Methods For

Analysis of Cottage Cheese

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The increasing production of cottage cheese and the increasing interest in control of its sanitary quality and composition emphasizes the need for rapid and reliable methods for use in dairy plants. A review of the literature on cottage cheese reveals that the methods of analyzing this product have received little attention compared with the data available on most other dairy products. It appears that some of the methods advocated need further study to make them applicable to cottage cheese.

The work herein reported was undertaken to evaluate methods of analyzing cottage cheese and to attempt to improve these methods or develop new ones which would be practical for use in dairy plants. The study included investigations of methods for determining yeasts and molds, fat, salt and moisture.

Review of Literature

The accepted methods for determining yeast and mold counts on cottage cheese are given in *Standard Methods for the Examination of Dairy Products* (16). These methods were modified somewhat by Morgan et al. (11) by employing sterile glass beads to assist in breaking up the curd and by using a modified plating medium. Later (11) these investigations used sodium citrate to help disperse the curd in the dilution blanks. Oklahoma Agricultural Experiment Station workers (12) used a method whereby the curd was dispersed by grinding the cheese in a sterile mortar with a sterile solution of sodium citrate. An additional modification involved the use of 100 ppm of aureomycin hydrochloride to suppress bacterial growth in the potato-dextrose agar plating medium.

Goss (4) presented a modification of the Babcock test for cottage cheese in which the test is run in 9 gm-50% cream test bottles. Other methods given for fat in cottage cheese which involve ether extraction include the ones given by the Association of Official Agricultural Chemists (2) and the Mojonnier Method (10).

Methods for analyzing cottage cheese for salt content have received more attention than those for other factors. Probably the most common method employed is the one recommended by the A. O. A. C. (2). This method involves digestion of the curd with nitric acid and potassium permanganate in the presence of silver nitrate and titration of the excess silver nitrate with potassium thiocyanate, using ferric alum as the indicator. Wilster et al. (19) gave a modification using a different strength standard solution as a convenience in calculating the results. Arbuckle (1) presented a method for direct titration with mercuric nitrate, using S-diphenylcarbazone as the indicator. Marquardt (8) gave two methods for direct titration which differed in the method of preparation of the samples; both involved direct titration with silver nitrate, using dipotassium chromate or dichlorofluorescein as the indicator.

The official method for moisture testing in cottage cheese (2) involves drying for several hours in a vacuum oven. Wilster et al. (19) recommended three methods for use in laboratory analysis and for use in routine analysis in cheese factories, depending on the degree of accuracy desired. All involved either oven or vacuum oven drying. Morgan et al. (11) reported on the use of toluene distillation in a Bidwell-Sterling tube for moisture analysis of cottage cheese. They also reported on the use of a Dietert-Detroit apparatus. Gould (5,6) presented comparative data on results obtained by using the Mojonnier and steam pressure oven methods and a method involving the use of olive oil. In this latter method, the moisture was driven off by heating the weighed samples in olive oil. Kosikowski et al. (7) reported on a rapid and fairly accurate method for determining moisture in cheddar cheese, processed cheese, and cheese food. The Mojonnier Method (10) for determining moisture in cottage cheese is used by many plants having the necessary equipment.

Control Methods

Certain methods were selected for use as control methods for the various analyses. These, in general, were commonly accepted methods with some modifications.

Yeast and Mold Counts

One gram of a thoroughly mixed sample of cottage cheese was weighed onto a piece of sterile parchment paper, transferred to a sterile mortar and thoroughly ground to a creamy consistency after adding 1 ml of sterile 20% sodium citrate. Eight ml of sterile water contained in a screw-capped test tube was mixed with the ground cheese and the mixture poured back into the test tube. This constituted a 1 to 10 dilution. Suitable serial dilutions were plated with potato-dextrose agar adjusted to a pH of 3.5 with sterile tartaric acid and the plates incubated at 21°C. for 5 days and then counted.

Fat Determinations

The Mojonnier Method (10) was used for the control fat determinations, using 5 gm of cottage cheese, and mixing with 5 ml of hot distilled water and 6 ml of ammonium hydroxide before proceeding with the fat extraction as for fat in milk.

Salt Determinations

The salt was determined by the Wilster et al. (19) modification of the A.O.A.C. method (2) which involved adding 10 ml of 0.1711 N silver nitrate to 3 gm of cheese and digesting with 15 ml of nitric acid, 50 ml of water and 15 ml of saturated potassium permanganate solution. The digested mixture was diluted with 100 ml water, decanted and precipitate washed with 100 ml water. The excess silver nitrate was titrated with 0.1711 N potassium thiocyanate, using ferrous ammonium sulfate as the indicator. The percent of salt was calculated by dividing the my of silver nitrate which combined with the salt by the weight of the sample.

Moisture Determinations

Control moisture determinations were made by the Mojonnier Method (10) which was briefly as follows: 0.5 gm of cottage cheese was weighed into a Mojonnier solids dish, mixed with 2 ml distilled water to spread evenly over the bottom of the dish, dried on a hot plate at 180°C. and then at 100°C. under 23 inches of vacuum for 20 minutes. After cooling in a desiccator for 5 minutes the dishes were reweighed and the moisture calculated.

Experimental

Yeast and Mold Counts

Aureomycin as a Bacterial Inhibitor

In earlier work at the Oklahoma Agricultural Experiment Station (12) it was found that yeasts and molds were able to grow well in high concentrations (more than 2,000 ppm) of aureomycin and other antibiotics, while bacteria were inhibited by low concentrations, generally 10 ppm or less. Aureomycin appeared to give the best results although other antibiotics including penicillin, streptomycin, terramycin and others, also were satisfactory. Since aureomycin did not precipitate the casein and did not inhibit the yeasts and molds, it appeared that this compound would be especially useful for yeast and mold counts on cottage cheese.

A concentration of 100 ppm of aureomycin hydrochloride was selected to compare the effectiveness of this compound with the counts obtained on acidified potato-dextrose agar, although considerably lower or higher concentrations could be used as effectively. Comparisons were run in which the curd was dispersed in various ways in preparing the dilutions and the dilutions plated on both acidified (pH 3.5) potato-dextrose agar and the agar with 100 ppm aureomycin added. The stock solution of aureomycin was prepared as a 1% solution in sterile water. One ml of the solution was added to 100 ml of the agar after melting and cooling to pouring temperature. The plates were incubated 5 days at 21° to 25°C. and then counted.

