COMMERCIAL UTILIZATION OF SOYBEAN MEAL FOR INDUSTRIAL CASEIN

by

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I. INTRODUCTION

Soybean production today is of considerable importance, in both agriculture and industry. The rapid rise of the soybean industry is shown by the following statistics. In 1924 seed production was 4,947,000 bushels with a total of 1,782,000 acres under cultivation. In 1938 the seed production was 57,665,000 bushels with a total of 7,789,000 acres under cultivation. The average market price dropped from $2.47 in 1924 to $0.76 per bushel in 1938. The rise of this industry is attributed to the development of the uses to which the soybean is applicable.

With such a rapidly growing industry, many problems arise which must be solved to have continued progress. The progress made in developing food and industrial uses for the soybean, the oil, and the meal has been remarkable. Though extensive work has been conducted to develop the industry, the possibilities are yet many.

The industrial uses of soybeans are many, with the use of soybean oil in the lead at present. Soybean oil is used satisfactorily as a core oil in foundry work. Much has been done in developing paint from soybean oil. Heat treatment produces a drying

oil which compares favorably with linseed oil. Remarkable progress has been made in developing high-temperature bake enamels from soybean oil for use in the automobile industry.

The meal remaining after the oil extraction has various industrial uses. Its value in the production of plastics is accounted for by the high protein content, approximately 44 per cent. Two general methods exist for the use of meal in plastic production. One is by the isolation and purification of the protein, with the addition of a filler and an indurating agent. The other method involves the use of whole meal in place of the isolated protein, with the remaining procedure being analogous. The use of the whole meal has given satisfactory results for some purposes. The extracted protein material, which is vegetable casein, has uses and properties similar to casein from skim milk. Among the uses are glues, sizing material, plastics and artificial wools.

It is the purpose of this work to study the various factors encountered in the process of isolating the industrial casein from the whole meal. Considerable work has already been done on this problem, but pertinent data which is necessary to industry has not yet been compiled and published.

II. LITERATURE REVIEW

General Method of Casein Extraction A.A. Horvath is the authority on soybeans and their industrial uses in the United States. Much of his information he has obtained from the Chinese chemist, Sato. The general method of casein extraction as given by him and others is as follows:[1][10][13][14] Solvent extraction of the oil is necessary prior to the casein extraction. This leaves a meal relatively free from oil and with the casein in an undamaged state. The solvents used for casein extraction are generally a basic salt or alkaline solution. Sodium hydroxide and Na_2SO_3 solutions are the more common. A weak alkaline solution and ground meal are then mixed, with the casein going into solution. Separation of the slurry resulting from the mixing is accomplished by frequent filterings[12] or by the use of a super centrifuge.[1][13] Casein is precipitated from the solution by any reagent capable of lowering the pH to the isoelectric point of casein, which is near 4.6. However, sulfurous, sulfuric, hydrochloric, and acetic acids are more common.[10] Care must be taken in controlling both the pH and temperature during precipitation to secure a curd convenient to handle.[1] The whey and curd are separated by centrifuging, filtration or settling. To reduce the impurities and acid content of the curd, it is first washed with a slightly alkaline solution, and last with water.[1] Depending on the

use of the casein, it is either dried or prepared in solution for some definite use. In the case of drying, it should be dried under reduced pressure, at the lowest temperature possible and in as short a period of time as possible.[10] Spray drying in hot air has been found successful.[2]

Chemistry of Soybean Casein Extraction As established by Osborn and Campbell[12] in 1897, nearly all of the protein material of soybeans consists of a globulin "glycinin," with small amounts of legumelin, proteose and phaseoline. The protein material is usually referred to as "soybean casein" which is the terminology used in this work. The composition of the glycinin is;[12] carbon 52.12 per cent, hydrogen 6.93 per cent, nitrogen 17.53 per cent, sulfur 0.79 per cent and oxygen 22.63 per cent.

Globulin proteins are soluble in dilute salt and alkaline solutions, and insoluble in water. The globulins are weakly acidic, thus having a neutralizing effect on the alkaline solution used in extraction. Casein from crushed soybeans may be extracted by leaching with a salt or alkaline solution and also water. The leaching of casein by water is attributed to the acid potassium phosphate present in the bean.[10] It is thought that the glycinin is present in the seed in the form of glycinates of alkaline earths, and also as absorption products of alkaline earth phosphates. Because of this

Sato recommends previous leaching with a 0.1 per cent acetic acid solution prior to casein extraction.(13) However, previous leaching with a 0.1 per cent acetic acid solution extracts about one tenth of the total casein present.(12)

Also contained in the soybean are phosphatides, which are substances consisting of phosphoric acid, glycerol, higher fatty acid and an organic base.(8)(9) With soybeans the organic bases are colamine and choline and the corresponding phosphatides are lecithin and cephalin respectively. Phosphatides are present in the bean from 1.6 per cent to 3 per cent, with 38 per cent of the phosphatides being lecithin and 62 per cent cephalin.(9) When the oil is removed by the hydraulic press or expeller method, phosphatides remain in the meal, but with solvent extraction they are removed with the oil.(1)

Glycinin is very sensitive to oxidation by air, especially in the moist state. When in alkaline solution it acquires a brownish coloration, which indicates decomposition. Sodium hydroxide of 0.1N results in the liberation of ammonia as well as liberation of one half the sulfur from the cystine in the form of H_2S.(11)

Solvents and Precipitating Agents In general, solvents used for dissolving the casein are either salts, alkalies or basic

salts. Since precipitation of the casein from neutral salt solutions requires dialysis, alkalies and basic salt are used more extensively which involves the simple process of neutralization. A table of the extracting agents and their relative qualities as given by Sato[13] are listed below.

Reagent	Concentration of Reagent %	Coloration	Plasticity
Na_2SO_3	0.3	Slightly yellowish white	Class I
Na_3PO_4	0.3	" " "	" II
Na_2CO_3	1.0	" brownish "	" III
$Na_2B_4O_7$	5.0	" " "	" III
$NaC_2H_3O_2$	5.0	White	" IV
NH_4OH	0.5	Slightly brownish white	" IV
NaOH	0.2	Brownish white	" V
$Ca(OH)_2$	0.5	White	" V
Na_2SiO_3	5.0	"	" VI
Na_2S	10.0	"	" VI
NaCl	10.0	"	" VI
Na_2SO_4	1.0	"	" VI
$(NH_4)_2SO_4$	5.0	"	" VI

The plasticity was divided into six classes, Class I being the best and Class VI the poorest. Alkaline solution which are too strong have a hydrolyzing action on the protein. To prevent oxidation and decomposition a weak alkaline solution possessing reducing properties is recommended.

