

A STUDY OF METHODS USED FOR ANALYSIS
OF COTTAGE CHEESE

A STUDY OF METHODS USED FOR ANALYSIS
OF COTTAGE CHEESE

By

Milford D. Bonner

Bachelor of Science

Mississippi State College

State College, Mississippi

1951

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

1954

OKLAHOMA
AGRICULTURAL & MECHANICAL COLLEGE
LIBRARY
JAN 10 1955

A STUDY OF METHODS USED FOR ANALYSIS
OF COTTAGE CHEESE

MILFORD D. BONNER

MASTER OF SCIENCE

1954

THESIS AND ABSTRACT APPROVED:



Thesis Adviser



Faculty Representative



Dean of the Graduate School

333814

ACKNOWLEDGMENT

The author wishes to take this opportunity to express his sincere appreciation to Dr. H.C. Olson for the many helpful suggestions and guidance throughout this study and during the preparation of this thesis.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. STATEMENT OF PROBLEM.....	2
III. REVIEW OF LITERATURE.....	3
IV. METHODS.....	9
A. Yeast and Mold Determinations.....	9
B. Fat Determinations.....	9
C. Salt Determinations.....	9
D. Moisture Determinations.....	10
V. EXPERIMENTAL.....	11
A. Yeast and Mold Counts.....	11
1. Methods for Dispersing Cottage Cheese Curd.....	11
a. Use of Chemical Compounds.....	11
b. Use of Mechanical Means.....	15
(1) Grinding with Food Mill.....	15
(2) Grinding with Mortar and Pestle.....	15
(3) Grinding with Mortar and Pestle and Sand.....	15
(4) Freezing and Thawing.....	15
2. Influence of Various Modifications on the Yeast and Mold Counts.....	16
a. Dispersion of Curd by Use of Chemicals.....	16
b. The Effect of Using Aureomycin Hydrochloride to Inhibit Bacterial Growth in Yeast and Mold Counts.....	23
c. The Influence of the Size of Sample Used on the Yeast and Mold Counts.....	24

Chapter	Page
B. Fat Tests.....	27
1. The Modified Babcock Method.....	27
2. The Modified Minnesota Method.....	29
3. The Modified Pennsylvania Method.....	30
4. Use of Detergents.....	32
C. Salt Tests-A Comparison of Methods of Preparation of Cottage Cheese Samples for Direct Titration.....	33
1. Method I. Cheese Ground in a Mortar.....	34
a. Not Filtered.....	34
b. Filtered.....	36
2. Method II. Cheese Ground in a Mortar with Sand....	36
a. Not Filtered.....	37
b. Filtered.....	37
3. Method III. Cheese Heated to 160°F.....	38
a. Not Filtered.....	38
b. Filtered.....	38
4. Method IV. Cheese Heated to Boiling.....	39
a. Not Filtered.....	39
b. Filtered.....	39
D. Moisture Tests-A Comparison of the Gencó Automatic Moisture Tester to the Mojonnier Method.....	41
VI. SUMMARY AND CONCLUSIONS.....	49
VII. WORKS CITED.....	53

INTRODUCTION

Cottage cheese is becoming an increasingly popular product with the consuming public, and therefore, the manufacture and sale of this product is of increasing importance to dairy plants. If a plant operator is to maintain his cottage cheese sales by manufacturing a uniform product of high quality, it is necessary to have adequate testing methods. The methods needed are for yeast and mold counts, and for fat, salt, and moisture determinations.

From a study of the existing methods of analysis used for cottage cheese, it was discovered that the methods are unsatisfactory and in some cases ambiguous. Many of the methods employed are methods that have been developed for other dairy products, primarily cheddar cheese. Many of the methods give results that are inaccurate, while others are time consuming and complicated or require the use of considerable equipment and reagents. There is a need for the development of a satisfactory method for yeast and mold counts, and also for the development of simple, rapid, and accurate methods for the determination of fat, salt, and moisture in cottage cheese.

STATEMENT OF PROBLEM

The objectives of the work herein reported are to develop satisfactory methods for yeast and mold counts, and to develop simple, rapid, and accurate methods for determination of fat, salt, and moisture in cottage cheese that will be of practical use in dairy plants.

REVIEW OF LITERATURE

The accepted method for determining the yeast and mold content of cottage cheese is found in Standard Methods for the Examination of Dairy Products, tenth edition, 1953 (13). Briefly, the procedure is as follows: weigh an 11 g. sample of cottage cheese, aseptically, into a sterile 99 ml. dilution blank containing water or some other non-toxic emulsifying agent. Mix the sample thoroughly before making suitable dilutions. Prepare dilutions and pour plates using potato dextrose agar which has been acidified to a pH of 3.5 with a sterile solution of 10% tartaric acid. Incubate the plates for 5 days at 21° or 25°C. (70° or 77°F.). Report the computed numerical estimate as the "Yeast and Mold Plate Count/ml.," of cheese.

The standard procedure has been modified by Morgan, et al, (11) in the following manner: weigh 1 g. of cheese onto sterile parchment paper and transfer to a sterile 99 ml. water dilution blank. Add a quantity of sterile beads to the sample and shake until thoroughly mixed. Make suitable dilutions and pour plates using acidified tomato-peptonized milk agar. The rest of the procedure is the same as the standard method. With this method the addition of sterile beads to the dilution water does aid in the breaking up of the lumps of cottage cheese curd.

Another modification of this method by these same workers (11) is as follows: weigh an 11 g. sample of cheese, aseptically, and emulsify in a sterile mortar with a portion from a 99 ml. dilution blank of 2% sodium citrate. Make further additions of the diluting solution to effect a smooth suspension of the cheese in the dilution blank. Pour the resulting mixture back into the dilution bottle and shake in the usual manner. Make suitable dilutions and pour plates using acidified potato dextrose agar. The rest of the procedure is the same as the

standard method.

Olson (12) used a modification of the standard method as follows: weigh 1 g. of cheese, aseptically, into a sterile mortar, add 1 ml. of a 20% sodium citrate solution and grind with a pestle. Then add 8 ml. of sterile water from an 8 ml. dilution blank, mix well, pour back into the dilution blank and shake in the usual manner. Make suitable dilutions and pour plates using potato dextrose agar to which has been added 100 parts per million of aureomycin hydrochloride, using a 1% solution in sterile water. This should be added just prior to the pouring of the plates. This method has two distinct advantages. One is the use of sodium citrate to emulsify the cheese in the dilution blank so that a uniform mixture of cheese and dilution water is obtained. The other advantage is the use of aureomycin hydrochloride in the agar instead of tartaric acid. With this method there is no precipitation of casein which often makes the plates poured from the lower dilutions difficult to count. The colonies grown on this medium are also larger and easier to count. It was found that the yeast and mold counts generally run slightly higher with this method than when acidified agar was used.

The Babcock method (4) is often used as a standard for determining the fat content of cheese. Briefly, this method is as follows: weigh 9 g. of cheese into a 50% Babcock cream test bottle, add 12 ml. of water at 160° F. (82°C.) and place the test bottle in a water bath at 160°F. (82°C.) or above for 5 minutes. Then add 17.5 ml. of sulphuric acid, specific gravity of 1.82 to 1.83, in installments, mixing well after each addition. After all lumps are dissolved, centrifuge for 5, 2, and 1 minute periods, adding water at 140°F. (60°C.) after the first and second whirlings. Place the test bottles in a 135° to 140°F. (57° to 60°C.)

water bath for 3 minutes, add glycol and read at once.

The method for determining fat in cheese as recommended by the A.O.A.C. (2) is as follows: mix 1 g. of cheese with 9 ml. of water and 1 ml. of ammonium hydroxide, digest the mixture at low heat until the casein is softened. Then neutralize the mixture with hydrochloric acid using litmus as an indicator, add 10 ml. more of hydrochloric acid and 0.5 g. of sand to prevent bumping, and boil gently for 5 minutes. Then cool the solution and transfer to a Mojonnier fat extraction flask. Extract the fat with petroleum benzene, evaporate the solvent on a hot plate, and then dry in an oven to constant weight. Then reweigh and calculate the percent of fat.

The Mojonnier method (10), which is a commonly used method for fat determinations, is briefly as follows: extract the fat from a 5 g. sample of cheese by the use of ammonium hydroxide, alcohol, petroleum ether, and ethyl ether. Then evaporate the solvents on a hot plate and dry to constant weight in a vacuum oven. Then reweigh and calculate the percent of fat. This method employs the same principle as the A.O.A.C. method.

The official method (2) for determining the amount of salt in cheese is as follows: weigh a 3 g. sample of cheese into a 300 ml. Erlenmeyer flask, add 25 ml. of 0.1 N silver nitrate, 10 ml. of C.P. nitric acid, and 50 ml. of water. Then heat, and as the solution boils, add 15 ml. of a 5% solution of potassium permanganate in 5 ml. portions. When the solution becomes clear, filter and make up to 200 ml. volume. Then titrate the excess silver nitrate with 0.1 N potassium thiocyanate solution using ferrous alum as an indicator. The percent of salt can then be calculated.

Wilster, et al, (17) used a slight modification of this method as follows: use silver nitrate and potassium thiocyanate of 0.1711 N and decant the liquid in preference to filtering. They also used ferrous ammonium sulphate as an indicator. Decanting is more rapid than filtering, thereby speeding up the operation. This modification also simplifies the calculations because the ml. of silver nitrate used divided by the weight of the sample equals the percent of salt. The results obtained are comparable to the results obtained with the A.O.A.C. method (17).

Arbuckle (1) reported a method of direct titration using mercuric nitrate to determine the salt content. His procedure is as follows: grind a 10 g. sample of cheese, add 250 ml. of water and heat to 160°F. (71°C.), cool to room temperature, remove a 25 ml. portion, and titrate with 0.1711 N mercuric nitrate using *s*-diphenylcarbazone as the indicator. The ml. of mercuric nitrate used equals the percent of salt. He reported results comparable to the official method.