The results show that in 105 counts on commercial cottage cheese higher counts were obtained in 82 instances on the plates with the aureomycin added. The average number of colonies per plate with aureomycin added was 115, while the average for the plates with acidified agar was 89, a difference of 25% in favor of the aureomycin agar.

In a number of instances in which large discrepancies occurred between the counts on the two media, colonies were picked from the plates which looked suspicious and examined microscopically, but in no instance were any bacterial colonies detected.

The advantages of the use of aureomycin hydrochloride added to the agar to inhibit bacterial growth are: (1) there is no precipitation of the casein to make counting difficult, (2) the colonies produced are larger and easier to distinguish, and (3) the counts are generally higher than those on acidified agar and appear to be more accurate. All evidence points to the fact that potato-dextrose agar with 100 parts per million aureomycin hydrochloride added just prior to pouring the plates is a superior medium to potato-dextrose agar which has been acidified to pH of 3.5 with tartaric acid.

Methods for Dispersing Cottage Cheese Curd

Use of Chemical Compounds

Since various chemical compounds are used as emulsifiers for making process cheese, it was thought that some chemical might be satisfactory for dispersing the curd for yeast and mold counts on cottage cheese. The general procedure was as follows: The cheese was ground in a food grinder which forced the cheeese through a screen with holes approximately 0.5 mm in diameter. The ground cheese was mixed well and 1 gm of the sample weighed into screw capped test tubes containing 9 ml of an aqueous solution of a chemical compound and a few glass beads. The mixture was agitated gently by continuous inversion at the rate of approximately 30 times per minute for three minutes, using a stop watch to time the procedure. The mixture was then observed for the extent of dispersion of the curd.

Various concentrations of a total of 23 chemical compounds were used in this experiment. Preliminary trials were run with each compound to determine the approximate concentration required to dissolve the curd. A compound was considered to be satisfactory if it dissolved the curd completely in a 3-minute period. On this basis the following compounds were considered unsatisfactory: ammonium citrate, calcium chloride, potassium monohydrogen phosphate, potassium dihydrogen phosphate, sodium acetate, sodium lactate, sodium oxalate, sodium dihydrogen phosphate, sodium sulphate, and tricalcium phosphate.

No further tests on these compounds were made. The minimum concentrations of the compounds that were satisfactory in dissolving or dispersing the curd were as follows: ammonium carbonate, 0.2%; ammonium hydroxide, 0.2%; ammonium phosphate, 0.5%; sodium monohydrogen phosphate, 1.25%; lithium hydroxide, 0.2%; Minnesota reagent, 1.5%; potassium hydroxide, 0.4%; sodium borate, 1.0%; sodium citrate, 2.0%; sodium hydroxide, 0.1%; sodium pyrophosphate, 1.5%; sodium sesquicarbonate, 1.0%; and trisodium phosphate, 1.25%.

With sodium citrate in effective concentrations, the mixtures were cloudy due to the suspension of fine particles of curd, while with the rest of the compounds that were satisfactory the solutions were practically clear.

The results indicate that the compounds which were satisfactory in dispersing or dissolving the curd would be satisfactory for yeast and mold counts if they did not have a toxic effect on the organisms or otherwise influence the accuracy of the counts. Accordingly, a series of trials was run to determine the influence on yeast and mold counts of these compounds used to disperse the curd. The same procedure as given above was used in dispersing the curd. At the end of 3 minutes, the necessary dilutions were made and duplicate plates were poured. Additional platings were made after the cheese and chemical mixture was allowed to stand for 3 minutes and for 6 minutes. One plate of each pair of duplicate plates was poured with potato-dextrose agar (pH 3.5) and the other plate was poured with potato-dextrose agar with 100 ppm of aureomycin hydrochloride added. The results are shown in Table 1. The results with lithium hydroxide, potassium hydroxide, sodium hydroxide, and Minnesota Reagent are not shown because they appeared to be rather toxic to yeasts and molds. Only two samples of cheese were run with these compounds as the dispersing agents.

The results on the 9 compounds shown in Table 1 indicate that,

Table 1.—The influence of various chemical compounds used to dissolve the curd on the yeast and mold counts on cottage cheese.

Compound	Concentration	Number of Colonies on Potato Dextrose Agar with: 100 ppm Aureomycin Added, Exposure Time (Minutes) 3	6	12	Tartaric Acid Added to pH 3.5, Exposure Time (Minutes) 3	6	12
		Series I (Average of 5 Samples)					
Ammonium carbonate	0.20%	149	156	146	70	55	58
Ammonium hydroxide	0.20%	152	133	131	52	52	58
Ammonium phosphate	0.50%	135	143	147	68	66	68
Control		169	168	167	120	102	100
		Series II (Average of 3 Samples)					
Sodium borate	1.0 %	132	130	118	96	96	116
Sodium citrate	2.0 %	133	128	140	121	121	116
Sodium mono-H phosphate	1.25%	128	144	147	120	131	138
Trisodium phosphate	1.25%	101	94	58	74	66	38
Sodium pyrophosphate	1.5 %	138	109	117	122	98	86
Sodium sesquicarbonate	1.0 %	127	125	108	122	122	93
Control		132			120		

when plated on aureomycin agar the compounds that appeared to be most satisfactory for dispersing the curd were ammonium carbonate, ammonium phosphate, sodium citrate and sodium monohydrogen phosphate. When plated on acidified agar, those that appeared most satisfactory were sodium citrate and sodium monohydrogen phosphate. Ammonium hydroxide and trisodium phosphate appeared to be rather toxic while the remaining compounds appeared to be fairly satisfactory, especially when the samples were plated on aureomycin agar. It should be noted that the counts obtained with aureomycin agar were considerably higher and appeared to be more consistent than those obtained with acidified agar.

The foregoing results indicate that sodium citrate and sodium monohydrogen phosphate were the most satisfactory for dispersing cottage cheese curd for running yeast and mold counts because they dissolve or disperse the cottage cheese curd satisfactorily and do not seem to be toxic to yeast and molds. It also appeared that ammonium carbonate, and ammonium phosphate, would be satisfactory when using aureomycin agar, but not when using acidified agar.