Any reagent capable of lowering the pH may be used for precipitating the casein from alkaline solutions. The quality and physical nature of the casein curd depends much upon the precipitating agent. Below is a table of the more general

precipitating agents and their relative merits.[14]

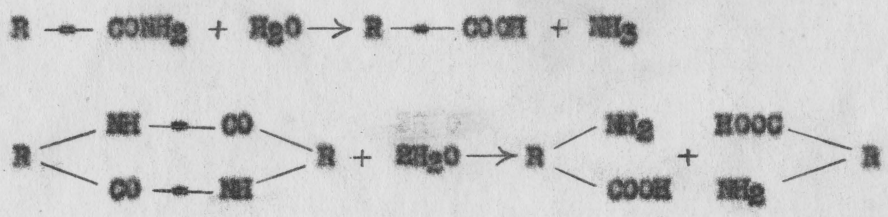
Precipitant	Form of Precipitate	Class	Remarks
H_2SO_3	Whitest, finest and lightest	I	Best in quality
H_3PO_4 H_2SO_4 CH_3COOH	Bulky and flocculent, settling	II	Medium in quality
HCl HNO_3	Dense aggregation	III	Inferior quality
Metallic salts of alkaline earths	Denser aggregation and more compact	IV	This class is not suitable for the manufacture of plastic celluloid-like substances
Salts of heavy metals	Heavy and compact, quickly settles		"
Formaldehyde	Compact, appears hard		"
Precipitate coagulated by heat and already precipitated by various means	Coagulated		"

Other factors affecting precipitation are pH, temperature, concentration of the casein solution and concentration of the precipitating agent.[15][8][1][14] The higher the temperature the more dense will be the curd,[16] though too high a temperature denatures the casein. The isoelectric point of the casein has been found by most workers to be a pH of 4.6 [8][5][12], but it ranges to a pH of 5.0.[10]

Denaturing of the Casein The soybean casein is very sensitive, being quite easily denatured by heat.[11] Solvent extraction of the oil, which is necessary for producing a meal satisfactory for casein production, denatures the casein only slightly, depending on the solvent, temperature and duration of extraction.[10] Azeotropic mixtures of benzine and methyl alcohol give a better protein than benzine alone.[13] The time of extraction should be as short as possible, the temperature as low as possible and meal freed from the solvent at a temperature not over 125° F. to give satisfactory casein.[11] Also it was found that the solvent extraction has less denaturing effect on the dry beans than wet beans.

The denatured casein has properties different from those of the natural protein.[10] From the ground beans, using consecutive extractions with water, 10 per cent NaCl, and 0.2 per cent NaOH respectively, approximately 80 per cent, 10 per cent and 50 per cent of the total nitrogen can be extracted. With benzine extracted meal, 25 per cent, 10 per cent and 50 per cent respectively can be extracted.[10] Denatured casein is much less soluble in water than is the natural casein. Less nitrogen and more hydrogen and oxygen is contained in denatured protein. The isoelectric point of the denatured casein is sharper, with less HCl being required to reach the isoelectric point, even with a lower isoelectric point.[11]

Accompanying the other changes of denaturing are the increase in free amino, carboxyl, and enol groups, and simultaneously becoming poorer in amide nitrogen. Deamination and hydration are suggested as partial explanation, according to the following reactions.



III EXPERIMENTAL

Purpose of Study. The purpose of this investigation was the development of a process for extracting the casein from soybean meal as a means of producing a cheap source of high grade casein, similar to milk casein.

The object of this research was to determine optimum conditions, equipment and procedure for the separation of commercial soybean casein.

Plan of Investigation

The investigation was planned to follow the steps listed below:-

1. Preparation of the meal prior to mixing with the extracting solution.

2. Preparation of the extracting solution; either a dilute NaOH or Na_2CO_3 solution.

3. Selection of proper equipment for mixing and stirring of the meal and extracting solution.

4. Separation of the slurry which is casein solution and meal residue. Three general methods of separation were planned; direct filtering, super-centrifuging, and a combination of screening, settling, and filtering using a filter aid.

5. Precipitation of the casein by addition of an acid until the proper pH is reached.

6. Separation of the casein curd and whey by settling with subsequent filtering.

7. Drying of the casein curd in a vacuum tray drier.

8. Grinding of the dried casein in the attrition mill.

Changes in the plan of investigation were contingent with the problems arising during the investigation.

Materials

Soybean Meal. Two specific types of soybean meal were used for this investigation. Meal A. Meal obtained from the Virginia variety of soybean, the oil of which had been extracted by C.C. Waddell by flaking, heating to a temperature near 120° F, and removing the oil by pressing under 2,000 pounds per square inch. By analysis, the meal contained 9.66 per cent oil.
Meal B. Flaked, white, 44 per cent protein meal obtained from the Saline Plant of the Ford Motor Company, delivered to the Chemical Engineering Department of V.P.I. March 12, 1941. The oil had been extracted using petroleum ether boiling at 145° F. By analysis, the meal contained 1.31 per cent oil.

Sodium Sulfite. Merck's Anhydrous Na_2SO_3, conforming to A.C.S. Specifications.

Sodium Hydroxide. Technical Grade Flake Caustic.

Hydrochloric Acid. C.P.-A.C.S. 35-37 per cent HCl.

Filter Cloth. 16 ounce duck filter cloth.

Filter Paper. Fisher rapid filter paper and Mosinee Kraft Towels, Daywest Paper Co., Green Bay, Wisconsin.

Apparatus

Sharples Super-Centrifuge. The centrifuge used was a laboratory size super-centrifuge, operated by steam with a maximum bowl speed of 50,000 R.P.M. Type T-41-2330-34, Serial No. 381A312, The Sharples Specialty Co., Philadelphia, Pa.

Basket Centrifuge. The basket centrifuge was electrically driven with an adjustable rheostat for speed control. The basket was a perforated brass bowl, 5 inches in diameter and 2-1/2 inches in depth. No. E-5457, International Chemical Centrifuge, Fisher Scientific Co., Pittsburg, Pa.

Stirring Apparatus. This apparatus was designed and constructed according to Fig. 1.

Attrition Mill. A single disc attrition mill driven by a direct connected electric motor. Mill size No. 1.5,

Robinson Mfg. Co., Muncy, Pa. Electric Motor - Type P.A., H.P. 5, R.P.M. 3450, Volts 220-440, Phase 3. The Master Electric Co., Dayton, Ohio.

