of concentrated hydrochloric acid until a dark, purplish-colored solution is produced. The mixture is now poured into the test bottle, portions of solution remaining in the dish rinsed with the hydrochloric acid fusel-oil mixture into the bottle, and, finally, enough strong hot acid added to fill the bottle up to the mark. It is then whirled for about a minute. The difficulty in this method is to get all the fat into the bottle. It is best to weigh the cheese in the bottle.

Bondzynksi applies the Werner-Schmid method to the determination of fat in cheese, as follows: A weighed quantity of the finely-shredded cheese is placed in the tube and decomposed with 20 c.c. hydrochloric acid of specific gravity 1.1, containing about 19 per cent. true acid. On cautiously warming over wire gauze, the melted fat rises to the surface. After cooling, 30 c.c. of ether are added and the tube warmed very gently until the acid and ethereal solution of fat separate sharply. Centrifugal force helps this, but is not essential. After the volume of ether has been read off, 20 c.c. are pipetted off into a weighed Erlenmeyer flask. From this, the quantity of fat in the entire solution may be calculated.

The fat of cheese may also be estimated by Cochran's method, page 48.

Lactose.—This may be estimated by boiling the finely divided cheese with water, filtering, and determining the reducing power of the filtrate on Fehling's solution.

Determination of Albuminoid Nitrogen (Stutzer's Method).—0.7 to 0.8 gram of the cheese are placed in a beaker, heated to boiling, 2 or 3 c.c. of saturated alum solution added to decompose alkaline phosphate, then copper hydroxid mixture (see below) containing about 0.5 gram of the hydroxid, and stirred in thoroughly; when cold, the mass is filtered, washed with

cold water, and, without removing the precipitate from the filter, the nitrogen determined by the Kjeldahl-Gunning method. Before distillation, sufficient potassium sulfid solution must be added to precipitate the copper.

The special reagent is prepared as follows: 100 grams of copper sulfate are dissolved in 5000 c.c. of water, 25 c.c. of glycerol added, and then a dilute solution of sodium hydroxid until the liquid is alkaline. The mass is filtered, the precipitate is mixed well with water containing 5 c.c. of glycerol per liter, and washed until the washings are no longer alkaline. It is then rubbed up with a mixture of 90 per cent. water and 10 per cent. glycerol in sufficient quantity to obtain a uniform magma that can be measured with a pipet. The quantity of copper hydroxid per c.c. should be determined. It should be kept in a well-closed bottle.

Ammonium Compounds.—About 5 grams of cheese are rubbed up in a mortar with water, transferred to a filter, and washed with a liter of cold water. The filtrate is concentrated by boiling (if alkaline, it must be neutralized before heating), barium carbonate added, the liquid distilled, and the ammonium hydroxid in the distillate estimated by titration with standard acid.

According to Stutzer, magnesia or magnesium carbonate (the latter usually contains some magnesia) should not be used to free the ammonia, as some of the amido-compounds may be decomposed.

Amido-compounds.—The nitrogen as amido-compounds is estimated by subtracting from the figure for total nitrogen the sum of the proteid and ammoniacal nitrogen. If nitrates are present, the nitrogen as such should also be determined and subtracted.

Van Ketel & Antusch propose the following methods for estimating the nitrogen compounds:

Ammonium Compounds.—The sample, powdered with the

addition of sand, is distilled with water and barium carbonate, and the distillate received in a measured quantity of standard sulfuric acid, and, after boiling, the excess of acid is neutralized with standard sodium hydroxid, using rosolic acid as indicator.

Amido-compounds.—These are estimated by macerating the powdered cheese in water for 15 hours at the ordinary temperature. After adding a little dilute sulfuric acid (1 : 4), the proteids and peptones are precipitated by phosphotungstic acid. The precipitate is filtered off and washed with water containing a little sulfuric acid. The filtrate is made up to a definite bulk, and the nitrogen is determined in an aliquot portion of the liquid by the Kjeldahl-Gunning process, allowance being made for the nitrogen existing as ammonium.

Peptones and Albumoses.—These are determined jointly by boiling the powdered cheese (mixed with sand as before) with water and filtering from the undissolved casein and albumin. In an aliquot portion of the filtrate the peptones and albumoses are precipitated by adding dilute sulfuric acid and phosphotungstic acid. After washing with acidulated water the nitrogen in the precipitate is determined by the Kjeldahl-Gunning process.

The total nitrogen of the cheese is also determined, and after allowing for the nitrogen existing as other forms, the balance is calculated to casein.

Poisonous Metals.—Lead chromate has been found in the rind of cheese, and finely divided lead in a number of Canadian cheeses. In England zinc sulfate has been employed under the name of cheese spice to prevent the heading and cracking. Arsenic has also been found; it may be detected by Reinsch's test. Lead, zinc, and chromium may be detected by ashing a portion of the sample in a porcelain crucible and applying the usual tests.