In order to obtain more supporting data for the yeast and mold determinations, a third series of trials was run with the 9 compounds used in Table 1. The same concentrations were used but a different procedure was followed. The cheese was ground in a food mill as before. The ground cheese was mixed well and 10 gm of the sample weighed into a 6-ounce screw-capped prescription bottle containing 90 ml of a sterile aqueous solution of the chemical compound and a teaspoon of glass beads. The mixture was agitated by vigorous shaking until the cheese was dissolved or dispersed to the extent that no curd particles settled out, and the time in seconds required to accomplish this recorded. The necessary dilutions were made, and duplicate plates were poured, one with aureomycin agar and the other with acidified agar. Controls were not run with these trials, because the plates containing sodium citrate had the same amount of this chemical compound as the controls used in previously reported trials, and therefore, served as the controls. The average, in 5 trials, of the number of colonies per plate and the time required to dissolve the curd with each compound are shown in Table 2.

The results indicate that on the plates poured with aureomycin added to the agar, ammonium carbonate, ammonium phosphate, and sodium monohydrogen phosphate, were the most satisfactory because they gave results comparable to the results obtained with the sodium citrate. With the plates poured with acidified agar, ammonium phosphate, sodium monohydrogen phosphate, sodium citrate, sodium pyrophosphate, and sodium sesquicarbonate appeared to be the most satisfactory. The results indicate further that ammonium hydroxide and trisodium phosphate were toxic to the yeasts and molds. The variations in the time required for the chemical compounds to dissolve the different samples of cottage cheese may be attributed to the differences in physical properties of the curd. Soft curd breaks down rather quickly,

Table 2.—The influence on yeast and mold counts and the time required to dissolve the curd with various chemical compounds.

Compound	Concentration	Colonies Per Plate (5 Trials) on Potato Dextrose Agar with: 100 ppm Aureomycin Added	Tartaric Acid Added to pH 3.5	Time Required to Dissolve the Curd (10 Samples) seconds: Range	Average
Ammonium carbonate	0.20%	146	57	45-150	85
Ammonium hydroxide	0.20%	111	64	30-150	73
Ammonium phosphate	0.50%	165	107	60-150	99
Sodium mono-H phosphate	1.25%	166	97	60-180	98
Trisodium phosphate	1.25%	112	88	45-90	67
Sodium borate	1.0 %	122	75	45-90	80
Sodium citrate	2.0 %	126	102	90-180	116
Sodium pyrophosphate	1.5 %	116	104	45-210	92
Sodium sesquicarbonate	1.0 %	119	112	30-100	62

while ehard or rubbery curd takes considerably more time. The average time required to dissolve the curd shows that ammonium hydroxide, trisodium phosphate, and sodium sesquicarbonate were the most effecetive. However, ammonium hydroxide and trisodium phosphate were rather tovic to yeasts and molds. Sodium citrate required the greatest length of time to dissolve the curd of any chemical compound used. In general, these results show that the compounds which dissolved the curd quickest were the most toxic.

The general results indicate that ammonium phosphate, sodium monohydrogen phosphate, and sodium citrate are the most satisfactory for dispersing cottage cheese curd for running yeast and mold count because they dissolve the curd and do not seem to be toxic to the organisms with either aureomycin or acidified agar. The order in which these compounds are recommended is (1) sodium monohydrogen phosphate, (2) ammonium phosphate, and (3) sodium citrate. Sodium monohydrogen phosphate and ammonium phosphate were placed above sodium citrate, because they dissolved cottage cheese curd more rapidly than sodium citrate, and sodium citrate seemed to give only a suspension of cheese curd in the solution.

Use of Mechanical Dispersion

Grinding with a food mill — Samples of cottage cheese can be dispersed by grinding in a food mill which forces the cheese through a screen with holes approximately 0.5 mm in diameter. It was observed that this operation breaks the cheese curd down into rather small particles but when plated out these small lumps of curd may be mistaken for yeast colonies. Other disadvantages of this method are the possibility of contamination. A rather large sample is required and the method is somewhat messy.

Grinding with mortar and pestle —Grinding cottage cheese with a mortar and pestle broke the cottage cheese curd into very small particles, forming a creamy paste. However, the particles were still large enough to be confused with yeast colonies. There is the possibility that yeasts may be trapped in these lumps, which would result in inaccurate counts. Grinding with sodium citrate added gives a suspension of the cottage cheese curd in the dilution blank that does not settle out. This method appears to be better than grinding in a food mill because it reduces the particles to a smaller size. It has the disadvantages of the possibility for contamination while grinding and is somewhat messy.

Grinding with mortar and pestle and sand — Cottage cheese was ground with a mortar and pestle with a small amount of sterile sea sand added. This method of grinding was effective in getting the cheese broken into small particles but it had the same disadvantages as grinding without sand.

Freezing and Thawing—It was thought that the action of hard freezing and then thawing might cause sufficient breaking up of the curd particles for plating. Samples of cheese were frozen at 0 to -10°F. and then thawed to note the effect on the curd. This operation caused a shattering of the curd, but was not sufficient to take the place of grinding with a mortar and pestle or a food mill. This procedure tends to lower the yeast and mold counts appreciably, presumably due to the crushing action incident to the freezing.

Whipping in a Waring Blendor —The Waring Blendor proved to be a useful means of breaking up the cottage cheese curd for plating. The container on the Blendor must be sterile and a relatively large sample (200 gm or more) should be used for effective whipping. This machine whips the cheese into a uniformly smooth mixture that can be handled with a cream pipette.Although the particles of cheese produced are very small, they are still large enough to cause confusion in counting the plates. Perhaps one great advantage in using the Waring Blendor is that the large sample required for proper mixing eliminates the errors inherent to the use of only a few grams from a package. Another advantage of the Waring Blendor is that the whipped samples can be used for other analyses, such as fat and total solids. It has certain disadvantages, namely that it is somewhat difficult to sterilize the container and a relatively large sample is required for effective whipping.