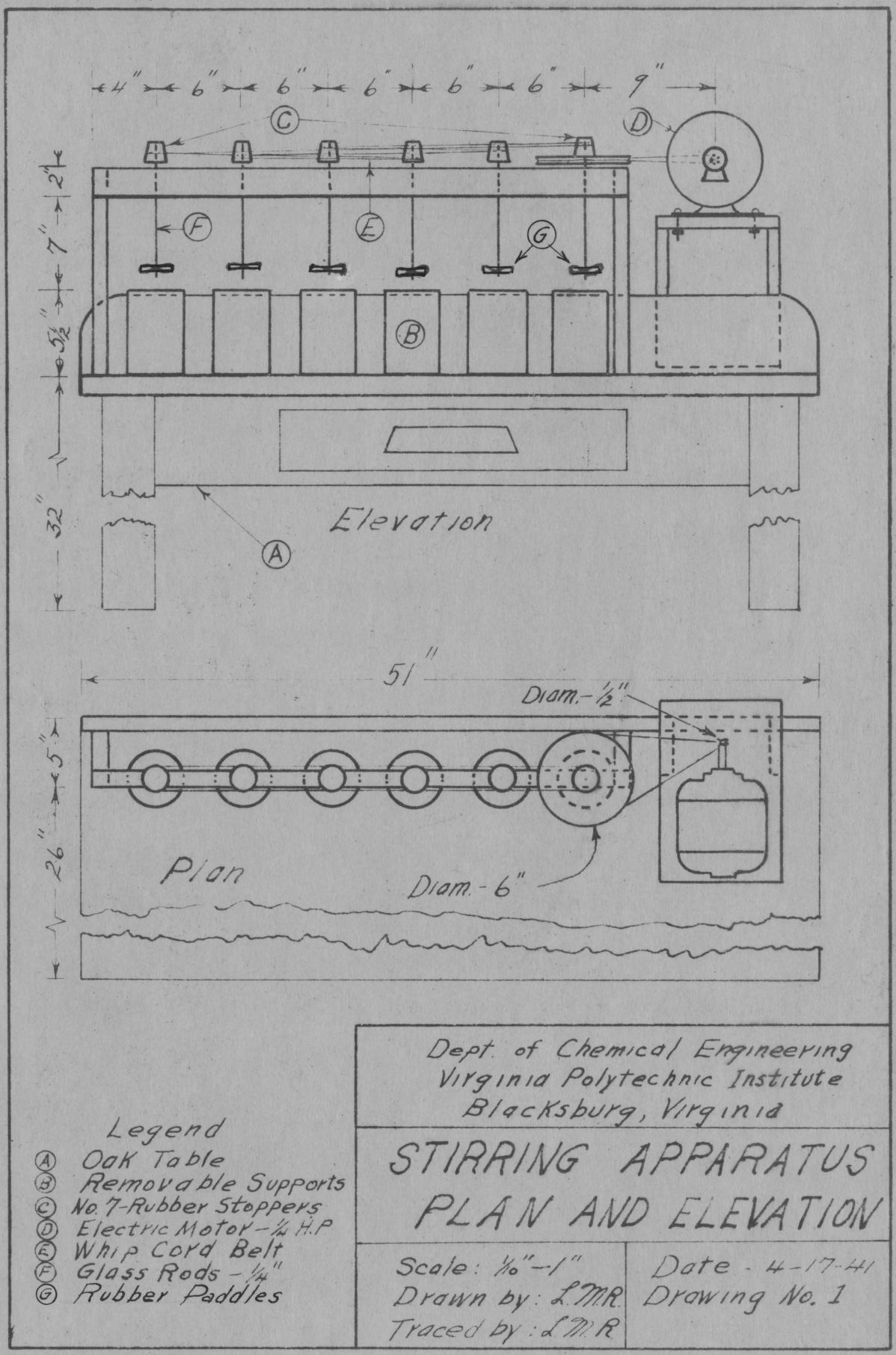
Eggbeater. An inexpensive 12 inch eggbeater purchased from F.W. Woolworth & Co.

Vacuum Tray Drier. F.J. Stokes Machine Co., Philadelphia, Pa. A-6628, 17282234. This is a laboratory size vacuum drier, having four shelves, 24 inches by 16 inches, and an allowable steam pressure within the shelves of 50 pounds per square inch gage.

Screen Analysis Apparatus. Combs Gyratory Riddle, Great Western Mfg. Co., Leavenworth, Kansas. U.S. Standard Sieve Series, Nos. 4, 16, 20, 40, 60, 80, and 100. Newark Wire Cloth Co., Newark, N.J.

pH Meter. Coleman Portable pH Meter, Model 3D, Inst. No. 2331.

Drying Oven. Sargent Electric Drying Oven. 110 Volts, 660 Watts. Pat. No. 1033592, Serial No. 02152. E.H. Sargent & Co., Chicago, Ill.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Legend
Ⓐ Oak Table
Ⓑ Removable Supports
Ⓒ No.7-Rubber Stoppers
Ⓓ Electric Motor-¼ H.P
Ⓔ Whip Cord Belt
Ⓕ Glass Rods-¼"
Ⓖ Rubber Paddles
Dept. of Chemical Engineering
Virginia Polytechnic Institute
Blacksburg, Virginia
STIRRING APPARATUS
PLAN AND ELEVATION
Scale: 1/10"-1"
Drawn by: L.M.R.
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Date - 4-17-41
Drawing No. 1

Fig. 1

Method of Procedure

Introduction. Though the procedure for the different runs varied considerably, the following procedure will, in general, cover all the operations. Details which are pertinent to only one run will be explained in the "Data and Results."

Meal Preparation. In the investigation using the Meal A as supplied by C.C. Waddell, which was composed of large, hard particles, it was necessary to grind the meal in the attrition mill. Because of the heat generated during the grinding operation, cold water was circulated through the water jacket of the stationary grinding plate. The fineness of grind was adjusted by varying the distance between the grinding plates. When using the Meal B, it was not necessary to grind, as the meal was flaked to a thickness varying from 0.0010 to 0.0012 of an inch. Screen analyses were made using Combs gyratory riddle and U.S. standard sieves.

Preparation of Extracting Solution. As a matter of convenience, solutions of 0.1 gram per milliliter were prepared of each NaOH and Na_2SO_3 from which they were diluted to the desired concentration.

Agitation. Stirring was accomplished mainly by the use of the stirring apparatus as shown in Fig. 1. The speed of rotation of the stirring paddles varied from 400 to 420 R.P.M., except for

special runs in which the speed of rotation was reduced to 90 R.P.M. by the addition of another reducing pulley. Paddles, were made by slitting 1/2 inch O.D. rubber hoses of various lengths and slipping over the rod at right angles to it. (See G, Fig. 2). When using the finely ground meal it was necessary to first use the hand eggbeater to break the lumps of wet dough, which were rather difficult to break.

Filtration. This step in the procedure is the bottleneck of the process and really the main substance of this work. Three general methods of separation were used.

1. Filtering the slurry directly without the aid of settling or filter aid. In this method, Buchner funnels, 7.5 cm., 11 cm., and 15 cm., with a vacuum of 27 inches of mercury produced by a water aspirator, were used. As a filter medium, rapid filter paper and 16 ounce filter cloth were used. Also the small basket centrifuge with 16 ounce filter cloth and paper toweling cut in the desired shape were used. Operation of the centrifuge varied in speed from 3400 to 3800 R.P.M.

2. Super-centrifuging of the slurry. Casein slurry was fed to the bottom of the centrifuge bowl with a hydraulic head of 18 inches, through the large jet, 1/8 inch

inside diameter. The centrifuge was operated at a speed of 36,000 R.P.M. Since all solids collected in the bowl, the casein solution flowed from the lower spout of the centrifuge.