Marquardt (8) reported two methods of direct titration using silver nitrate. His first method is briefly as follows: weigh a 10 g. sample of cheese, dilute with 250 ml. of water and heat to the boiling point. Then filter and titrate 25 ml. portions of the filtrate using 0.1711 N silver nitrate with dipotassium chromate or dichloroflourescein as the indicator. The ml. of silver nitrate used equals the percent of salt. His second method is as follows: grind a 10 g. sample of cheese with fine sand in a mortar, dilute with 250 ml. of water, then filter and titrate 25 ml. portions of the filtrate with the same solutions as above. The ml. of silver nitrate used equals the percent of salt.

The official method (2) for determination of moisture in cheese is

briefly as follows: weigh a 2 to 3 g. sample of cheese into a round, flat bottomed dish with cover, dry in a vacuum oven at 100°C. (212°F.) until constant weight is reached, which usually requires about 4 hours. During drying, the pressure shall not be allowed to exceed 100mm. of mercury. After drying, cool and reweigh. The percent of moisture can then be calculated from the weight lost.

Wilster, et al, (17) recommends three procedures for moisture determinations as follows: for accurate laboratory analysis, weigh accurately 2 to 3 g. of cheese into a previously dried and weighed moisture dish with cover. Then dry the sample in an oven at 100°C. (212°F.). If a vacuum of 20" or more is available, 10 hours of drying should suffice. If no vacuum is used, 24 hour drying is recommended. Then cool in a desiccator for 1 hour, reweigh, and calculate the percent of moisture. For routine laboratory work, weigh accurately 10 g. of cheese into a moisture dish 3" in diameter and 1" deep by use of a tare beam balance having a sensibility reciprocal of 15 mg.. Then dry in an oven as above, cool in a desiccator, place back on the balance, and read the percent of moisture. For cheese factories, weigh accurately 10 g. of cheese into a dry aluminum dish by use of a tare beam balance as above. Then dry in an oven at 220°F. to 230°F. (104.4°C to 110.0°C.) for 24 hours. If an oven with a steam jacket with 40 to 50 pounds of steam pressure and a temperature of 290°F. (143.3°C.) is available, they report drying can be completed in 4 to 6 hours. After drying, cool in a desiccator, place back on the balance and read the percent of moisture. The two latter methods do have the advantage of being able to read the percent of moisture directly from the beams on the balance.

Morgan, et al, (11) reported moisture determinations using a Bidwell-

-Sterling tube with toluene distillation. They recommend the use of a 5 g. sample of cheese and the procedure recommended for dried milk. They also reported moisture determinations using a Mojonnier solids dish and drying in a Diert-Detroit oven at 255°F. (124°C.) for 20 minutes. The toluene distillation method is not desirable because of the chance of error due to the small size of sample necessary in a product such as cottage cheese. (15)

Goold (5,6) presented a comparison of the Mojonnier and steam pressure oven methods to an olive oil method for the determination of moisture. The olive oil method is briefly as follows: weigh a 5 g. sample of cheese, add 20 ml. of olive oil, heat over an open flame to drive off the moisture, reweigh, and calculate the percent of moisture. He reported results that were slightly lower than the steam pressure oven method and slightly higher than the Mojonnier method.

Kosikowsky, et al, (7) used a semi-automatic moisture tester for cheddar cheese, processed cheese, and cheese food. Their results were comparable to standard atmospheric moisture tests conducted at 101°C. (214°F.). The method was rapid and fairly accurate.

The Mojonnier method (10) for determining moisture in cheese is briefly as follows: weigh accurately approximately 0.5 g. of cheese, dilute with 1.5 ml. of water, and dry on a hot plate until the first brown color appears. Then dry in a vacuum oven for 20 minutes, cool in a desiccator, reweigh, and calculate the percent of moisture from the weight lost.

METHODS

A. Yeast and Mold Determinations.

The method used was Olson's modification (12) of the standard method and is run as follows: thoroughly mix the sample of cheese and weigh 1 g. carefully onto sterile parchment paper, transfer to a sterile mortar and add 1 ml. of a sterile 20% solution of sodium citrate. Grind until the sample is of a smooth consistency, add 8 ml. of sterile water contained in a screw capped test tube and grind until the cheese is completely suspended in the solution. Pour the mixture back into the screw capped test tube, shake the mixture 25 times through an up and down movement of 1 foot in a time not to exceed 7 seconds, make suitable dilutions and pour the plates with potato dextrose agar. Use either potato dextrose agar acidified to a pH of 3.5 with tartaric acid or potato dextrose agar to which 100 parts per million of aureomycin hydrochloride has been added just prior to pouring. The aureomycin is added as a 1% solution in sterile water. This solution was prepared by weighing 100 mg. of aureomycin hydrochloride onto sterile parchment paper and then transferring to 10 ml. of sterile water contained in a screw capped test tube.

B. Fat Determinations.

The Mojonnier method used in the fat determinations is as follows: weigh approximately 5 g. of cottage cheese into a Mojonnier fat extraction flask, add 5 ml. of hot distilled water and 6 ml. of ammonium hydroxide. Mix until the cheese is dissolved and proceed as for the fat determination in milk.

C. Salt Determinations.

The method used was the A.O.A.C. method as modified by Wilster, et al,

(17). The method is as follows: weigh 3 g. of cheese into a 300 ml. Erlenmeyer flask, add 10 ml. of 0.1711 N silver nitrate, 15 ml. of C.P. nitric acid, and 50 ml. of water. Boil the solution and as it boils add 15 ml. of a saturated solution of potassium permanganate in 5 ml. portions. Boil until all cheese particles are digested, and then dilute the solution to about 100 ml. and decant off the liquid into a beaker. Then wash the precipitate by adding 100 ml. of water and decanting into the same beaker as before. Add 3 ml. of ferrous ammonium sulphate as an indicator and titrate the excess silver nitrate with 0.1711 N potassium thiocyanate. The ml. of silver nitrate used minus the titration value divided by the weight of the sample equals the percent of salt in the sample.

D. Moisture Determinations.

The method used for moisture determinations was the Mojonnier method for cheese (10) which is briefly as follows: weigh approximately 0.5 g. of cheese into a previously weighed Mojonnier solids dish, mix with 2 ml. of water to spread the cheese evenly over the bottom of the dish, place on a hot plate at 100°C. (212°F.) and drive off most of the moisture, then place in a vacuum oven at 100°C. (212°F.) for 20 minutes. Remove from the oven and place in a desiccator for 5 minutes, then reweigh and calculate the percent of moisture.

EXPERIMENTAL

A. Yeast and Mold Counts.

In routine analysis of cottage cheese at the Oklahoma Agricultural Experiment Station it was observed that the recommended methods for yeast and mold counts (13) were rather unsatisfactory in certain respects. One of the problems was that of satisfactory dispersion of the curd in preparation of the sample for plating and another was that of the difficulty of counting the plates poured from low dilutions with acidified agar. In the work herein reported attempts were made to develop satisfactory methods for dispersing the curd and to eliminate the precipitation of the curd in the plates due to the use of an acid medium.

1. Methods For Dispersing Cottage Cheese Curd

a. Use of Chemical Compounds

Since various chemical compounds are used as emulsifiers for the making of process cheese, it was thought that some chemical might be satisfactory for dispersing the curd for yeast and mold counts. The general procedure was as follows: The cheese was ground in a food grinder which forced the cheese through a screen with holes approximately 0.5 mm. in diameter. The ground cheese was well mixed and 1 g. 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. With each compound, preliminary trials were run to determine the approximate concentration required to dissolve the curd. The double dilution method was employed in arriving at the various concentrations. This method consists of diluting a given volume of a solution with an equal volume of water to reduce the concentration in half. This can be repeated any number of times to get the concentrations desired and is very valuable in establishing an effective range.

The effectiveness of various concentrations of certain chemical compounds in dispersing cottage cheese curd are shown in Table 1. Since it is desirable to use a minimum of time for running yeast and mold counts, it was thought that the time allowed for the dissolving of the curd should not exceed 3 minutes. A compound was considered to be satisfactory if it dissolved the curd completely in a 3 minute period. The following compounds were considered unsatisfactory because at the end of the 3 minute period of shaking, the curd was not dissolved or dispersed enough for the satisfactory running of yeast and mold counts: ammonium citrate, calcium chloride, potassium monohydrogen phosphate, potassium dihydrogen phosphate, sodium acetate, sodium lactate, sodium oxalate, sodium dihydrogen phosphate, sodium sulphate, and tricalcium phosphate. These compounds were discarded and no further tests involving them were made. The minimum concentrations of the compounds that were satisfactory in dissolving or dispersing the curd are 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%.

TABLE 1

THE EFFECTIVENESS OF VARIOUS CHEMICAL COMPOUNDS
IN DISSOLVING OR DISPERSING COTTAGE CHEESE CURD

CHEMICAL COMPOUND USED	RANGE OF CONCENTRATIONS USED	MINIMUM CONCENTRATION TO DISSOLVE THE CURD IN 3 MINUTES
Ammonium citrate ($(\text{NH}_4)_3 \text{C}_6 \text{H}_5 \text{O}_7$)	1.25% to 10%	Unsatisfactory in concentrations used
Calcium chloride (CaCl_2)	1.25% to 10%	"
Potassium mono-H phosphate (K_2HPO_4)	0.50% to 20%	"
Potassium di-H phosphate (KH_2PO_4)	1.25% to 20%	"
Sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2$)	1.25% to 10%	"
Sodium lactate ($\text{NaC}_3\text{H}_5\text{O}_3$)	1.25% to 10%	"
Sodium Oxalate ($\text{Na}_2\text{C}_2\text{O}_4$)	1.25% to 10%	"
Sodium di-H phosphate ($\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$)	1.25% to 20%	"
Sodium Sulphate (Na_2SO_4)	1.25% to 10%	"
Tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$)	1.25% to 10%	"
Ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$)	0.50% to 10%	0.20%
Ammonium hydroxide ($\text{NH}_4 \text{OH}$)	0.50% to 10%	0.20%
Ammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$)	0.25% to 10%	0.50%
Sodium mono-H phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)	0.50% to 20%	1.25%

TABLE 1 (CONTINUED)

CHEMICAL COMPOUND USED	RANGE OF CONCENTRATIONS USED	MINIMUM CONCENTRATION TO DISSOLVE THE CURD IN 3 MINUTES
Lithium hydroxide (LiOH)	0.10% to 1.60%	0.20%
Minnesota reagent	0.50% to 10%	1.50%
Potassium hydroxide (KOH)	0.10% to 1.60%	0.40%
Sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)	0.50% to 10%	1.00%
Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)	0.50% to 20%	2.00%
Sodium hydroxide (NaOH)	0.20% to 1.60%	0.10%
Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$)	0.50% to 20%	1.50%
Sodium sesquicarbonate ($\text{Na}_3\text{H}(\text{CO}_3)_2 \cdot 2\text{H}_2\text{O}$)	0.50% to 10%	1.00%
Trisodium phosphate ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$)	0.50% to 20%	1.25%

With sodium citrate in effective concentrations, the mixtures were cloudy due to the suspension of fine particles of curd in the mixture 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 in the running of yeast and mold counts if they do not have a toxic effect on the yeasts and molds or otherwise influence the accuracy of the counts.

b. Use of Mechanical Means.