From observations at this Station, the methods of grinding with a mortar and pestle and of whipping in a Waring Blendor are thought to be the best mechanical means of breaking up the cottage cheese curd. However, it is believed that none of the mechanical means alone is sufficient for breaking up the curd particles for running yeast and mold counts and that dispersion by the use of chemicals to dissolve the curd is necessary.

Influence of Size Sample on Yeast and Mold Counts

One gm samples were used in obtaining the data above because they were convenient to handle. However, a 10 gm sample would probably give a more representative number of the yeasts and molds in the cottage cheese. In order to test the influence of the size of the sample on the accuracy of the yeast and mold counts, a trial was run in which ten 1 gm samples and one 10 gm sample were used on the same sample of cottage cheese. The procedure used was as follows: ten 1 gm samples of cottage cheese were plated out using the modification of the standard method as shown under methods. Then one 10 gm sample was plated from the same cheese using the same procedure except that 10 ml of a 20% solution of sodium citrate and an 80 ml water dilution blank were used. Plates were poured with both aureomycin and with acidified agar.

On aureomycin agar, the number of colonies on the ten 1 gm samples ranged from 84 to 123 and averaged 102, while the count on the 10 gm sample was 116. Two of the 1 gm samples had higher and eight had lower colony counts than the 10 gm sample. On acidified agar the colonies on the ten 1 gm samples ranged from 34 to 110 and averaged 74, while the colony count on the 10 gm sample was 82. Four of the 1 gm samples had higher colony counts than the 10 gm sample while the remainder had lower counts. Results with acidified agar appeared to be more variable than those with aureomycin agar. The results indicate that 1 gm samples of cottage cheese do not give reliable counts and that a larger sample, such as 10 gm, should be used. It should be noted that these counts were made on a sample of cheese without disintegrating the curd prior to sampling. The variation due to the relatively large size of the particles is evident. Cheese which has been prepared by whipping in a Waring Blendor or which was otherwise broken down into fine particles probably would not show this much variation.

Fat Tests

Although numerous modifications for various fat tests have been applied to other dairy products, particularly milk and ice cream, not many such tests have been applied to cottage cheese. The Modified Babcock test (4) has been used by some investigators. The Mojonnier Method (10) is satisfactory except that it is time consuming and requires considerable skill and equipment. Since there appeared to be a need for simple and practical tests for fat in cottage cheese, an investigation was made to evaluate various modifications of the Babcock test.

Preliminary Investigations

Various modifications of the Babcock test were investigated in a search for tests that would be applicable to cottage cheese. With each test, numerous trials were run in which varying amounts and proportions of reagents and variations in operational procedures were used to determine the influence of these factors on the accuracy of the tests. Much of the preliminary work was done with both raw and homogenized-pasteurized milk as the test materials. These materials are much easier to handle than is cottage cheese. Since Wildasin et al. (18) have shown that the addition of a small amount of a cationic surface-active quaternary ammonium compound (alkyl dimethyl benzyl ammonium chloride) is effective as a fat de-emulsifier and improves the Babcock test, various such compounds were tested to determine their influences in improving the accuracy of the tests investigated.

The general results obtained

with the modified Babcock tests are as follows:

Babcock test. Because of the texture and consistency of cottage cheese, even after thorough grinding in a mortar and pestle or whipping in a Waring Blendor, the ordinary Babcock test is not readily applicable to cottage cheese. Trials were run in which 9 gm of ground cheese were weighed into 9 gm-50% test bottles and into Paley test bottles. Ten ml water and 17.5 ml sulfuric acid were added in three installments and the test completed as for cream and for cheese, respectively. Only a few trials were run because the tests were difficult to perform and the results rather erratic, averaging considerably lower than the Mojonnier tests. The tests were generally difficult to read because of excessive charring or because of undigested curd at the base of the fat column.

Pennsylvania method. A modification of the Pennsylvania method (17) was employed for cottage cheese as follows: 27 gm of cottage cheese was weighed into a bottle or flask and 6.7 ml (6 gm) of concentrated ammonium hydroxide were added. The mixture was heated in a water bath at 120°F. and mixed occasionally. After the cheese was dissolved, 11 gm (9 gm of cheese plus 2 gm of NH_4OH) were weighed into an 8% Babcock milk test bottle. Because of the irritating effect of the ammonia fumes, a rubber bulb was used to create the suction and pressure on the pipette to transfer the sample to the test bottles. Three ml of normal butyl alcohol were added and mixed with the sample and then 17.5 ml sulfuric acid (sp. gr. 1.72-1.74) were added and the test completed as for milk, doubling the reading because of the 9 gm sample used. This method gave good, clear tests but the tests on 16 samples ranged from 0.64 lower to 0.70% higher than the Mojonnier tests and averaged 0.185% higher. Only 5 of the 16 tests checked within 0.10% of the Mojonnier tests and only 7 within 0.20%.

Illinois Method. A number of samples of cottage cheese were run with the Illinois Method developed by Overman and Garrett (13). The cheese was prepared by mixing in a Waring Blendor, with Minnesota Reagent added at the rate of 14.5 ml to 100 gm of cheese. Twenty-one gm of the mixture, representing 18 gm of cheese were weighed into 18 gm-8% milk test bottles, using a 17.6 ml milk pipette for the transfer. The tests were then completed as recommended for the method, except that 5 ml of Reagent A and 18 ml of Reagent B were used. The Illinois method gave cheese tests that were easy to read, but the results were rather variable as compared to the Mojonnier tests. In one trial with 11 samples of cottage cheese, the tests by the Illinois Method ranged from 0.11% lower to 0.85% higher than the Mojonnier tests and averaged 0.13% higher. Only three of the 11 tests were within 0.10% of the Mojonnier tests and only six were within 0.20%. Modifications in the proportions of the compounds used in the reagents failed to bring about an improvement in the Illinois test as applied to cottage cheese.