3. A combination of screening, settling, and filtering, using a filter aid, Celite No. 545. In all cases of screening, the slurry was made from a coarse meal and screened through a No. 16, U.S. standard screen. The screen was in the form of a cylinder, 3 inches in diameter and 18 inches long, with a metal plate soldered to one end. The solution thus obtained was allowed to settle either in test tubes 25 mm. x 250 mm. in the case of small samples, or large two-liter jars 12 inches in height in the case of larger portion. Filtration was done by adding filter aid, Celite No. 545, and filtering through a Buchner funnel with rapid filter paper as the medium. A pressure difference of 27 inches of mercury was maintained by a water aspirator.

Precipitation. In all cases, the casein was precipitated by adding 10 per cent HCl until the correct pH was obtained. The Coleman pH meter was used in obtaining the correct pH, between 4.2 and 4.6. Later it was necessary to only watch the precipitation carefully and at the point where the curd would precipitate coarsely and leave a clear yellow whey the pH would

be 4.6 ∓ 0.2.

Separation of Whey and Casein Curd. Casein curd precipitated with HCl, being heavier than the whey, settles readily. Decanting and final separation by filtering through a Buchner funnel or the small basket centrifuge was the procedure followed. Filter mediums were either 16 ounce duck, rapid filter paper, or paper toweling. Care had to be taken not to operate the basket centrifuge at too high a speed, or the curd would press through in the paper or cloth in the form of a paste.

Drying. Drying was done in the vacuum drier at a vacuum of 24 inches of mercury and 5 lbs. per square inch gage steam pressure. The casein was spread in a thin layer, 1/8 inch in thickness, on a plate glass which was placed in the drier. To prevent the casein from being heated excessively, above 125° F., the glass plate was raised from the tray by pieces of chalk, which left a dead air space of approximately 1/2 inch between the hot surface of the shelf and the glass.

Analysis for Total Solids. For determining the total solids in both the casein solution and whey, roughly 10 gms. of liquid were placed in a 50 ml. beaker and weighed.

The contents were then dried in an oven at 220° F. to constant weight and the per cent total solids calculated.

Analysis for Filterable Solids. When only a small amount of fine and mucilaginous material was present in a casein solution, a special method was employed to determine the percentage present. Fifty gram samples were weighed into a test tube which contained a weighed quantity of filter aid. This slurry was filtered through a Buchner with a weighed filter paper. It was necessary to add the filter aid to make filtration and washing of the cake possible. The paper and cake were removed from the funnel, dried in an oven for 30 minutes at 220° F., and weighed. The increase in weight of paper and filter aid is the weight of filterable solids in the solution filtered.

Experimental Data and Results

All of the work in this investigation was done with meal which may be identified by the three following screen analyses.

TABLE I
Screen Analysis, Sample A.
Meal A (Ground)

		Weight Grams	Per cent
Wt. on Screen No. 16,		0.0	0.0
" " " No. 20,	through No. 16	1.2	1.2
" " " No. 40,	" No. 20	20.0	20.3
" " " No. 60,	" No. 40	22.1	22.5
" " " No. 80,	" No. 60	11.3	11.5
" " " No.100,	" No. 80	6.9	7.0
" " bottom pan,	" No.100	36.9	37.5
	Total	98.4	100.0

TABLE II
Screen Analysis, Sample B.
Meal A (Ground)

		Weight Grams	Per cent
Wt. on Screen No. 16,		313	10.7
" " " No. 20,	through No. 16	615	21.1
" " " No. 40,	" No. 20	1110	38.0
" " " No. 60,	" No. 40	392	13.4
" " bottom pan,	" No. 60	490	16.8
	Total	2920	100.0

TABLE III
Screen Analysis, Sample C.
Meal B (Unground)

	Weight Grams	Per cent
Wt. on Screen No. 4,	8.	1.2
" " " No.16, through No. 4	384.	55.6
" " " No.20, " No.16	86.	12.6
" " " No.40, " No.20	127.	18.4
" " " No.60, " No.40	46.	6.7
" " " No.80, " No.60	17.	2.5
" " bottom pan, " No.80	21.	3.0
Total	689.	100.0

Extracting Solution Concentration. To determine the concentration of solution to use, runs were made with both NaOH and Na_2SO_3. With NaOH, 20 grams of Sample A were stirred for 30 minutes with 200 ml. portions of NaOH solution of varying concentration. A small portion of the solution was filtered through a Buchner funnel and analyses made for total solids. Also, analysis of the whey after precipitating the casein was made.

TABLE IV

Effect of NaOH Concentration on Casein Concentration

NaOH conc. %	NaOH sol., 0.1 gr.ml., to make 200 ml. extract. sol. ml.	pH of Casein Solution	Solids Casein sol. %	Solids Whey %	Casein %
0.0	0.0	6.1	3.32	3.00	0.32
0.025	0.50	6.4	3.94	3.00	0.94
0.05	1.0	6.8	4.70	3.00	1.70
0.10	2.0	7.8	5.32	3.00	2.32
0.20	4.0	9.3	6.04	3.00	3.04
0.30	6.0	10.0	6.38	3.00	3.38
0.40	8.0	10.3	6.70	3.00	3.70
0.50	10.0	10.5	6.78	3.00	3.78

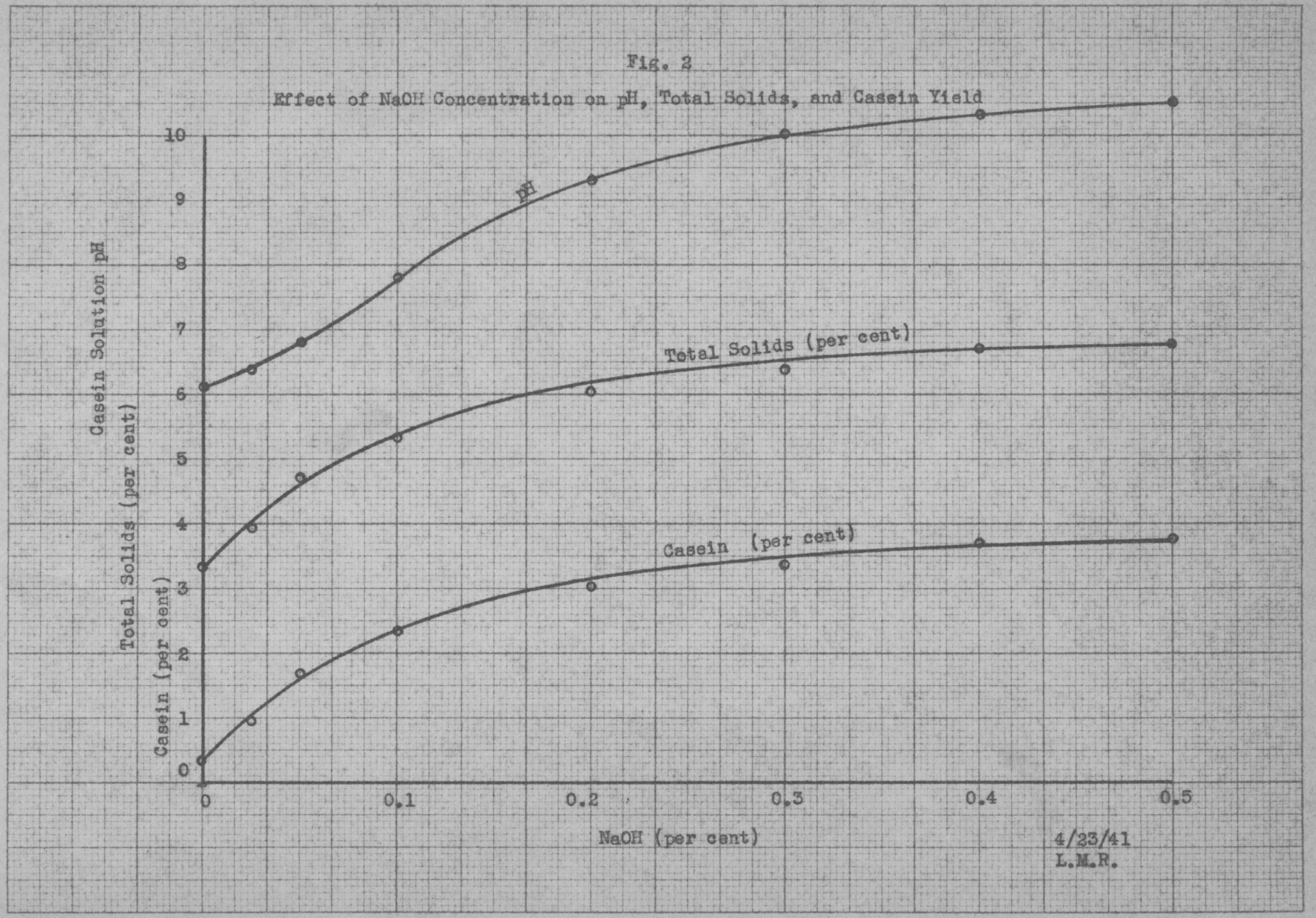
On plotting these data, concentration vs. pH, per cent total solids, and per cent casein, it will show clearly the variation of casein extracted with concentration.