(1) Grinding with 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 there are still small lumps of curd which may be mistaken for yeast colonies. Other disadvantages of this method are the possibility of contamination, the waste of cheese, and the method is somewhat messy.

(2) Grinding with Mortar and Pestle

Grinding cottage cheese with a mortar and pestle was found to break the cottage cheese curd down into very small particles, forming a creamy paste. However, the particles are 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. It was observed that this method of 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.

(3) Grinding with Mortar and Pestle and Sand

Cottage cheese was ground with a mortar and pestle with a small amount of sea sand added. It was observed that this method of grinding is effective in getting the cheese broken down into small particles but it has the same disadvantages as grinding without sand.

(4) 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 hard and then thawed to note the effect on the curd. It was observed that this operation does cause a shattering of the curd, but not sufficient to take the place of grinding with a mortar and pestle or a food mill. It was also found that this operation tends to lower the yeast and mold counts appreciably, presumably due to the crushing action incident to the freezing.

From observations at this station the method of grinding with a mortar and pestle is 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 are sufficient for breaking up the curd particles for the running of yeast and mold counts.

2. Influence of Various Modifications on the Yeast and Mold Counts.

a. Dispersion of Curd by use of Chemicals

Having established that cottage cheese curd can be dissolved or dispersed by various chemical compounds, it was necessary to determine the influence of the use of these compounds on yeast and mold counts. The general procedure was as follows: The cheese was ground with a food mill which forced the cheese through a screen with holes approximately 0.5 mm. in diameter. The ground cheese was well mixed and 1 g. of the sample weighed into a screw capped test tube 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 3 minutes, using a stop watch to time the procedure. At the end of 3 minutes, necessary dilutions were made and duplicate plates were poured. The cheese and chemical mixture was allowed to stand for 3 minutes more at which time the necessary dilutions were made again and duplicate plates were poured. Then the cheese and chemical mixture was

allowed to stand for 6 minutes more at which time the necessary dilutions were made again and duplicate plates were poured. One plate of each pair of duplicate plates was poured with potato dextrose agar which had been acidified to pH of 3.5 with a sterile solution of 10% tartaric acid. The other plate was poured with potato dextrose agar to which had been added 100 parts per million of aureomycin hydrochloride. Both the tartaric acid and the aureomycin hydrochloride were added to the agar just prior to the pouring of the plates. The plates were incubated at 70°F. for five days, then counted and the results recorded. This procedure was followed with the 13 compounds that were satisfactory in dispersing or dissolving the cottage cheese curd. A control was run on each sample of the cottage cheese using Olson's modification of the standard method as shown under methods.

The influence of various chemical compounds used to dissolve or disperse cottage cheese curd on the yeast and mold counts is shown in Table 2. The results of the counts on cottage cheese when using lithium hydroxide, 0.20%; potassium hydroxide, 0.40%; sodium hydroxide, 0.10%; and Minnesota reagent (9), 1.5% are not shown in Table 2, because it was found that the number of colonies on the plates poured with both aureomycin and tartaric acid added were considerably lower than the controls. The number of colonies was considerably lower than the controls, even with 3 minutes exposure time, which indicated that the compounds in the concentrations used were toxic. Only 2 samples of cottage cheese were run using these compounds to dissolve the curd because of their toxicity.

A series of trials were run in which three compounds, ammonium hydroxide, 0.20%; ammonium carbonate, 0.20%; and ammonium phosphate, 0.50% were used.

TABLE 2

THE INFLUENCE OF VARIOUS CHEMICAL COMPOUNDS
USED TO DISSOLVE THE CURD ON THE YEAST AND
MOLD COUNTS ON COTTAGE CHEESE

COMPOUND USED	CONCENTRATION USED	NUMBER OF COLONIES ON POTATO DEXTROSE AGAR WITH					
		100 I.P.M. AUREOMYCIN ADDED			TARTARIC ACID ADDED TO PH 3.5		
		EXPOSURE TIME 3 (minutes) 6 12			EXPOSURE TIME 3 (minutes) 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	113	117	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	114	117	100	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		

The average number of colonies per plate in five trials in these determinations is shown under Series I, Table 2.

The results indicate that ammonium hydroxide, in the concentration used, was toxic to yeasts and molds because the counts were lower than the control and decreased further with the increased holding time. Ammonium phosphate did not seem to be toxic in the concentration used.

Although the counts were lower than the controls, there was no decrease in count as the holding time was increased. Ammonium carbonate seemed to give satisfactory counts with aureomycin agar, but showed a decrease in count upon holding when plated on acidified agar. It was observed

that considerably higher counts were obtained on the plates containing 100 parts per million aureomycin than on the plates acidified with tartaric acid.

A second series of trials were run in which six compounds, sodium borate, 1.0%; sodium citrate, 2.0%; sodium monohydrogen phosphate, 1.25%; trisodium phosphate, 1.25%; sodium pyrophosphate, 1.5%; and sodium sesquicarbonate, 1.0% were used. The average number of colonies per plate in three trials in these determinations is shown under Series II, Table 2.

The results indicate that on the plates poured with potato dextrose agar with 100 parts per million aureomycin added, sodium monohydrogen phosphate, 1.25%; sodium citrate, 2.0%; and sodium borate, 1.0% were satisfactory for dispersing cottage cheese curd for yeast and mold counts, because they gave counts comparable to the controls. In some cases they even showed a slight increase in count with increased holding time. Sodium pyrophosphate, 1.5% and sodium sesquicarbonate, 1.0% seemed to be somewhat toxic because they showed decreases in counts with increased holding time. Trisodium phosphate, 1.25% seemed to be fairly toxic because the counts were considerably lower than the controls and decreased further with increased holding time.

The results on the plates poured with acidified potato dextrose agar indicated the same general trends as those on the agar plates with aureomycin added except that the counts were generally lower. It also appeared that with this medium, sodium borate was somewhat toxic as the average count was lower than that on the control, but there actually appeared to be an increase in count with increased holding. A possible explanation of this discrepancy is that the acid in the agar reacted with the sodium borate carried into the plates with the dilution medium to

form a slightly toxic compound.

The foregoing results indicate that sodium citrate, 2.0% and sodium monohydrogen phosphate, 1.25% are satisfactory for dispersing cottage cheese curd for the running of yeast and mold counts because they dissolve or disperse the cottage cheese curd satisfactorily and do not seem to be toxic to yeasts and molds. It also appeared that ammonium carbonate, 0.20% and ammonium phosphate, 0.50% 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 Series I and Series II using the same concentrations but using the following procedure: The cheese was ground in a food mill as before. The ground cheese was well mixed and 10 g. 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 was recorded. The necessary dilutions were made, and duplicate plates were poured. One of the plates was poured with aureomycin agar and the other was poured with acidified agar. The plates were incubated at 70°F. for 5 days, then counted and the results recorded. Controls were not run with these trials, because the plates containing sodium citrate have the same amount of this chemical compound in them as the controls used in previously reported trials have, therefore, the sodium citrate plates were considered 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 is shown in Table 3.

The results indicate that on the plates poured with aureomycin added to the agar, ammonium carbonate, 0.20%; ammonium phosphate, 0.50%; sodium monohydrogen phosphate, 1.25%; sodium borate, 1.0%; and sodium citrate 2.0% seemed to be satisfactory, because the first three compounds gave results comparable to the results obtained with sodium citrate.

The results indicate that on the plates poured with acidified agar, ammonium phosphate, 0.50%; sodium monohydrogen phosphate, 1.25%; sodium citrate, 2.0%; sodium pyrophosphate, 1.5%; and sodium sesquicarbonate, 1.0% seemed to be satisfactory, because they all gave satisfactory results, assuming that sodium citrate was the control.

The results show further that ammonium hydroxide and trisodium phosphate, in the concentrations used, were toxic to the yeasts and molds as the counts obtained with these compounds were considerably lower than the counts obtained by using sodium citrate as the dispersing medium.

The results also show that there is a considerable variation in the time required for the chemical compounds to dissolve the different samples of cottage cheese. This can be attributed to the differences in physical properties of the curd. The soft curd breaks down rather quickly, while the hard or rubbery curd takes considerably more time. The average time required, in 10 trials, to dissolve the curd shows that ammonium hydroxide, trisodium phosphate, and sodium sesquicarbonate were the most effective. However, two of these compounds, ammonium hydroxide and trisodium phosphate were fairly toxic to yeasts and molds. Sodium citrate, which apparently was not toxic, required the greatest length of time to dissolve the curd of any chemical compound used. Generally, these results show

TABLE 3

THE INFLUENCE ON YEAST AND MOLD COUNTS AND
THE TIME REQUIRED TO DISSOLVE THE CURD
WITH VARIOUS CHEMICAL COMPOUNDS

COMPOUND USED	CONCENTRATION USED	AV. NO. OF COLONIES PER PLATE (5 TRIALS) ON POTATO DEXTROSE AGAR WITH		TIME REQUIRED TO DISSOLVE THE CURD (10 TRIALS) SECONDS	
		100 PPM AUREOMYCIN ADDED	TARTARIC ACID ADDED TO PH 3.5	RANGE	AVERAGE
Ammonium carbonate	0.20%	116	57	45-150	85
Ammonium hydroxide	0.20%	111	61	30-150	73
Ammonium phosphate	0.50%	165	107	60-150	99
Sodium mono-H phosphate	1.25%	166	97	60-130	98
Trisodium phosphate	1.25%	112	68	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	101	45-120	92
Sodium sesquicarbonate	1.0%	119	112	30-100	62

that the more toxic the chemical compound is to yeasts and molds, the more quickly that it will dissolve cottage cheese curd.