Minnesota - Ammonium hydroxide method. A modification of the Minnesota Method (9) for fat in dairy products was applied to cottage cheese. Briefly, the method was as follows: The cottage cheese was prepared for analysis by the method used for the Pennsylvania Method which involved the mixing of the cheese with ammonium hydroxide to give a product with a creamy consistency which could be handled with a pipette. The same technique was used as with the Pennsylvania Method in weighing out 11 gm samples of the cheese —NH_4OH mixture into 18 gm-8% milk test bottles. Ten ml Minnesota Reagent were added to each bottle and the test completed as for the Minnesota test on milk except that the fat test reading was doubled to compensate for the 9 gm sample used. This test, except for the irritating effect of the ammonia fumes, was rather satisfactory from the standpoint of ease of performance and appearance of the fat columns. The results, compared to the Mojonnier tests, were somewhat variable. The tests ranged from 0.24% lower to 1.68% higher than the Mojonnier tests and averaged 0.24% higher. Only five of the 16 tests were within 0.10% of the Mojonnier tests and 10 of the tests were within 0.20%. The fact that the reading of the test was doubled because a 9 gm sample was used may have been an important influence in the accuracy of the test.

"Sal" method. The "Sal" method (3) was applied to numerous samples of milk and cottage cheese with varying degrees of success. Briefly, this method is as follows: the "Sal" reagent is composed of 80 gm sodium hydroxide, 40 gm sodium chloride made up to 1 liter and then 450 ml ethyl alcohol and 45 ml amyl alcohol are added. Pipette 18 gm (17.5 ml) of milk into milk test bottles, add 17.5 ml reagent and shake vigorously. Digest in a water bath at 140°F. to 160°F. for 7 to 8 minutes, mix well by shaking and complete as for the ordinary Babcock test for milk.

This test gave good, clear fat tests on milk, but the tests were rather variable. The tests on unhomogenized milk agreed fairly well with the Babcock tests but the tests on homogenized milk were considerably lower. The tests on cottage cheese ran about 0.70% lower than the Mojonnier tests. Various modifications in the amounts of reagent used and the proportion of the constitutents failed to improve the test. On the basis of these preliminary results, the "Sal" test was eliminated as a possibility for a test for fat in cottage cheese.

Detergent tests. Various detergent tests have been proposed for dairy products. A few tests were run with some of these. The D.P.S. test (14) showed considerable promise in that it gave clear tests with cottage cheese but the preliminary tests were somewhat variable. This test deserves further investigation. The Schain test (15) and the "Banco" test* were ineffective on cottage cheese in that the tests were considerably lower than the Mojonnier tests.

Recommended Tests

After considerable experimentation with various modifications of the Babcock test, a modified Babcock and a Modified Minnesota test seemed to best fulfill the requirements for a simple, rapid, and

*"Banco Reagent" was obtained from Anderson Laboratories Inc., Fort Worth, Texas

accurate method for fat in cottage cheese. With each test various modifications were made in the procedure to determine the best method to use on cottage cheese.

Acid-QAC Method. The preliminary results indicated that the ordinary Babcock test with sulfuric acid was unsatisfactory because of the difficulty of weighing the cheese into the test bottles and because the tests were difficult to read due to excessive charring when enough acid was added for complete digestion. Wildasin et al. (18) showed that the addition of a small amount of a quaternary ammonium compound to the acid used for testing homogenized milk resulted in a considerable improvement in the accuracy of the test. Numerous trials were run on raw milk, homogenized milk, and cottage cheese, using various amounts of a number of different quaternary ammonium compounds to determine the influence of these variations on the tests. The results indicated that the procedure recommended for the Connecticut test developed by Wildasin et al (18) gave consistently satisfactory results and that caution should be used in the selection of the QAC to use. In the Connecticut test, alkyl dimethyl benzyl ammonium chloride was used. In our tests several QAC compounds gave satisfactory results with unhomogenized milk but some of these same compounds lowered the tests considerably on homogenized milk and on cottage cheese. Alkyl dimethyl benzyl ammonium chloride was satisfactory for either unhomogenized or homogenized products.

The authors (18) recommend the addition of 9 ml of 50% alkyl dimethyl benzyl ammonium chloride to a liter of sulfuric acid adjusted to a specific gravity of 1.82. Wide variations were made in the amount of the QAC used and the results confirmed the observations of the authors in recommending 9 ml per liter, although considerable variations can occur without affecting the accuracy of the test. It was also found that the addition of 0.20 ml of the 50% QAC directly to the test bottle after adding the acid was very satisfactory in producing good tests.

Our results showed that the use of QAC resulted in a great improvement in the quality of the tests on cottage cheese by more completely dispersing the curd and eliminating dark colored fat columns due to charring.

Since the preliminary tests indicated considerable difficulty in weighing the cheese into the test bottles, various methods were tried in an attempt to put the curd in such a condition that it could be transferred with a pipette. These included dissolving the curd with ammonium hydroxide, sodium salicylate, Minnesota reagent, and combinations of sodium hydroxide and sodium salicylate. The following objections to the use of these materials were noted:

Ammonium hydroxide—The mixtures were difficult to handle because of the irritating effect of the ammonia fumes.

Sodium hydroxide—The mixtures were too viscous to pipette.

Sodium salicylate — Fat separated during mixing in a Waring Blendor.

Sodium hydroxide-Sodium salicylate mixture—The fat tended to separate during whipping in a Waring Blendor and precipitate formed on adding sulfuric acid. Minnesota reagent—With 10 to 20 ml added per 100 gm cheese, the mixtures were very smooth and of the right viscosity to prevent fat separation. Too much foaming occurred when sulfuric acid was added in conducting the test.

Another mixture was tried in which various proportions of distilled water were added to the cheese during whipping but the mixtures of the right viscosity for pipetting were not stable and there was whey and fat separation within a short time after preparation.

The method finally selected for preparing the cheese and running the test is as follows:

1. Empty a carton of cottage cheese into a Waring Blendor container or place a large enough sample for effective whipping (about 200 gm) in the Blendor and whip to a uniform, creamy consistency.
2. Weigh 18 gm of the cheese into a 125 ml Phillips beaker or Erlenmeyer flask.
3. Add 18 ml acid-QAC mixture (prepared by adjusting sulfuric acid to a specific gravity of 1.82 at 68°F. and adding 9 ml of 50% alkyl dimethyl benzyl ammonium chloride per liter.) If desired, 17.5 ml of acid may be used and then 0.20 ml of the QAC added after adding the acid to the curd.
4. Mix acid and cottage cheese completely.
5. After the reaction has proceeded enough for complete digestion (dark brown color) pour the mixture into an 18 gm milk test bottle. Rinse the beaker or flask with about 10 ml hot water (135°F.) and add the rinsings to the test bottle.
6. Centrifuge for 5 minutes and add hot water (140°F.) to bring the contents to the base of the neck of the bottle.
7. Centrifuge 2 minutes.
8. Add water until fat column is contained within the graduated scale of the bottle neck.
9. Centrifuge 1 minute.
10. Temper the fat columns by placing the bottles in a water bath at 135°-140°F. for 5 minutes. The water should be deep enough to immerse the fat columns.
11. Read the test to the nearest 0.1%.