In Fig. 2 these data are plotted.

For determining the effect of Na_2SO_3 concentration, similar runs were made, using Sample C, 4-16 mesh. Two hundred and fifty milliliter portions of Na_2SO_3 solution varying in concentration were stirred for 30 minutes with 25 grams of the meal. Stirring was done in 400 ml. beakers by paddles 1/2 in. x 1-3/4 in. rotating at 400 R.P.M. The slurry was then separated by first decanting, filtering the decanted solution in the basket centrifuge, and then the solution removed from the saturated meal residue by use of the basket centrifuge. Analyses were made on the casein

Fig. 2

Effect of NaOH Concentration on pH, Total Solids, and Casein Yield



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solution and on the whey after precipitation. The pH was controlled with the pH meter.

TABLE V

Effect of Na_2SO_3 Concentration on Casein Concentration

Na_2SO_3 conc. %	Wt. of Casein Sol. grams	pH of Sol.	Total solids in casein Sol. %	Total solids in whey %	Casein %	Casein Yield %
0.0	224	6.5	6.00	3.14	3.94	35.4
0.025	216	6.6	6.55	3.26	3.40	30.2
0.05	225	6.65	6.64	3.18	3.57	32.7
0.10	226	6.75	6.82	3.50	3.54	32.3
0.20	228	7.1	7.16	3.12	4.16	37.1
0.30		7.1				
0.40	222	7.2	7.29	3.84	4.29	37.4
0.50	217	7.3	7.37	3.12	4.37	39.0

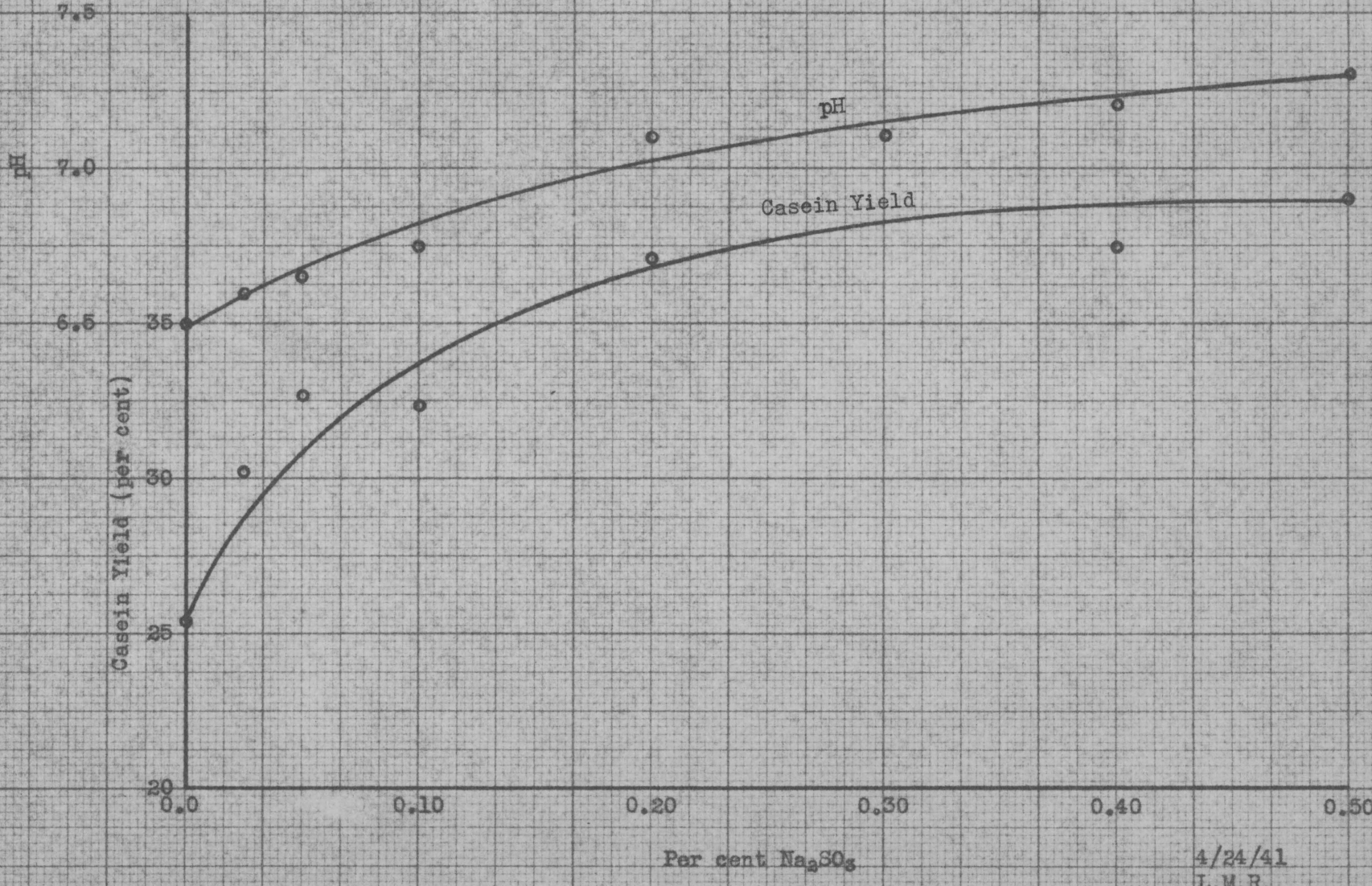
In calculating the per cent casein yield, the average of the per cent total solids in the whey was used rather than rely on an individual analysis. Also, the average weight of casein solution was used.