The general results indicate that ammonium phosphate, 0.50%; sodium monohydrogen phosphate, 1.25%; and sodium citrate, 2.0% are satisfactory for dispersing cottage cheese curd for the running of yeast and mold counts because they dissolve the curd and do not seem to be toxic to the yeasts and molds 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.

b. The Effect of the Use of Aureomycin Hydrochloride to Inhibit Bacterial Growth on Yeast and Mold Counts.

It has been pointed out earlier that one of the main faults of the standard method (13) for the running of yeast and mold counts is the precipitation of curd in the plates due to the use of an acid medium. The purpose of this acid medium is to inhibit bacterial growth and allow the yeasts and molds to grow. It was thought that if some material other than acid could be used to inhibit bacterial growth the precipitation of curd in the plates which makes counting very difficult could be avoided. The general procedure used has already been given under "Dispersion of Curd by Use of Chemicals". The effect of using aureomycin hydrochloride to inhibit bacterial growth on the yeast and mold counts on cottage cheese is shown in Table 4.

TABLE 4

A COMPARISON OF THE NUMBER OF YEAST AND MOLD COLONIES DEVELOPING ON AUREOMYCIN AGAR AND ON ACIDIFIED AGAR (105 COMPARISONS)

	PLATES Poured WITH POTATO DEXTROSE AGAR WITH	
	100 PARTS PER MILLION AUREOMYCIN ADDED	TARTARIC ACID ADDED TO PH 3.5
Average number of colonies	115	89
Number of comparisons having the higher number of colonies per plate	82	17

The results show that, of the 105 comparisons between the number of colonies per plate, in 82 instances higher counts were obtained with potato dextrose agar to which 100 parts per million aureomycin had been added than with the acidified agar. The average number of colonies on the plates in 105 comparisons with 100 parts per million aureomycin

added to the agar was 115, while the average number on the plates with acidified agar was 89. This is an increase in the count of 29% in favor of the plates with the aureomycin added over the plates acidified to pH of 3.5 with tartaric acid.

It appeared that where there were large discrepancies between the counts on the two media, the difference could be due to the failure of the aureomycin to inhibit all the bacteria. This was not true, however, because in several trials colonies were picked from the plates which looked suspicious, 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 seem 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.

c. The Influence of the Size of Sample Used on the Yeast and Mold Counts.

Although 1 g. samples were used in the data heretofore presented because they were easier to handle, it was thought that a 10 g. sample would probably give a more representative number of the yeasts and molds in the cottage cheese because of the possible uneven distribution of the yeasts and molds in the 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 g. samples and one 10 g. sample were used on the same sample of cottage cheese. The procedure used was as follows:

ten 1 g. samples of cottage cheese were plated out using Olson's modification of the standard method as shown under methods. Plates were poured with both potato dextrose agar with 100 parts per million aureomycin added and potato dextrose agar which has been acidified to pH 3.5 with tartaric acid. Then one 10 g. 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. Again plates were poured with both media. The effect of the size of the sample on the yeast and mold counts on cottage cheese is shown in Table 5.

TABLE 5
THE EFFECT OF THE SIZE OF SAMPLE USED ON THE
YEAST AND MOLD COUNTS ON COTTAGE CHEESE

NUMBER OF 1 g. SAMPLES:	NUMBER OF COLONIES DEVELOPING ON POTATO DEXTROSE AGAR WITH	
	100 PPM AUREOMYCIN ADDED	TARTARIC ACID ADDED TO PH 3.5
1	86	93
2	84	65
3	102	110
4	86	34
5	113	90
6	100	45
7	120	53
8	123	80
9	110	73
10	100	96
AVERAGE	102	74
10 g. SAMPLE	116	82
DIFFERENCE	14	8

The results of the counts on the plates poured with potato dextrose agar which had aureomycin added show that the colonies on the ten 1 g.

samples ranged from 84 to 123 and averaged 102, while the colony count on the 10 g. sample was 116. Two of the 1 g. samples had more colonies than the 10 g. sample, while the remaining 3 had fewer colonies than the 10 g. sample. On the plates poured with acidified potato dextrose agar, the colonies on the ten 1 g. samples ranged from 34 to 110 and averaged 74, while the colony count on the 10 g. sample was 82. Four of the 1 g. samples had more colonies than the 10 g. sample, while the remaining had fewer colonies than the 10 g. sample.

The results seem to show that the use of 1 g. samples would give rather wide variations in the yeast and mold counts on cottage cheese and that the counts would be as reliable as they would be if a 10 g. sample were used. The variations seemed to be more pronounced with the acidified agar than with the agar with aureomycin added. The results also show that higher counts are obtained with potato dextrose agar with 100 parts per million aureomycin hydrochloride added than with potato dextrose agar acidified to pH of 3.5 with tartaric acid.

B. Fat Tests.

In the past, satisfactory methods for fat determinations in cottage cheese have been somewhat neglected. The modified Babcock method (1) for fat determination in cheddar cheese has been applied to cottage cheese by some workers. The Mojonnier method (10), which is an accepted method for fat determinations, has also been applied to cottage cheese. The modified Babcock method has some disadvantages which will be discussed later. The Mojonnier method, as listed under methods, is an accurate method but is time consuming and requires several reagents. This method was used as the standard for comparison for the other methods. There is a need for the development of a simple, rapid, and accurate method for fat determination in cottage cheese, particularly for use in dairy plants.

In an attempt to evaluate various methods for fat determination which might be applied to cottage cheese, 16 samples of cottage cheese were run by various methods and the results obtained compared with the results obtained by the Mojonnier method. The results obtained by the tests which looked promising, which included modifications of the Babcock, the Minnesota, and the Pennsylvania methods, are shown in Table 6. The detailed methods and results obtained are as follows:

1. The Modified Babcock Method.

The modified Babcock method was performed as follows: 9 g. of cottage cheese were weighed into a 50% Babcock cream test bottle, 12 ml. of water at 180°F. was added, and then the bottle was placed in a water bath at 180°F. for 5 minutes. Then 17.5 ml. of sulphuric acid, specific gravity of 1.82 to 1.83, was added in 3 installments, mixing well after each installment. After all the lumps were dissolved, the test bottles were centrifuged for 5, 2, and 1 minute periods, adding water at 140°F.

after the first and second whirlings. The bottles were then placed in a water bath at 140°F. for 3 minutes, glycol was added and the fat percentage obtained by doubling the reading. This method has the advantages of being simple, rapid and requiring a minimum of reagents and equipment.

TABLE 6

A COMPARISON OF VARIOUS METHODS FOR THE DETERMINATION
OF FAT IN COTTAGE CHEESE
(ALL RESULTS SHOWN ARE THE AVERAGES OF DUPLICATE DETERMINATIONS)

SAMPLE NUMBER	MOJONNIER METHOD (CONTROL) % FAT	MODIFIED BABCOCK METHOD		MODIFIED MINNESOTA METHOD		MODIFIED PENNSYLVANIA METHOD	
		% FAT	VAR. FROM CONTROL	% FAT	VAR. FROM CONTROL	% FAT	VAR. FROM CONTROL
1	4.75	3.8	-0.95	4.8	+0.05	4.8	+0.05
2	4.24	3.1	-1.14	4.2	-0.04	3.6	-0.64
3	5.44	4.0	-1.44	5.2	-0.24	5.6	+0.16
4	3.61	3.8	+0.19	3.6	-0.01	3.8	+0.19
5	4.62	4.0	-0.62	5.0	+0.38	5.2	+0.58
6	2.45	2.8	+0.35	2.6	+0.15	2.8	+0.35
7	2.08			2.45	+0.37	2.4	+0.32
8	3.90			4.5	+0.60	4.6	+0.70
9	6.52			8.2	+1.68	6.8	+0.28
10	3.53			4.4	+0.87	4.0	+0.47
11	1.29			1.4	+0.11	1.3	+0.01
12	1.67			1.6	-0.07	1.6	-0.07
13	1.38			1.5	+0.12	1.6	+0.22
14	1.68			1.5	-0.18	1.6	-0.08
15	1.77			1.7	-0.07	1.8	+0.03
16	2.21			2.4	+0.19	2.6	+0.39
AVERAGE	3.20			3.44	+0.24	3.38	+0.185
AV. OF 6 SAMPLES	4.18	3.58	-0.60				

However, it has the disadvantages of the difficulty in getting the sample into the test bottle and the difficulty in reading the fat test due to the large graduations on the 50% cream test bottle. Excessive charring also results, making it difficult to read the fat percentages. From Table 4 it can be seen that the percent of fat as determined by the modified Babcock method does not compare favorably with the Mojonnier method.