This test requires no special equipment or reagents except for the QAC compound. It is easy to perform and gives reasonably accurate results. Comparisons between fat tests run on cottage cheese by the acid-QAC method and the Mojonnier test are shown in Table 3. The data on 15 samples of commercial cottage cheese show that the variations in fat tests by the acid-QAC method ranged from 0.157% lower to 0.060% higher than the Mojonnier tests and averaged 0.0690% lower. The mean deviation from the Mojonnier tests was 0.0803% and 11 of the 15 tests were within 0.10% of the Mojonnier tests.

Minnesota Babcock Method. Preliminary investigations with the Minnesota test on cottage cheese samples dissolved with ammonium hydroxide showed the best promise of any of the tests used. Since subsequent trials showed that the mixing of cottage cheese and Minnesota reagent in the Waring Blendor gave a uniformly creamy mixture in which the fat remains in suspension indefinitely, the Minnesota Method was applied to samples of cottage cheese prepared by this method. Mixing from 10 to 20 ml of Minnesota reagent with 100 gm of cottage cheese gave mixtures that were sufficiently viscous to prevent fat separation and sufficiently fluid to permit handling with a milk pipette. For convenience in weighing out the samples, 14.5 ml of the reagent per 100 gm of cheese was selected, as 21 gm of this mixture represents 18 gm of cheese as determined from the specific gravity (1.15) of the Minnesota reagent. The amount of Minnesota reagent used per 18 gm sample (21 gm of mixture) was varied from 6 to 24 ml. Results showed that as little as 12 ml could be used and that increasing the amount of the reagent did not alter the test. As a matter of convenience in measuring, 17.5 ml was selected.

The recommended method for preparing the sample and running the test is as follows:

1. Weigh a carton of cottage cheese, empty contents into a Waring Blendor container and reweigh the empty carton to determine weight of cheese; or weigh a convenient amount, such as 200 to 300 gm, of a well-mixed sample and place in the Waring Blendor container.
2. Add 14.5 ml of Minnesota Reagent per 100 gm of cheese and mix to a uniform, creamy consistency. This mixture may be stored in screw-capped jars or other air-tight containers for future analysis.
3. Using a 17.6 ml milk pipette, weigh 21 gm of the prepared mixture into a milk test bottle. If the sample is too viscous, the viscosity may be reduced by warming to about 120°F.
4. Add 17.5 ml Minnesota reagent and mix thoroughly.
5. Digest by placing the bottles in a water bath at a temperature of about 200° F. for 10 minutes and mixing the contents of the bottles by shaking 3 or 4 times during the digestion period.
6. Add hot water to bring contents to the base of the neck of the bottle.
7. Centrifuge for ½ minute in a regular Babcock Centrifuge.
8. Add hot water to bring the fat up into the graduated neck of the test bottle.
9. Centrifuge for ½ minute.
10. Temper bottles in a water bath at 135°F. for 5 minutes.
11. Read test as for milk.

This modified Minnesota test gives very clear, easy to read, fat columns. This test is rapid and requires no special reagents or equipment except for the Minnesota reagent, which is generally readily available.

This test gave results which were closer to those by the Mojonnier tests than the acid-QAC test as shown by the results presented in Table 3. Each test is the average of duplicate determinations. The tests on the 15 samples ranged from

Table 3.—Comparison of fat tests on commercial cottage cheese by the Mojonnier, Acid-QAC and Minnesota Babcock Methods.

(Averages of duplicate determinations with each test)

Sample No.	Mojonnier Test	Acid-QAC Test	Minnesota Test	Deviation from Mojonnier Test: Acid-QAC	Deviation from Mojonnier Test: Minnesota
	Percent Fat			Percent Fat	
1	5.910	5.85	5.85	—0.060	—0.060
2	9.195	8.10	8.05	—0.095	—0.145
3	5.190	5.25	5.20	+0.060	+0.010
4	3.116	3.05	3.15	—0.066	+0 034
5	4.846	4.80	4.75	—0.046	—0.096
6	2.339	2.25	2.35	—0.089	—0.011
7	3.542	3.40	3.45	—0.142	—0.092
8	5.625	5.65	5.55	+0.025	—0.075
9	4.885	4.75	4.85	—0.135	—0.035
10	3.989	3.85	3.95	—0.139	—0.039
11	6.066	6.05	5.95	—0.016	—0.116
12	4.642	4.55	4.55	—0.092	—0.092
13	3.615	3.55	3.50	—0.065	—0.115
14	6.368	6.35	6.50	—0.018	+0.132
15	5.207	5.05	5.20	—0.157	—0.007
Means	4.9023	4.8333	4.8567		
Difference from Mean of Mojonnier		—0.0690	—0.0456		
Maximum deviation from Monjonnier				0.157	0.145
Mean of deviations from Mojonnier				0.0803	0.0706

0.145% lower to 0.132% higher than the Mojonnier tests and averaged 0.0456% lower. The mean deviation from the Mojonnier test was 0.0706% and 11 of the 15 samples were within 0.10% of the Mojonnier tests.