These data, concentration of Na_2SO_3 solution vs. pH of casein solution and per cent yield of casein are plotted in Fig. 3.

Rate of Solution. The time required for extraction of casein was initially investigated by making several short runs of meal of Sample A. Casein was extracted from the meal by stirring 50 grams of meal with 0.2 per cent NaOH for

Fig. 3

Effect of Na_2SO_3 Concentration on pH and Casein Yield



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different length of time and determining the yield.

TABLE VI
Effect of Stirring Time on Casein Yield

Meal	Time min.	Casein yield grams	Casein yield %
Sample A	5	20.	40.
" A	25	16.5	33.
" B, 40-60 mesh	30	9.5	19.
" B, " " "	60	10.0	20.

A more comprehensive investigation was made on the solvent extracted meal, Sample C, 4-16 mesh. Extractions were made on 50 gram samples with 500 ml. of 0.5 per cent Na_2SO_3 by stirring for varying lengths of time. Stirring was done by a paddle 1/2 in. x 1-3/4 in. rotating at 410 R.P.M. in an 800 ml. beaker. Separation was carried out by first straining the slurry through a 16 mesh screen, then filtering with the basket centrifuge, and finally precipitating and drying the casein.

TABLE VII
Effect of Stirring Time on Casein Yield from Solvent Extracted Meal, Sample C, 4-16 mesh

Time min.	Casein Yield grams	Casein Yield %
5	9.5	19.0
10	13.8	27.6
15	11.9	23.8
20	14.5	29.0
40	16.6	33.2
60	15.8	31.6

A plot of the data in Table VII as shown in Fig. 4 indicates that 25 minutes time is sufficient for complete extraction.

Temperature Effect on Rate of Solution.

Extractions were made on 50 gm. portions of solvent extracted meal, Sample C, 4-16 mesh, using 500 ml. of 0.3 per cent Na_2SO_3 solution. The mixtures were stirred for a period of 10 minutes at various temperatures ranging from 55° F to 125° F.

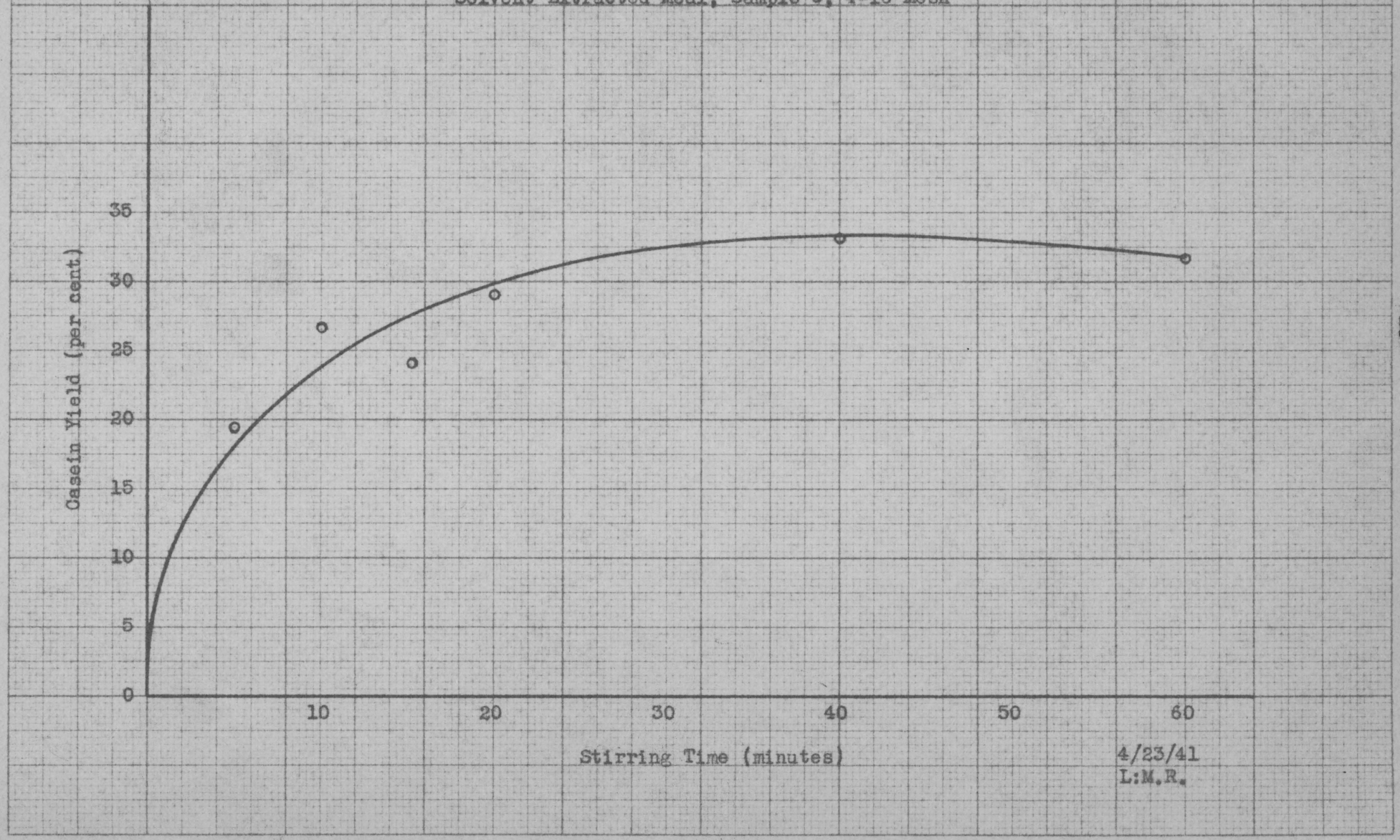
TABLE VIII
Relation of Temperature to Rate of Solution of Casein

Temperature of	Casein Yield grams	Casein Yield %
55	13.8	27.6
75	11.8	23.6
100	13.8	27.6
125	13.5	27.0

The slight change in casein yield shows that the temperature has little effect on the rate of solution under these conditions.

Fig. 4

Effect of Extracting Time on Casein Yield from Solvent Extracted Meal, Sample C, 4-16 Mesh



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Effect of Particle Size on Rate of Solution. Samples of solvent extracted meal, Sample C, were taken from the different sieves. Extractions were made on 50 gram samples, using 500 ml. of 0.3 per cent Na_2SO_3 solution, stirring with a 1/2 in. x 1-3/4 in. paddle at 400 R.P.M. in 800 ml. beakers. The stirring time was for a period of 10 minutes.