The method was tried with only 6 samples of cottage cheese and was considered unsatisfactory because of the considerable variations from the Mojonnier method. The average of the 6 samples was 0.60% lower than the average of the 6 samples as determined by the Mojonnier method. Only 1 out of the 6 determinations checked within 0.20% of the Mojonnier method.

b. The Modified Minnesota Method

Since so much trouble is encountered in getting the cottage cheese into the test bottles, it was thought that some material might be used to dissolve the cottage cheese curd so that it could be pipetted into the test bottles. It was found that this could be accomplished by adding concentrated ammonium hydroxide (27%-30%) to the cottage cheese. It was also thought that the Minnesota method of fat determination might be applied to this dissolved cheese. The test was performed as follows: 27 g. of cottage cheese were weighed into a 2 ounce cream sample jar, then 6.7 ml. of concentrated ammonium hydroxide were added to the cheese. This gave 27 g. of cheese plus 6 g. of ammonium hydroxide, which made 11 g. of the mixture contain 9 g. of cottage cheese. The jar was then placed in a water bath at 120°F. and mixed occasionally until the curd was dissolved. Then 11 g. of the cheese mixture was weighed into an 8% Babcock milk test bottle using a milk pipette. A bulb syringe or pipette pump was used in drawing the cheese mixture into the pipette because of the irritating effect of the ammonia fumes. Then 10 ml. of Minnesota reagent (9) were added to the test bottle and the bottle placed in a water bath at 180°F. for 5 minutes, mixing occasionally. Water at 180°F. was added to bring the mixture up to the base of the neck of the test bottle. The bottle was then centrifuged for 30 seconds and water at 140°F. added to bring the fat column up into the graduated portion of the neck. Then the bottle

was centrifuged for 30 seconds, removed from the centrifuge and the reading doubled to get the percent of fat.

The results show that the greatest variation from the Mojonnier method was 1.68% too high, while the smallest variation was 0.01% too low, with the average variation 0.24% too high. There was a general tendency for the results to be higher than the Mojonnier method, because 10 samples showed results which were higher than the Mojonnier method, while 6 samples showed results which were lower than the Mojonnier method. Ten out of the 16 determinations gave results which checked within 0.20% of the Mojonnier method; this margin of 0.20% is about as close as you can read the tests as the bottles used were graduated in 0.10% and it was necessary to double the readings.

This method has the advantages of being simple, rapid, and easy to run. It does not require a great deal of equipment and reagents not normally found in a dairy plant. It has the particular advantage of dissolving the cottage cheese so that it can be pipetted into the test bottles. It also makes reading the test considerably easier due to the use of the 8% Babcock milk test bottle. The method does not cause any charring of the sample and leaves the fat column clear and easy to read.

The results show that the method is not accurate enough to be considered as a replacement for the Mojonnier method. However, the results compare favorable enough in most cases that the method might have some practical application in a cottage cheese plant.

c. The Modified Pennsylvania Method.

Since the Pennsylvania method for fat determination in ice cream and some other dairy products involves the use of ammonium hydroxide, it was thought that a modification of this method could possibly be applied to cottage cheese. The method, which was compared to the Mojonnier method,

was run as follows: 27 g. of cottage cheese were weighed into a 2 ounce cream sample jar. Then 6.7 ml. of concentrated ammonium hydroxide (27%-30%) were added to the cheese. This was only slightly more ammonium hydroxide than the Pennsylvania method calls for. This mixture gave 27 g. of cheese plus 6 g. of ammonium hydroxide, which made 11 g. of the mixture contain 9 g. of cottage cheese. The jar was then placed in a water bath at 120°F. and mixed occasionally until the cheese was dissolved. Then 11 g. of the cheese mixture were pipetted into an 8% Babcock milk test bottle using a milk pipette. A bulb syringe or pipette pump was used in drawing the cheese mixture into the pipette because of the irritating effect of the ammonia fumes. Then 3 ml. of normal butyl alcohol were added to the test bottle and mixed well with the cheese. Then 17.5 ml. of dilute sulphuric acid, specific gravity of 1.72 to 1.74, were added. This acid was made by adding three and one-half parts of acid to one part of water. The contents of the test bottle were well mixed and then centrifuged as for milk. The test bottle was removed from the centrifuge and the reading doubled to get the percent of fat.

From Table 6 it can be seen that the greatest variation from the Mojonnier method was 0.70% too high, while the smallest variation was 0.01% too high, with the average variation 0.185% too high. There was a tendency for this method to give results slightly higher than the Mojonnier method, because 13 samples showed results which were higher, while only 3 showed results lower than the Mojonnier method. Seven out of the 16 determinations gave results which checked within 0.20% of the Mojonnier method.

This method has the same advantages as the modified Minnesota method

but the results indicate that it is not as accurate. While the average variation from the Mojonnier method was slightly less than with the modified Minnesota method, a smaller number of determinations gave results that checked within 0.20% of the Mojonnier method. This method was not considered as satisfactory as the modified Minnesota method.

d. Use of Detergents.

The possibility of the use of detergents for fat determinations in cottage cheese was considered. The Banco* and Shain** methods were tried using cottage cheese with nothing added and also using cottage cheese which had been dissolved with ammonium hydroxide as in the two previous methods. The rest of the procedures were run according to the instructions given with the reagents. These methods were used on only 4 samples of cottage cheese and the results are not given because they were too erratic. In all cases the results were considerably lower than the Mojonnier method. This was probably due to the acidity of the cottage cheese making it difficult for the detergents to act upon the fat in the cheese. All the results indicate that detergents are unsatisfactory for the determination of fat in cottage cheese.

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

**Shain Reagent was obtained from Dr. Philip Shain, Staten Island, New York.

C. Salt Tests-A Comparison of Methods of Preparation of Cottage Cheese Samples for Direct Titration.

The A.O.A.C. method (2) for the determination of salt in cheese is an accurate method 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, and 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. In comparison of the methods of direct titration for salt in cottage cheese, 10 g. of cheese were mixed with 250 ml. of distilled water. Additional treatments of grinding, heating to 160°F., boiling and filtering were also employed in attempts to get results which check closely with the control method which was the A.O.A.C. method as modified by Wilster, et al, (17). In all cases 25 ml. portions of the cheese and water mixtures were used in the titrations with silver nitrate and mercuric nitrate. Titrations were made on the supernatant liquid before it was filtered. The remainder of the liquid was then filtered through rapid filter paper (E.H. Sargent, number 501) and titrations were made on the filtrate. The titrations were made with 0.1711 N silver nitrate and with 0.1711 N mercuric nitrate using 1 ml. of a 10% solution of potassium chromate as the indicator for silver nitrate and using 12 drops of a 0.10% solution of s-diphenylcarbazone in neutral ethyl alcohol as the indicator for mercuric nitrate. At the end point with silver nitrate the color changed from a yellow to a yellowish orange, while with mercuric nitrate a distinct permanent purple color developed. With these titrations the percent of salt was read directly

from the burette because each ml. of the silver nitrate or mercuric nitrate used represented 1% salt.

The results of the titrations with silver nitrate and mercuric nitrate for salt determinations on cottage cheese prepared by four different methods are shown in Table 7. The results shown in this Table are the average of duplicate determinations. Of the 160 sets of duplicate determinations shown, other than the control, 130 checked exactly with each other, while 29 varied only 0.10%, and only 1 varied as much as 0.20%.

1. Method I. Cheese Ground in a Mortar.

Since it is rather obvious that cottage cheese cannot be titrated without breaking up the curd, it was thought that by grinding the cheese, diluting with water, and then titrating the mixture that the percent of salt might be determined. It was also thought that the cheese should be ground finely to give a uniform mixture of water and cheese. The procedure used was as follows: 10 g. of cottage cheese were weighed onto parchment paper, transferred to a mortar and ground with approximately 10 ml. of distilled water to a fine paste. The mixture was then transferred to a 400 ml. beaker and the mortar rinsed several times with distilled water to make sure all the cheese was transferred. Then enough distilled water was added to make the total amount used 250 ml..

a. Not Filtered.

The results show that with silver nitrate on samples of cottage cheese ground in a mortar and not filtered, the greatest variation from the control was 0.23% too high, with the average variation 0.056% too high. Six of the 10 determinations checked within 0.10% of the control.

TABLE 7

A COMPARISON OF METHODS OF PREPARATION OF COTTAGE CHEESE FOR DIRECT TITRATION WITH SILVER NITRATE AND MERCURIC NITRATE
(ALL RESULTS SHOWN ARE THE AVERAGES OF DUPLICATE DETERMINATIONS)

SAMPLE NUMBER	CONTROL SALT (%)	METHOD I GROUND IN MORTAR				METHOD II GROUND IN MORTAR WITH SAND				METHOD III HEATED TO 160°F.				METHOD IV HEATED TO BOILING			
		NOT FILTERED		FILTERED		NOT FILTERED		FILTERED		NOT FILTERED		FILTERED		NOT FILTERED		FILTERED	
		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH		SALT (%) OBTAINED WITH	
		AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂	AgNO ₃	Hg (NO ₃) ₂
1	1.22	1.20	1.10	1.20	1.10	1.20	1.10	1.15	1.15	1.10	1.10	1.10	1.10	1.20	1.20	1.20	1.20
2	1.66	1.55	1.45	1.50	1.40	1.50	1.50	1.45	1.45	1.45	1.30	1.40	1.30	1.70	1.45	1.65	1.40
3	1.85	1.90	1.80	1.90	1.80	1.90	1.80	1.85	1.85	2.00	1.90	1.90	1.90	2.10	2.10	2.10	2.10
4	2.08	2.05	2.00	2.10	2.05	2.10	2.00	2.10	2.00	2.10	2.10	2.10	2.10	2.20	2.20	2.20	2.20
5	1.74	1.80	1.70	1.80	1.70	1.80	1.70	1.75	1.70	1.75	1.70	1.75	1.70	1.80	1.80	1.80	1.80
6	1.05	1.20	1.10	1.20	1.10	1.20	1.10	1.18	1.10	1.30	1.10	1.25	1.10	1.20	1.20	1.20	1.20
7	0.52	0.70	0.60	0.70	0.60	0.60	0.60	0.60	0.60	0.70	0.55	0.65	0.60	0.70	0.55	0.65	0.60
8	1.52	1.75	1.60	1.70	1.60	1.70	1.60	1.70	1.60	1.70	1.55	1.70	1.60	1.80	1.70	1.80	1.70
9	1.15	1.20	1.10	1.20	1.10	1.20	1.10	1.15	1.12	1.20	1.18	1.15	1.15	1.18	1.20	1.20	1.20
10	1.20	1.20	1.10	1.20	1.10	1.20	1.20	1.20	1.20	1.20	1.15	1.20	1.20	1.25	1.25	1.30	1.20
AVERAGE	1.399	1.455	1.355	1.45	1.355	1.44	1.37	1.413	1.377	1.450	1.363	1.420	1.375	1.513	1.465	1.510	1.460
AV. VAR. FROM CONTROL		+0.056	-0.044	+0.051	-0.044	+0.041	-0.029	+0.014	-0.022	+0.051	-0.036	+0.021	-0.024	+0.114	+0.066	+0.111	+0.061

The greatest variation from the control when titrating with mercuric nitrate was 0.21% too low, with the average variation 0.044% too low. Eight of the 10 determinations checked within 0.10% of the control.

b. Filtered.