Salt Determinations— Methods of Preparing Cheese for Direct Titration

The A.O.A.C. method (2) for the determination of salt in cheese is accurate but it has the disadvantages of being time consuming, complicated, and requiring several reagents. Since the test is based upon the reaction of silver nitrate with sodium chloride to give silver chloride (mercuric nitrate also reacts the same way), it was thought that a method of direct titration using silver nitrate or mercuric nitrate might be applied to cottage cheese. The general procedure involved weighing out 10 gm of sample, mixing with distilled water, treating by grinding or heat and making up to a total volume of 250 ml. Twenty-five ml portions of the supernatant liquid or of filtrate after filtering through rapid filter paper were titrated with 0.1711 N silver nitrate using 1 ml of 10% potassium chromate as the indicator or with 0.1711 N Mercuric nitrate, using 12 drops of a 0.10% solution of S-diphenylcarbazone in neutral ethyl alcohol as the indicator. The percent of salt is read directly from the burette as each ml of standard solution equals 1% of salt.

The results of direct titration of cottage cheese for salt percentage with silver nitrate and with mercuric nitrate after various treatments to prepare the samples are shown in Table 4. The salt percentages shown are the means of duplicate determinations on 10 samples. Of the 160 sets of duplicate determinations run, other than the controls, 130 checked exactly with each other, 29 varied only 0.10% and only one varied as much as 0.20%.

Grinding with a Mortar and Pestle

Grinding with a mortar and pestle involved weighing 10 gm of cottage cheese, transferring to a mortar, adding 10 ml distilled water and grinding to a uniformly fine paste. The mixture was then transferred to a beaker or flask by rinsing the mortar and pestle sev-

Table 4.—A comparison of methods of preparing cottage cheese for direct titration for salt.

Means for duplicate determinations on 10 samples (Mean % salt by control method = 1.3 9)

	Not Filtered		Filtered	
	$AgNO_3$	$Hg(NO_3)_2$	$AgNO_3$	$Hg(NO_3)_2$
METHOD I Ground in Mortar				
Mean % Salt	1.455	1.355	1.450	1.355
Difference from Mean of Control	+0.076	—0.024	+0.076	—0.024
Maximum Deviation from Control	+0.23	—0.12	+0.18	—0.12
Mean Deviation from Control	0.084	0.066	0.075	0.066
% of Samples within 0.10% of Control	70	90	70	90
METHOD II Ground in Mortar with Sand				
Mean % Salt	1.440	1.370	1.413	1.377
Difference from Mean of Control	+0.061	—0.009	+0.034	—0.002
Maximum Deviation from Control	+0.18	—0.12	+0.18	+0.08
Mean Deviation from Control	0.065	0.059	0.050	0.044
% of Samples within 0.10% of Control	80	90	80	100
METHOD III Heated to 160°F.				
Mean % Salt	1.450	1.363	1.420	1.375
Difference from Mean of Control	+0.071	—0.016	+0.041	—0.004
Maximum Deviation from Control	+0.25	—0.16	+0.20	—0.16
Mean Deviation from Control	0.097	0.058	0.077	0.060
% of Samples within 0.10% of Control	50	60	60	80
METHOD IV Heat to Boiling				
Mean % Salt	1.513	1.465	1.510	1.460
Difference from Mean of Control	+0.134	+0.086	+0.131	+0.081
Maximum Deviation from Control	+0.28	+0.25	+0.28	+0.25
Mean Deviation from Control	0.138	0.092	0.135	0.097
% of Samples within 0.10% of Control	40	60	40	60

eral times with distilled water and adding distilled water to make the total volume 250 ml.

The results indicate that this is a reasonably satisfactory method of preparing cottage cheese for direct titration. Seventy percent of the unfiltered samples, titrated with silver nitrate, checked within 0.10% of the controls and the mean deviation from the controls was 0.084%, while 90% of the samples titrated with mercuric nitrate checked within 0.10% of the controls and the mean deviation was 0.066%.

Grinding with a Mortar and Pestle and Sand

The second method was identical to the first except that a teaspoonful of fine sea sand was added to assist in disintegrating the cheese. The results with this method indicate that it was the most satisfactory of the four methods used. Eighty percent of the unfiltered samples titrated with silver nitrate checked within 0.10% of the controls and the mean deviation from the controls was 0.065%, while 90% of the samples titrated with mercuric nitrate checked within 0.10% of the controls and the mean deviation from the controls was 0.059%. Filtration appeared to improve the accuracy of the test, especially with the mercuric nitrate titrations. Eighty percent of the filtered samples titrated with silver nitrate checked within 0.10% of the controls and the mean deviation was 0.050%, while all of the filtered samples titrated with mercuric nitrate checked within 0.10% of the controls, and the mean deviation 0.044% from the controls.

Heating to 160°F.

It was thought that the proteins of cottage cheese might interfere with the accuracy of direct titrations for salt percentage and that these proteins could be eliminated by heating. Accordingly, 10 gm samples of ground cottage cheese were placed in a beaker and distilled water added to a volume of 250 ml. The mixture was heated over an open flame to 160°F., cooled to room temperature, and unfiltered and filtered portions titrated. The results indicate that this method of preparation gave less satisfactory results than either Methods 1 or 2 in that there were fewer samples that checked within 0.10% of the controls and the maximum and mean deviations were considerably greater. The best results with this method were obtained on the filtered samples titrated with mercuric nitrate in which 80% of the samples checked within 0.10% of the controls; the maximum deviation was -0.16% and the mean deviation 0.060%.

Heating to Boiling

The fourth method involved the same procedure as Method 3 except that the mixture was heated to boiling. The results with this method were generally unsatisfactory and appeared to be the poorest of the four methods used. Only about half of the samples checked within 0.10% of the controls, and the maximum and mean deviations from the controls were greater than with the other methods.

The above results indicated that grinding with sand and filtering is the best procedure for preparing cottage cheese for salt determinations, although grinding in a mortar without sand and not filtering also appeared to be reasonably satisfactory and involved fewer manipulations. In each of the methods, the titrations with mercuric nitrate gave results that checked closer with the controls than did the titrations with silver nitrate. The titrations with mercuric nitrate have a further advantage in that the end point (change from colorless to purple) is much more distinct than it is with the titrations with silver nitrate.

Moisture Tests

The Mojonnier method (10) and the A.O.A.C. method (2) for the determination of moisture in cheese are accurate, but they require considerable equipment usually not found in dairy plants and they are also time consuming. There is a need for the development of a simple, rapid, and accurate method for determination of moisture in cottage cheese.

Since Kosikowsky et al. (7) used a semi-automatic moisture tester for cheddar cheese, processed cheese and cheese food, it was thought that the Cenco automatic moisture tester* might be applied to cottage cheese.