TABLE IX
Effect of Particle Size on Rate of Solution of Casein

Meal mesh	Casein Yield grams	Casein Yield %
4-16	14.5	29.0
16-20	14.4	28.8
20-40	14.3	28.6
40-60	14.4	28.8

These data indicate that particle size of flaked meal has no affect on the rate of solution from mesh 4 to mesh 60.

Effect of Stirring Rate on Rate of Solution. Casein extractions from 25 grams of meal Sample C, 4-16 mesh, with 250 ml. of 0.3 per cent Na_2SO_3 solution were carried out with various rates of stirring. Stirring time was 10 minutes.

TABLE X

Effect of Stirring Rate on Per Cent Yield when Stirring Time is 10 Minutes

Stirring	R.P.M.	Casein Yield grams	Yield %
2-1/2" x 1-3/4" paddles	400	5.5	22.0
1-1/2" x 1-3/4" "	410	5.0	20.0
1-1/2" x 1/2" "	410	4.1	16.4
1-1/2" x 1-3/8" "	400	4.5	18.0
Slowly stirring by hand with tablespoon. 50 circular motions per minute		4.5	18.0

Settling Time of Unscreened Particles in Casein Solution.

A casein solution was prepared by stirring 50 grams of solvent extracted meal, Sample C, 4-16 mesh, and 500 ml. of 0.3 Na_2SO_3 solution for 30 minutes. Stirring was done in 600 ml. beakers, using a paddle 1/2 in. x 1-3/4 in. rotating at 400 R.P.M. The slurry was strained through a 16 mesh screen and samples were placed in test tubes, 25 mm. x 200 mm., for settling. After various time intervals, analyses were made for per cent filterable solids. Analysis of the casein solution showed 7.3 per cent total solids and analysis of the whey after precipitating gave 3.17 per cent solids.

TABLE XI
Per Cent Filterable Solids, Less than 16 Mesh, Remaining in Casein Solution after Settling

Time of Settling min.	Filterable Solids in 50 gms. sol. grams	Filterable Solids %
0	0.2501	0.50
10	0.1556	0.31
20	0.1347	0.27
30	0.1128	0.23
40	0.0952	0.19
50	0.0877	0.18
60	0.0829	0.17
70	0.0786	0.16
80	0.0759	0.15

These data show the greater portion of settling to occur in the first 20 minutes with 0.27 per cent filterable solids remaining that additional settling is slower with 0.15 per cent filterable solids remaining after 80 minutes time of settling.

A plot of the data is made in Fig. 5.

Effect of Meal Size on Settling Rate of Filterable Solids. Twenty-five gram samples of meal, Sample C, of different sizes were stirred in a 400 ml. beaker by a paddle 1/2 in. x 1-3/4 in. at 400 R.P.M. with 250 ml. of 0.3 per cent Na_2SO_3 solution. Stirring was continued for 30 minutes, at which time the slurry was strained through a 16 mesh screen and placed in test tubes, 25 mm. x 200 mm., and allowed to settle for 20 minutes. Analyses for the per cent filterable solids were made on each of the samples.

Fig. 5

Settling Rate of Less Than 16 Mesh Particles
in a 4.1 Per cent Casein Solution

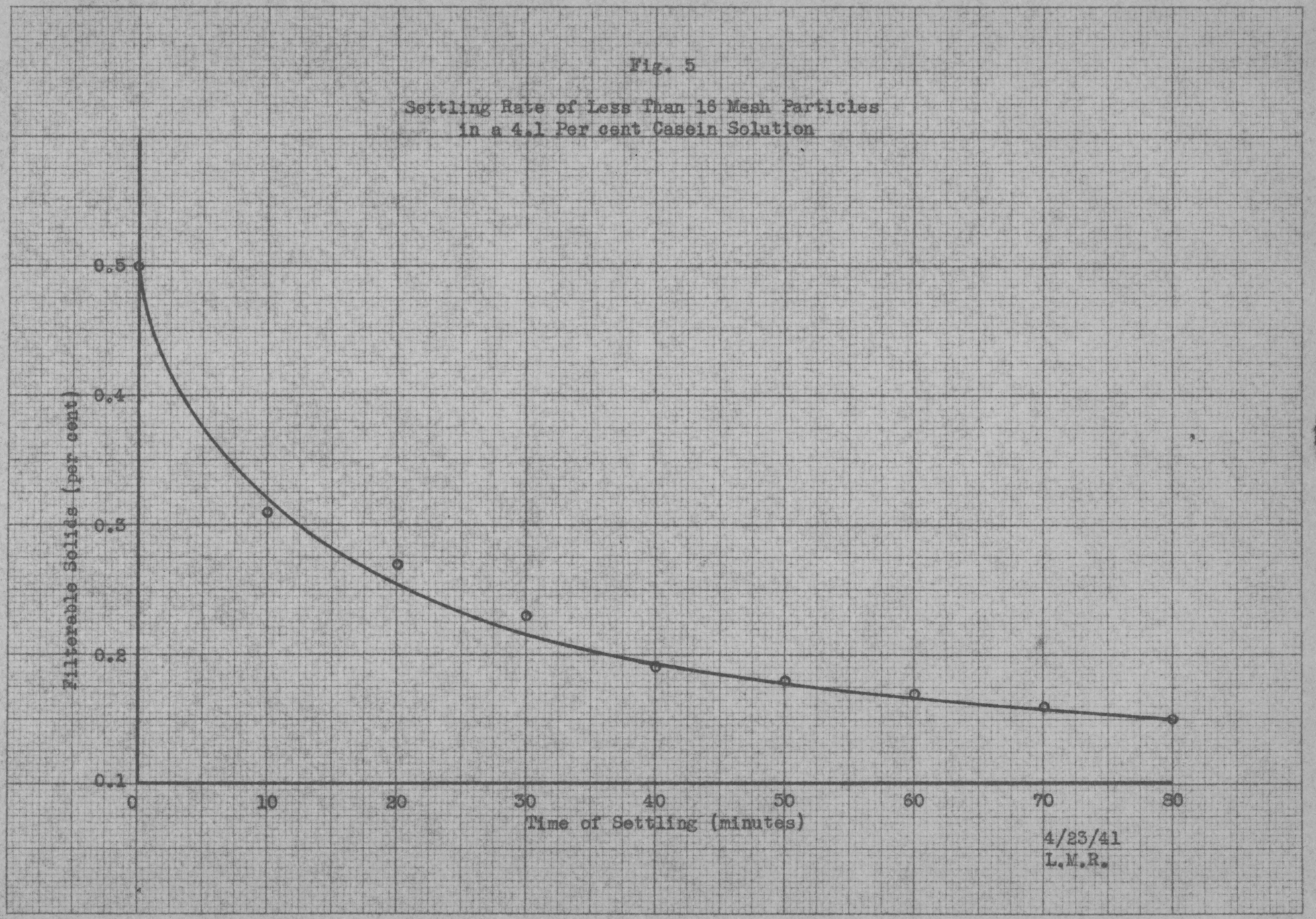

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TABLE XII
Effect of Meal Size on Filterable Solids in Casein Solution after 20 Minutes Settling

Meal mesh	Wt. of Filterable Solids in 50 grams Solution gms.	Filterable Solids %
4-16	0.1104	0.22
16-20	0.1625	0.32
20-40	0.1981	0.40
40-60	0.1930	0.39

The data indicates that the smaller size meal, mesh 20-60, produces a casein solution with considerable more filterable solids than does a meal of mesh 4-16.