The results show that with silver nitrate on samples of cottage cheese ground in a mortar and filtered, the greatest variation from the control was 0.18% too high, with the average variation 0.051% too high. Six of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.16% too low, with the average variation 0.044% too low. Eight of the 10 determinations checked within 0.10% of the control.

The above results indicate that grinding the cottage cheese with a mortar and mixing with water is a satisfactory method of preparing cottage cheese for salt determination by direct titration. It appeared that filtering of the sample improved the accuracy of the method only slightly. Titration with mercuric nitrate seemed to give more accurate results than titration with silver nitrate. Mercuric nitrate has the additional advantage of having an end point that is very easy to read.

2. Method II. Cheese Ground in a Mortar with Sand.

It was thought that grinding the cottage cheese in a mortar with the addition of sea sand might aid in getting the curd particles broken down more finely and would give a more uniform mixture of water and cheese than grinding without sand. The procedure used was as follows: 10 g. of cottage cheese were weighed onto parchment paper, transferred to a mortar and ground to a fine paste with 1 teaspoon of sea sand and approximately 10 ml. of distilled water. The mixture was then transferred to a 400 ml.

beaker and the mortar rinsed several times to make sure all the cheese was transferred. Then enough distilled water was added to make the total amount used 250 ml..

a. Not Filtered.

The results show that with silver nitrate on samples of cottage cheese ground in a mortar with sand and not filtered, the greatest variation from the control was 0.18% too high, with the average variation 0.041% too high. Seven of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.16% too low, with the average variation 0.029% too low. Eight of the 10 determinations checked within 0.10% of the control.

b. Filtered.

The results show that with silver nitrate on samples of cottage cheese ground in a mortar with sand and filtered, the greatest variation from the control was 0.21% too low, with the average variation 0.014% too high. Seven of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.21% too low, with the average variation 0.022% too low. Nine of the 10 determinations checked within 0.10% of the control.

The above results indicate that grinding the cottage cheese with a mortar with sea sand added and mixing with water is a satisfactory method of preparing cottage cheese for salt determination by direct titration. It appeared that there was a slight advantage in favor of filtering the sample. Titration with mercuric nitrate seemed to give more accurate results than titration with silver nitrate. This method

gave results that checked more closely with the control than grinding without sand, but the difference was slight.

3. Method III. Cheese Heated to 160°F.

It was thought that some method of eliminating most of the protein portion from the cheese and water mixture might give results which check more closely with the control. It was thought that heating to 160°F. might accomplish this protein precipitation. The procedure used was as follows: 10 g. of cottage cheese were weighed into a 400 ml. beaker, 250 ml. of water were added and the mixture well mixed. The mixture was heated to 160°F. and then cooled to room temperature in a water bath.

a. Not Filtered.

The results show that with silver nitrate on samples of cottage cheese heated to 160°F. and not filtered, the greatest variation from the control was 0.25% too high, with the average variation 0.051% too high. Four of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.36% too low, with the average variation 0.036% too low. Eight of the 10 determinations checked within 0.10% of the control.

b. Filtered.

The results show that with silver nitrate on samples of cottage cheese heated to 160°F. and filtered, the greatest variation from the control was 0.26% too low, with the average variation 0.021% too high. Five of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.36% too low, with the average variation 0.24% too low. Eight of the 10 determinations checked within 0.10% of the control.

The above results indicate that mixing cottage cheese with water

and heating to 160°F. is a fairly satisfactory method of preparing cottage cheese for salt determination by direct titration. It appeared that there was a slight advantage in favor of filtering the sample. Titration with mercuric nitrate seemed to give more accurate results than titration with silver nitrate. This method did not give results that checked as closely with the control as method I or method II.

4. Method IV. Cheese Heated to Boiling.

It was thought that heating the cheese and water mixture to a temperature higher than 160°F. might eliminate more of the protein portion of the cheese and give results which check more closely with the control. The procedure used was as follows: 10 g. of cottage cheese were weighed into a 400 ml. beaker, 250 ml. of water were added, and the mixture well mixed. The mixture was heated to the boiling point and then cooled to room temperature in a water bath.

a. Not Filtered.

The results show that with silver nitrate on samples of cottage cheese heated to boiling and not filtered, the greatest variation from the control was 0.28% too high, with the average variation 0.114% too high. Five of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.25% too high, with the average variation 0.066% too high. Five of the 10 determinations checked within 0.10% of the control.

b. Filtered.

The results show that with silver nitrate on samples of cottage cheese heated to boiling and filtered, the greatest variation from the control was 0.28% too high, with the average variation 0.111% too high. Five of the 10 determinations checked within 0.10% of the control.

The greatest variation from the control when titrating with mercuric nitrate was 0.26% too low, with the average variation 0.061% too high. Five of the 10 determinations checked within 0.10% of the control.

The above results indicate that mixing cottage cheese with water and heating to boiling is not a satisfactory method of preparing cottage cheese for salt determination by direct titration. There did not appear to be any advantage to filtering the sample. Neither silver nitrate nor mercuric nitrate seemed to give any particular advantage to accuracy of results. This method was the least accurate of the four methods tried.

D. Moisture Tests-A Comparison of the Cenco Automatic Moisture Tester with the Mojonnier Method.

The Mojonnier method (10) for the determination of moisture in cheese is an accurate method, but it requires the use of the Mojonnier milk tester which is not available in many dairy plants. The A.O.A.C. method (2) is also an accurate method, but it requires considerable equipment usually not found in dairy plants and is 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 to determine the percent of moisture.

The Cenco automatic moisture tester was designed primarily for low moisture materials such as wheat, oats, barley, rice, corn, starch, flour, dry milk powder, and other materials having a low moisture content. With these materials it is satisfactory to place the material directly into the drying pan. However, with cottage cheese there will be a dried layer of cheese in the pan which would be difficult to remove. For this reason it was considered desirable to use a filter paper in the bottom of the pan to spread the cheese over to avoid this dried cheese in the drying pan. The procedure used was as follows: a piece of filter paper (E.H. Sargent, Number 501) $12\frac{1}{2}$ cm. in diameter was placed in the drying pan and the lamp turned on to remove any moisture from the filter paper.

*The Cenco automatic moisture tester is manufactured by the Central Scientific Company, Chicago 13, Illinois.

The pan and paper were then balanced at 100%. The tester dial was then turned to 0%. Enough cottage cheese was placed on the filter paper and spread evenly over the surface to balance the pan at 0%. Approximately 5 g. of cheese are required to balance the tester. The cheese must be spread evenly over the paper to keep the pan from tilting. 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, which included the use of 115, 100, 85, and 70 volts of current for the entire drying process, and the use of a 225 watt drying lamp with the different voltages, were tried on samples of cottage cheese to determine the most satisfactory way of drying the sample with the Cenco automatic moisture tester. It was found that 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 making the use of it undesirable. It was found that by using the 125 watt drying 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, the cottage cheese could be dried to a weight that would remain constant for a 2 minute period without excessive charring

of the sample.

It was thought that it would be desirable to establish a drying curve for cottage cheese on the Cenco automatic moisture tester. To accomplish this, 10 samples of cottage cheese were used for moisture determinations, and the reading of the tester dial observed at 2 minute intervals for a period of 20 minutes. In a few trials the observations were made for as long as 30 minutes, but it was noted that there was no significant change past the 20 minute period.

The drying curve which was determined by averaging quadruplicate determinations read at 2 minute intervals on 10 samples of cottage cheese is shown in Table 8 and presented graphically in Chart I. The results show that there is a rather rapid loss of moisture for the first 8 minutes, the tester dial showing an average reading of 77.2% at the end of this period. There is a considerably decreased rate of loss for the next 6 minutes, the tester dial showing an average reading of 80.9% at the end of this period. From this point on the loss of moisture is very slight through the 20 minute period, the tester dial showing an average reading of 81.2% at the end of this period.

In order to determine what degree of accuracy could be expected by the Cenco method for moisture determination, the maximum variation among the 4 replicate determinations on each sample of cottage cheese at each time interval indicated above was calculated. The results are shown in Table 9. The results show that as the drying time proceeds, the maximum variation among replicate determinations on the same sample of cottage cheese at 2 minute time intervals becomes increasingly less. At 16 minutes the maximum average variation is 0.36% and at 18 and 20 minutes the maximum average variation is 0.35%. These results include

sample number 8 which had 1 replicate that was considerably higher than the others indicating that an error had been made. Excluding this sample, the results show that the maximum average variation among replicate determinations at 16 and 18 minutes was 0.32%, and at 20 minutes 0.31%. These results bear out the observation that with the Cenco method of moisture determination, results can be expected that will check within approximately 0.30% among replicate determinations.