The Cenco automatic moisture tester was designed primarily for low moisture materials such as various grains, starch, flour and dried milk. With these materials, it is satisfactory to place the material directly into the drying pan. The general procedure used with cottage cheese was as follows:

A filter paper was placed in the bottom of the pan and the cheese spread evenly over the paper. The cover was closed and the drying lamp turned on at 115 volts. The percent moisture removed, as shown on the dial of the tester by moving the dial to balance the pan, was recorded at 2-minute intervals. The cheese was dried to a weight that would remain constant for a 2-minute period, because this conforms to the instructions with the tester for other products.

A great deal of preliminary work was done with the Cenco automatic moisture tester to determine the voltage and size of drying lamp necessary to dry the cheese to a weight that would remain constant for a 2-minute period without charring the sample. Various modifications included use of a 125-watt and a 225-watt drying bulb at voltages of 115, 100, 85, and 70 for the entire drying process, to determine the most satisfactory conditions for drying the sample. Lowering the voltages increased the drying time, making this modification undesirable. The use of the 225-watt drying lamp caused excessive charring of the sample. Using the 125-watt lamp with 115 volts of current until the reading on the tester dial showed 75% and then cutting the current down to 85 volts for the remainder of the drying period was the most satisfactory method for drying the cottage cheese, although other combinations of voltage and bulb size were also satisfactory.

To establish the rate of moisture loss and the time required for complete drying, quadruplicate deter-

*The Cenco automatic moisture tester is manufactured by the Central Scientific Company, Chicago 13, Illinois minations were made on 10 samples of cottage cheese, with readings taken at 2-minute intervals. The 125-watt lamp and 115-volt current were employed until the moisture scale showed 75% and then the voltage was cut back to 85 for the remainder of the drying period. After 8 minutes the mean moisture reading on the 4 replicates of 10 samples was 77.2%. Following this rapid initial loss in moisture the following mean readings were observed: 10 minutes, 80.1%; 12 minutes, 80.5%; 14 minutes, 80.9%; 16 minutes, 81.1%; 18 minutes, 81.2% and 20 minutes, 81.2%. The results showed further that at the end of 16 minutes drying the 4 replicate determinations checked within 0.30% with each other with 6 of the 10 samples and that at the end of 18 minutes, 8 out of 10 checked within 0.30%. In an analysis of the 4 replicate determinations on the 10 samples, 24 of the 40 tests were dried to within 0.10% of their final moisture percentages in 16 minutes, and 39 of the 40 were within 0.10% in 18 minutes. These results indicate that with a 125-watt bulb heated at 115 volts for 7-8 minutes and then at 85 volts, a drying time of 18 minutes is sufficient for cottage cheese.

Using the drying conditions just mentioned, 4 replicate determinations were made on each of 17 samples of cheese and the results compared with the means of duplicate tests by the Mojonnier method. The mean of the moisture content by the Mojonnier method on the 17 samples was 81.82%; while that for the 68 tests (4 replicates on 17 samples) with the Cenco method was 81.90%, a difference of 0.08% higher for the latter method. The maximum deviation among the 68 tests by the Cenco method from the mean of the Mojonnier was 0.3121%. Of the 68 individual tests, 19% checked within 0.10% of the controls; 43% within 0.20%; 62% within 0.30%; 71% within 0.40%; 78% within 0.50%; 84% within 0.60%; 91% within 0.70%; 97% within 0.80% and 100% within 0.90%.

The results indicate that the Cenco method, carefully operated, will give rapid and reasonably accurate moisture tests on cottage cheese. The moisture percentages should check within 0.30% of those obtained by the Mojonnier method which may be close enough for routine plant work. These moisture tests were run on the curd as it came from the packages. Better results should be obtained by running the tests on cheese prepared by whipping in a Waring Blendor or some other means to give a uniform sample.

Summary and Conclusions

A study was made of methods of analyzing cottage cheese to determine which were the most satisfactory and to attempt to improve the methods in current use. The methods studied were those for yeast and mold counts, and fat, salt, and moisture contents.

The addition of 100 ppm of aureomycin to potato-dextrose agar just before pouring the plates effectively suppressed bacterial growth. The counts on the aureomycin agar were slightly higher than those on the same samples poured with acidified agar. The aureomycin agar was particularly useful for plating cottage cheese and other dairy products containing a considerable amount of casein. The casein did not precipitate as it does with acidified agar and, therefore, the plates were much easier to count.

In plating cottage cheese, the curd can be satisfactorily dispersed by dissolving with (1) a 1.25% solution of sodium monohydrogen phosphate, (2) a 0.5% solution of ammonium phosphate or (3) a 2.0% solution of sodium citrate. These solutions dissolved the curd rapidly and did not seem to affect the yeast and mold counts.

Whipping cottage cheese in a Waring Blendor is an effective method for preparing the cheese for analysis if a large enough sample is available. Small samples can be prepared by grinding in a mortar and pestle. Freezing and thawing was not effective in breaking of cottage cheese curd for analysis. Small samples (1 gm) of cottage cheese as it comes from the package are likely to give erroneous results in yeast and mold counts.

Two fat tests proved to be satisfactory for cottage cheese. One is a modification of the acid Babcock method in which the cheese is weighed into a small flask, a sulfuric acid-QAC mixture added and the contents then transferred to a milk test bottle for completion of the test. The other method involves whipping the cheese in a Waring Blendor after adding a small amount of Minnesota reagent to partially dissolve the curd and then running the Minnesota test on a representative sample of the mixture. Both of these tests checked reasonably close with the Mojonnier test and are recommended for routine plant tests.

The salt content of cottage cheese can be determined rapidly by whipping or grinding the cheese, mixing with water and titrating the supernatant liquid or the filtrate after filtering. Titration with a standard mercuric nitrate solution appeared to give better results than did titration with silver nitrate. Heating the cheese-water mixture to 160°F. or to boiling to precipitate the proteins appeared to be detrimental to the accuracy of the test.

The moisture content of cottage cheese can be determined quickly and reasonably accurately by the use of the Cenco moisture tests if the rate of drying is carefully controlled.

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