ANALYSES OF VARIOUS CHEESES

(Reports by W. A. Chattaway, T. M. Pearmain, and C. G. Moor)

Name.	Water.	Ash	Fat.	Reichert-Meissl Number.	N.
Cheddar,	33.0	4.3	29.5	24.2	4.31
Gorgonzola,	40.3	5.3	26.1	22.1	4.36
Dutch,	41.8	6.3	10.6	27.0	5.11
Gruyère,	28.2	4.7	28.6	30.0	4.93
Stilton,	19.4	2.6	42.2	29.0	4.73
Cheshire,	37.8	4.2	31.3	31.6	4.03
Gloucester,	33.1	5.0	23.5	31.4	4.99
Camembert,	47.9	4.7	41.9	31.0	3.83
Parmesan,	32.5	6.2	17.1	28.0	6.86
Roquefort,	29.6	6.7	30.3	36.8	4.45
Double Cream,	57.6	3.4	39.3	31.2	3.14
Filled (United States),	30.6	3.6	27.7	3.0	4.84

The common American cheese is known as Cheddar. According to Van Slyke, this has, when ripe, about the following average composition:

Water,	31.50 per cent.
Fat,	37.00 "
Proteids,	26.25 "
Ash, sugar, etc.,	5.25 "

FERMENTED MILK PRODUCTS

The usual fermentation of milk is the conversion of the lactose into lactic acid, but by special methods other changes may be substituted. These modified fermentations are of rather ancient origin, and being produced by mixture of organisms, the products are complex and irregular. The proteids are more or less changed into proteoses and peptones.

Kumiss is milk which has undergone alcoholic fermentation. The inhabitants of the steppes of Russia prepare it from mares' milk. When cows' milk is used, cane-sugar must be added. It is often made by adding cane-sugar and yeast to skim-milk.

P. Vieth gives the following analysis of kumiss at successive stages of fermentation:

KUMISS FROM COWS' MILK

	One Day.	One Week.	One Month.	Three Months.
Alcohol,	1.1	0.9	1.0	1.1
Solids,	11.3	8.9	8.6	8.5
Fat,	1.6	1.4	1.5	1.5
Casein,	2.0	2.0	1.9	1.7
Albumin,	0.3	0.2	0.2	0.1
Sugar,	6.1	3.1	2.2	1.7
Lactic acid,	0.2	0.9	1.3	1.9
Lactoproteid and peptone,	0.3	0.5	0.7	0.9
Soluble ash,	0.1	0.2	0.2	0.2
Insoluble ash,	0.4	0.3	0.3	0.3

The item "lactoproteid and peptone" refers to the substances precipitated by tannin after removal of the casein and albumin.

KUMISS FROM MARES' MILK

At the End of:	Alcohol.	Fat.	Nitrogenous Matters.	Lactic Acid.	Sugar.	Ash.
1 day,	2.47	1.08	2.25	0.64	2.21	0.36
8 days,	2.70	1.13	2.00	1.16	0.69	0.37
22 "	2.84	1.27	1.97	1.26	0.51	0.36

Kefyr.—This is usually made from cows' milk. It has been used in the Caucasus for centuries. For its preparation a peculiar ferment is used, which is contained in the kefyr grains. These are first soaked in water, by which they are caused to swell and are rendered more active, and then added to the milk. If taken out of the milk and dried, the grains may be used repeatedly.

The following are analyses of kefyr:

	König.	Hammarsten.
Alcohol,	0.75	0.72
Fat,	1.44	3.08
Casein,	2.88	2.94
Albumin,	0.36	0.18
Hemialbumose,	0.26	0.07
Peptone,	0.04	
Sugar,	2.41	2.68
Lactic Acid,	1.02	0.73
Ash,	0.68	0.71

According to König, good kefyr will not contain more than 1 per cent. of lactic acid.

Analytic Methods.—Fixed solids and ash are determined by evaporations of a weighed amount in a platinum basin as described on page 11. Acidity is determined by filtration with $\frac{N}{10}$ alkali, using phenolphthalein or methyl-orange as an indicator. The amount of acidity is expressed in terms of lactic acid. The Kjeldahl-Gunning method will give the total nitrogen. For further examination of the nitrogenous bodies, the methods given on pages 71 and 73 may be applied. Total reducing sugars may be estimated as given on page 27. If sucrose and common yeast have been added, the fermented material will be likely to contain invert-sugar, with unchanged lactose and sucrose, and the method of examination of sweetened condensed milk may be applicable. Fat can, probably in all cases, be determined with sufficient accuracy by the L-B. process. If it be desired to make polarimetric readings, the liquid should be clarified with acid mercuric nitrate solution (page 30), as some partly hydrolyzed proteids which have rotatory power may not be precipitated by other reagents. The determination of alcohol accurately is difficult, as the quantity is usually small. The cautious distillation of a considerable volume of the material previously neutralized with a little sodium hydroxid will yield a distillate in which alcohol may be determined by specific gravity.

Preservatives are not likely to be used, since they would interfere with the fermentation, but attempts may be made to secure better keeping by adding some preservative after the fermentation has occurred. In some cases, therefore, tests for boric acid, formaldehyde, and salicylic acid should be made, as these will be most likely to be used.

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