TABLE 8

THE RATE OF LOSS OF MOISTURE FROM COTTAGE CHEESE
DURING DRYING BY THE CENCO METHOD
(RESULTS ARE THE AVERAGES FOR 4 REPLICATE DETERMINATIONS)

SAMPLE NUMBER	DRYING TIME IN MINUTES									
	2	4	6	8	10	12	14	16	18	20
	AVERAGE READINGS ON THE CENCO DIAL FOR 4 REPLICATE DETERMINATIONS									
1	20.2	46.6	69.8	76.0	80.7	81.4	81.8	81.9	81.9	82.0
2	18.5	44.1	65.5	75.5	78.6	80.1	80.6	80.9	81.0	81.0
3	18.8	44.8	66.8	76.2	79.2	80.2	80.6	80.8	80.9	80.9
4	18.7	43.9	63.2	75.8	78.7	80.6	81.0	81.4	81.5	81.6
5	26.9	51.6	72.2	78.9	80.3	80.8	81.1	81.4	81.5	81.5
6	23.1	48.8	68.5	76.6	78.8	79.6	79.9	80.1	80.3	80.3
7	19.3	46.1	68.4	76.9	78.6	79.3	79.8	80.1	80.2	80.2
8	18.7	45.0	67.5	77.8	80.2	81.1	81.3	81.5	81.5	81.6
9	19.5	46.3	69.1	78.1	80.4	81.0	81.4	81.6	81.7	81.7
10	19.0	46.1	68.0	77.5	80.1	80.9	81.1	81.3	81.4	81.5
AVERAGE	20.3	46.6	68.0	77.5	80.1	80.5	80.9	81.1	81.2	81.2

The results further show that at the end of 16 minutes of drying, the replicate determinations on each sample of cottage cheese checked within 0.30% of each other with 6 out of the 10 samples. With an additional 2 minutes of drying, 8 out of the 10 samples checked within 0.30% of each other. In analysis of the individual determinations on the 4 replicates on 10 samples, 24 out of the 40 determinations were dried to within 0.10% of their final moisture after 16 minutes of drying.

CHART 1
DRYING CURVE FOR COTTAGE CHEESE

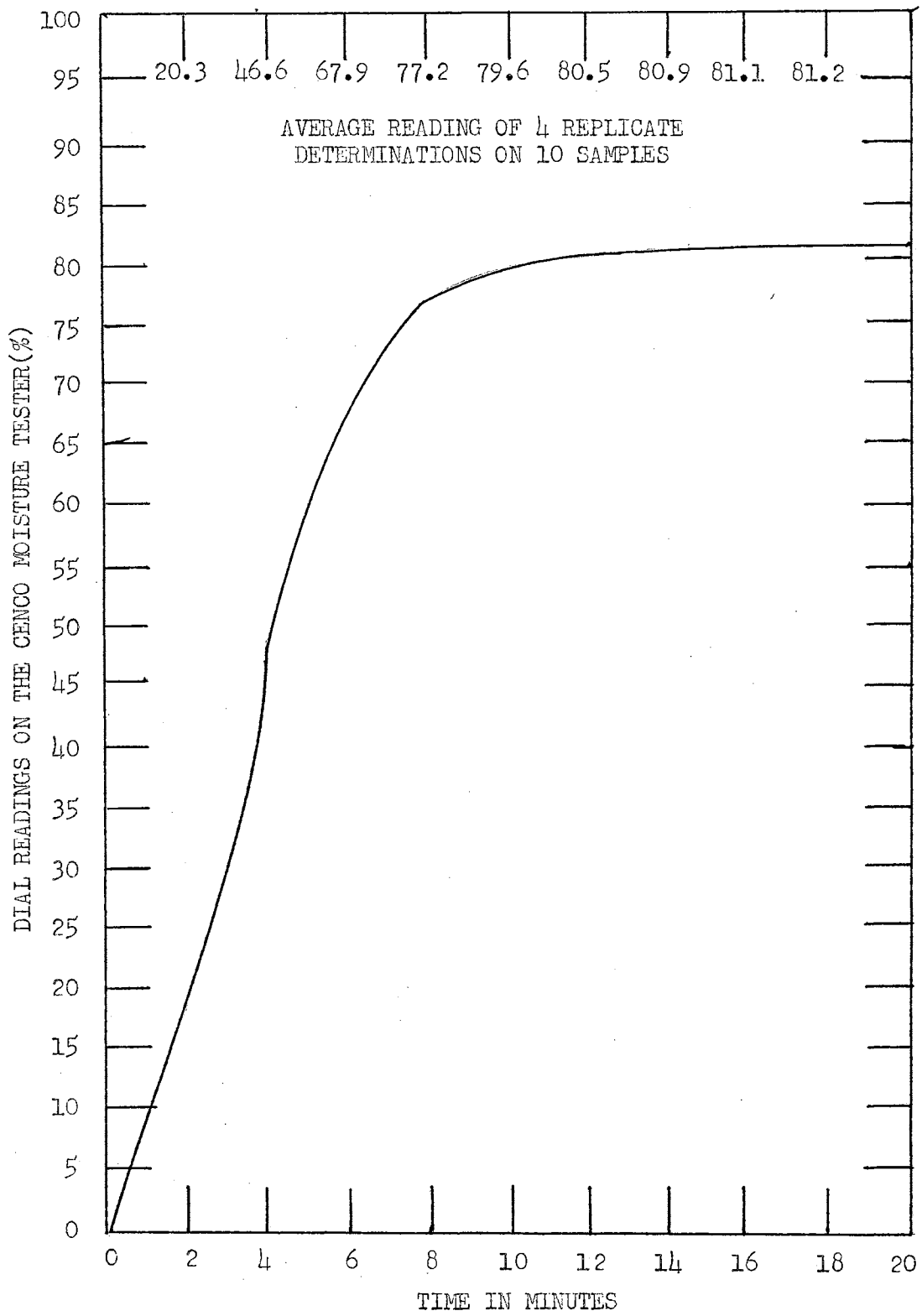


TABLE 9

THE MAXIMUM VARIATION AT 2 MINUTE INTERVALS
AMONG FOUR REPLICATE DETERMINATIONS FOR MOISTURE IN
COTTAGE CHEESE BY THE CENCO METHOD

SAMPLE NUMBER	TIME IN MINUTES									
	2	4	6	8	10	12	14	16	18	20
	MAXIMUM VARIATIONS IN READINGS ON THE CENCO DIAL AMONG FOUR REPLICATE DETERMINATIONS									
1	4.8	7.6	2.2	2.4	1.3	0.8	0.4	0.3	0.3	0.3
2	3.0	7.2	6.0	5.4	3.5	1.6	1.0	0.5	0.3	0.3
3	3.4	4.0	3.6	3.2	1.2	0.9	0.6	0.3	0.3	0.3
4	4.4	8.4	4.8	7.1	2.4	0.7	0.3	0.2	0.3	0.4
5	2.0	6.8	4.2	2.3	1.8	1.2	0.7	0.5	0.3	0.3
6	7.0	5.0	2.2	0.6	0.4	0.5	0.4	0.4	0.5	0.3
7	1.0	3.2	4.0	1.2	1.0	0.6	0.3	0.1	0.2	0.3
8	1.5	1.6	1.6	0.4	1.4	1.0	0.9	0.7	0.7	0.7
9	1.0	2.6	1.8	0.7	0.2	0.2	0.3	0.3	0.3	0.3
10	0.9	1.4	0.4	0.7	0.6	0.3	0.3	0.3	0.3	0.3
AVERAGE	2.90	4.78	3.08	2.40	1.38	0.78	0.52	0.36	0.35	0.35
AV. EX- CLUDING SAMPLE NO. 8	3.05	5.13	3.38	2.62	1.38	0.75	0.48	0.32	0.32	0.31

With an additional 2 minutes of drying, 39 out of the 40 determinations were dried to within 0.10% of their final moisture.

The results show that cottage cheese can be dried by the Cenco moisture tester in 18 minutes without charring, if 115 volts of current are used until the reading on the Cenco dial is about 75%, which requires about 8 minutes, and then the current is cut to 85 volts for the remainder of the drying time.

In order to determine the relative accuracy of the method, tests were run on 18 samples of cottage cheese using the above method and the results obtained compared to the results obtained from the Mojonnier method. The Cenco determinations were made in quadruplicate, while the Mojonnier determinations were made in duplicate.

The results obtained with the Cenco moisture tester and with the

TABLE 10

A COMPARISON OF THE CENCO METHOD WITH THE MOJONNIER
METHOD FOR THE DETERMINATION OF MOISTURE
IN COTTAGE CHEESE

SAMPLE NUMBER	MOJONNIER METHOD AVERAGE OF DUPLICATES %	CENCO METHOD REPLICATE DETERMINATIONS					VAR. FROM MOJON- NIER METHOD %
		1	2	3	4	AV.	
		%	%	%	%	%	
1	81.52	81.4	81.8	81.5	81.6	81.56	+0.06
2	81.61	81.1	81.0	80.8	80.9	80.95	-0.66
3	81.51	81.5	81.7	81.8	81.4	81.60	+0.09
4	81.74	81.4	81.8	81.5	81.6	81.57	-0.17
5	81.50	81.5	81.7	81.7	81.8	81.67	+0.17
6	81.33	81.4	81.5	81.7	81.6	81.55	+0.22
7	79.97	80.4	80.3	80.7	80.3	80.42	+0.45
8	80.34	80.4	80.4	80.2	80.6	80.40	+0.06
9	77.86	78.8	78.6	78.6	78.7	78.67	+0.81
10	80.02	80.7	80.8	80.5	80.6	80.65	+0.63
11	81.45	81.8	81.8	81.6	81.6	81.70	+0.25
12	77.76	78.5	78.4	78.6	78.5	78.50	+0.74
13	84.33	84.1	84.3	84.2	84.2	84.20	-0.13
14	83.91	83.8	84.0	83.8	83.9	83.87	-0.04
15	83.89	83.3	83.6	83.6	83.4	83.47	-0.42
16	83.40	82.7	83.1	82.8	82.9	82.87	-0.53
17	83.90	83.9	84.2	84.0	84.1	84.05	+0.15
18	82.77	83.0	83.6	83.4	83.2	83.30	+0.53
AVERAGE VARIATION FROM THE MOJONNIER METHOD OF ALL SAMPLES							+0.12

Mojonnier method for moisture in 18 samples of cottage cheese are shown in Table 10. Of the 18 samples of cottage cheese run on the Cenco moisture tester and compared to the Mojonnier method, 4 samples checked within 0.10%, 8 within 0.20%, 10 within 0.30%, 12 within 0.50%, 14 within 0.60%, 16 within 0.70%, 17 within 0.80%, and all the samples checked within 0.90% of the Mojonnier method. The greatest variation from the Mojonnier method was 0.81% too high, with the average variation 0.12% too high. Twelve of the 18 determinations gave results that were higher

than the results obtained with the Mojonnier method, indicating that there is a tendency for the method to give results that are too high. The greatest variation of 0.81% from the Mojonnier method is an error of only 1.04%. A comparison of each individual determination (4 replicates each on 18 samples) to the average results obtained by the Mojonnier method shows that, of a total of 72 comparisons, in the majority (40 of 72) of the determinations the maximum variation was 0.30% or less. All determinations checked within 1.0% of the average for the Mojonnier determinations.

These results indicate that the majority of the moisture tests by the Genco method will be within 0.30% of the Mojonnier method.

The results indicate that the method is accurate enough to be used for the determination of moisture in cottage cheese in a dairy plant. The method had the advantages of being simple and rapid. It does have the disadvantage of fairly high initial cost for the tester.

SUMMARY AND CONCLUSIONS

A study was made of methods of analysis of cottage cheese in order to determine which were the most satisfactory and to attempt to develop new methods or to improve on methods in current use. The methods studied were those for yeast and mold counts, fat content, salt content and moisture content.

A study was made on methods of preparing cottage cheese curd for yeast and mold counts involving chemical and mechanical means to disperse or dissolve the curd. A total of 23 chemical compounds were used in an attempt to disperse or dissolve the curd for yeast and mold counts. Briefly the procedure was to weigh 1 g. of ground cottage cheese into 9 ml. of an aqueous solution of the chemical compound containing a few glass beads. The mixture was mixed by inverting at the rate of 30 times per minute. A compound was considered satisfactory if it dissolved or dispersed the curd completely in 3 minutes. Ten of the compounds were unsatisfactory because they did not dissolve or disperse the curd in 3 minutes of mixing. The remaining 12 compounds were satisfactory because they dissolved or dispersed the cottage cheese curd in 3 minutes. The mechanical means included: grinding with a food mill, grinding with a mortar and pestle, grinding with a mortar and pestle with sand, and freezing and thawing. These methods were placed in the following order with respect to their ability to break down the cottage cheese curd: (1) grinding with a mortar and pestle with sand, (2) grinding with a mortar and pestle, (3) grinding with a food mill, and (4) freezing and thawing. Grinding with a mortar and pestle and also the food mill proved to be somewhat messy, while freezing and thawing considerably lowered the

yeast and mold counts. None of the mechanical means alone was considered satisfactory for breaking down the cottage cheese curd for yeast and mold counts.

Cottage cheese was plated out using the 12 chemical compounds that were satisfactory for dissolving or dispersing the curd to see if they were toxic to yeasts and molds. It was found that lithium hydroxide, potassium hydroxide, sodium hydroxide, and Minnesota reagent were highly toxic, ammonium carbonate, ammonium hydroxide, sodium borate, trisodium phosphate, sodium pyrophosphate, and sodium sesquicarbonate seemed to be slightly to considerably toxic, while ammonium phosphate, sodium citrate, and sodium monohydrogen phosphate did not seem to be toxic to yeasts and molds. Sodium monohydrogen phosphate and ammonium phosphate are recommended above sodium citrate, because they seemed to dissolve the curd, while sodium citrate seemed to give a suspension of cheese in the solution.

A comparison was made between potato dextrose agar with 100 parts per million aureomycin hydrochloride added and potato dextrose agar acidified to pH 3.5 with tartaric acid. It was found that higher counts were obtained with the agar with aureomycin added, indicating that these counts were more accurate than those obtained with the acidified agar. Another advantage of the aureomycin agar was that there was no precipitation of casein as was the case with the acidified agar.

A trial was run to determine the influence of the size of the sample used on the yeast and mold counts. The counts on ten 1 g. and one 10 g. samples from the same sample of cottage cheese were compared. It was found that the 1 g. samples varied considerably and it appeared that the use of a 10 g. sample gave results that were more reliable.

A study was made of methods for the determination of the fat content of cottage cheese. The modified Babcock, the Minnesota, and the Pennsylvania methods were tried along with the Banco and the Shain methods. The two latter methods and the modified Babcock method were unsatisfactory, because they did not give results that checked favorably with the Mojonnier method. With the modified Minnesota and the modified Pennsylvania methods, the cheese was dissolved with concentrated ammonium hydroxide, pipetted into an 8% Babcock milk test bottle, and then the recommended procedures were followed. It was found that the modified Minnesota method gave results that checked more closely with the Mojonnier method than did those obtained with the modified Pennsylvania method, but it was not considered entirely satisfactory.

A study was made of various methods of preparation of cottage cheese for direct titration with silver nitrate and mercuric nitrate for salt determination. The methods used were as follows: (1) cheese ground in a mortar, (2) cheese ground in a mortar with sand, (3) cheese diluted with water and heated to 160°F., and (4) cheese diluted with water and heated to boiling. All samples were diluted with 250 ml. of distilled water and 25 ml. portions titrated, with and without filtering, with silver nitrate and with mercuric nitrate. It was found that the most accurate method was grinding in a mortar with sand, diluting with 250 ml. of distilled water, filtering, and titrating with mercuric nitrate. However, it appeared that the advantage gained by the use of sand and filtering over just grinding with a mortar was not worth the extra trouble and time involved.

A study was made of the Cenco automatic moisture tester to determine its relative accuracy for the determination of moisture in cottage cheese. A drying curve was established for cottage cheese for the Cenco tester.

This curve shows that there is a rapid loss of moisture for the first 8 minutes of drying, with a considerably decreased rate of loss for the next 6 minutes. From this point on the loss is very slight for the remainder of the drying process. The results indicated that from 14 to 18 minutes are required for the drying to be completed. It was found that results can be expected that will check within about 0.30% of the Mojonnier method in 18 minutes if the 125 watt drying lamp with 115 volts of current are used for the first 8 minutes of drying, and then cutting the current to 85 volts for the remainder of the drying operation.

WORKS CITED

1. Arbuckle, W.S. Mercurimetric Method for Salt Determination in Butter and New Cheese, *J. Dairy Sci.*, 29:431-436. 1946.
2. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 6th ed. Washington, D.C.: A.O.A.C., 1945.
3. Foter, Milton J., E.O. Anderson, and L.R. Dowd. A Survey of Commercial Cottage Cheese. *J. Dairy Sci.*, 24:544-545. 1941.
4. Goss, Emery F. Techniques of Dairy Plant Testing. 1st. ed. Ames, Iowa.: The Iowa State College Press. 1953.
5. Gould, I.A. A comparative study of Methods of Determining the Moisture Content of Cheddar Cheese. I. The Modified Mojonnier and Olive Oil Methods. *J. Dairy Sci.*, 20:625-635. 1937.
6. Gould, I.A. A Comparative Study of Methods of Determining the Moisture Content of Cheddar Cheese. II. The Steam Oven Method at High Pressure and the Olive Oil Method. *J. Dairy Sci.*, 21:379-383. 1938.
7. Kosikowsky, F.V., A.C. Dahlberg, and B.L. Herrington. The Use of A Semi-automatic Moisture Tester for Cheese and Cheese Products. *Food Technology*, 3:320-322. 1949.
8. Marquardt, J.C. Methods for Determining Salt in Various Cheeses. *N.Y. Agr. Exp. Sta. Bul.* 249. 1938.
9. Matt, Morris C. Kimble Manual on Dairy Testing. 4th ed. Kimble Glass Co., Toledo, Ohio. 1951.
10. Mojonnier, T. and H.C. Troy. Mojonnier Bros. Instruction Manual for Setting Up and Operating the Mojonnier Milk Tester. Mojonnier Bros. Co., Chicago, Illinois. 1925.
11. Morgan, Max E., E.O. Anderson, L. Hankin, L.R. Dowd, and Milton J. Foter. Chemical Bacteriological and Organoleptic Characteristics of Retail Cottage Cheese. *Storrs Agr. Exp. Sta. Bul.* 284. Univ. of Conn.. 1952.
12. Olson, H.C. Unpublished Data. *Okla. Agr. Exp. Sta.* 1954.
13. Standard Methods for the Examination of Dairy Products. 10th. ed. American Public Health Association, Inc. New York, N.Y. A.O.A.C. Washington, D.C.. 1953.
14. Turner, A.W., B. Rogers, and V. Conquest. Orthophosphoric acid as a Cheese Solvent. *J. Dairy Sci.*, 25:777-778. 1942.
15. Triebold, Howard O. Quantitative Analysis with Applications to Agricultural and Food Products. 3rd printing. New York. D. Van Nostrand Co., Inc., 1949.

16. Troy, H .C. A Cheese Moisture Test. Cornell Agr. Ext. Bul. 17. 1917.
17. Wilster, G.H., W.V. Price, A.J. Morris, E.F. Goss, and G.P. Sanders. Determination of Fat, Moisture, and Salt in Soft Cheese. J. Dairy Sci., 23:197-200. 1940.

VITA

Milford Dean Bonner
candidate for the degree of
Master of Science

Thesis: A STUDY OF METHODS USED FOR ANALYSIS OF
COTTAGE CHEESE

Major: Dairy Manufacturing

Biographical and Other Items:

Born: June 12, 1929 at Reform, Alabama

Undergraduate Study: Mississippi State College,
1947-1951

Graduate Study: Oklahoma A & M College, 1951-
-1954

Experiences: Farming in summers, 1944-1947;
Employed part time by Dairy Department,
Mississippi State College, 1949-1951;
Army, 7th Infantry Division in Korea,
1951-1953; Graduate assistant, Oklahoma
A & M College Dairy Department, 1953-1954.

Member of Alpha Zeta, Beta Beta Beta, and Masonic
Order.

THESIS TITLE: A STUDY OF METHODS USED FOR ANALYSIS
OF COTTAGE CHEESE

AUTHOR: Milford Dean Bonner

THESIS ADVISER: Dr. H.C. Olson

The content and form have been checked and approved
by the author and thesis adviser. The Graduate
School Office assumes no responsibility for errors
either in form or content. The copies are sent to
the bindery just as they are approved by the author
and faculty adviser.

TYPIST: Phyllis Poole