Effect of Stirring Rate on Per Cent Filterable Solids. Extractions were made on 25 gram portions of Sample C, 4-16 mesh, by stirring for 20 minutes at various rates of agitation in 400 ml. beakers with 250 ml. of 0.5 per cent Na_2SO_3 solution. The slurry was screened through a 16 mesh screen. Analyses for the per cent filterable solids immediately after screening and after 20 minutes of settling were made.

TABLE XIII

Influence of Stirring Rate on Per Cent Filterable Solids

Stirring	R.P.M.	Filterable Solids	
		Unsettled %	Settled 20 min. %
2-1/2" x 1-3/4" paddles	400	0.53	0.35
1-1/2" x 1-3/4" "	410	0.53	0.34
1-1/2" x 1-3/4" "	420	0.43	0.26
Slowly stirred by hand with tablespoon, 50 circular motions per min.	430	0.35	0.19

Effect of Per Cent Filter Aid on Time of Filtration.

Casein solution was prepared by extracting casein from 300 gms. of Sample C, 4-16 mesh, with 3 liters of 0.3 per cent Na_2SO_3 solution. Stirring was done in a can, 6-1/2 in. in diameter x 7-1/2 in. high, by two paddles, 1/2 in. x 3-1/2 in. rotating at 400 R.P.M. The solution was screened through a 16 mesh screen and allowed to settle for 30 minutes in a bottle 10 inches high. The top 8 inches was siphoned out without disturbing the settlings. Analysis of the solution showed 50 grams to contain 0.1015 grams filterable solids, or 0.203 per cent. Two hundred and fifty milliliter portions of the solution, with the addition of various amounts of Johns Manville Celite No. 545, were filtered through a 7.5 cm. Buchner funnel with rapid filter paper as the filter medium. A pressure difference of 27 inches of mercury was applied by a water aspirator.

TABLE XIV

Filtering Time of Casein Solution vs. Per Cent Filter Aid

Filter Aid Celite No. 545 gms.	Filter Aid Celite No. 545 %	Filtering Time sec.
7.5	5.0	60
6.25	2.50	45
5.0	2.00	50
3.75	1.5	95
2.50	1.0	240
1.88	0.75	600

A plot of per cent filter aid vs. filtering time is made in Fig. 6.

The identical procedure as followed to obtain the data for Table XIV, with the exception of stirring rate, was followed to obtain the following data. The speed of rotation was changed to 90 R.P.M. Analysis of the solution for per cent filterable solids gave 0.17 per cent.

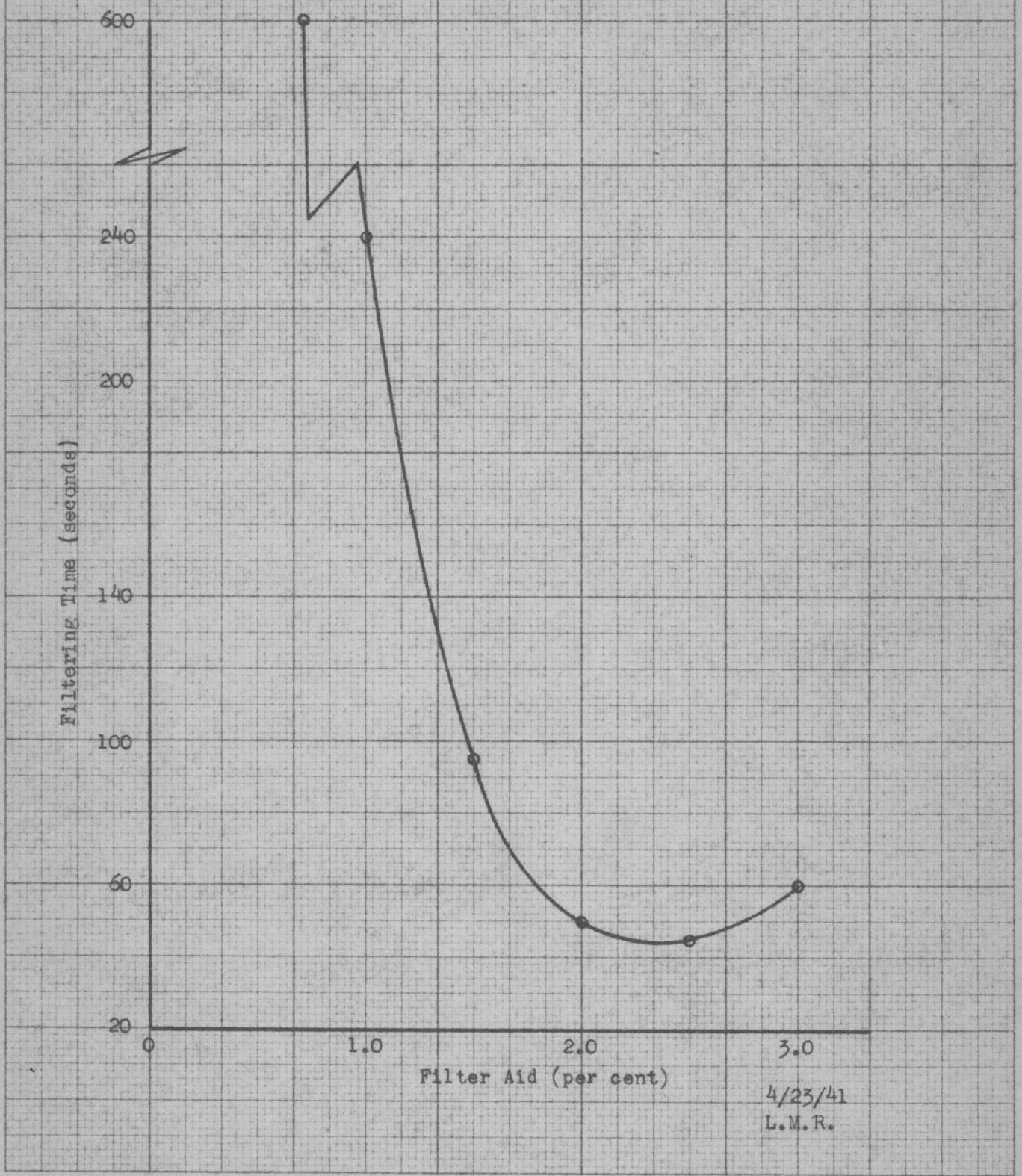
TABLE XV

Filtering Time of Casein Solution vs. Per Cent Filter Aid

Filter Aid Celite No. 545 gms.	Filter Aid Celite No. 545 %	Filtering Time sec.
7.5	5.0	30
5.0	2.0	32
3.75	1.5	45
2.50	1.0	60
1.88	0.75	70
1.25	0.50	120
0.63	0.25	plugged completely

Fig. 6

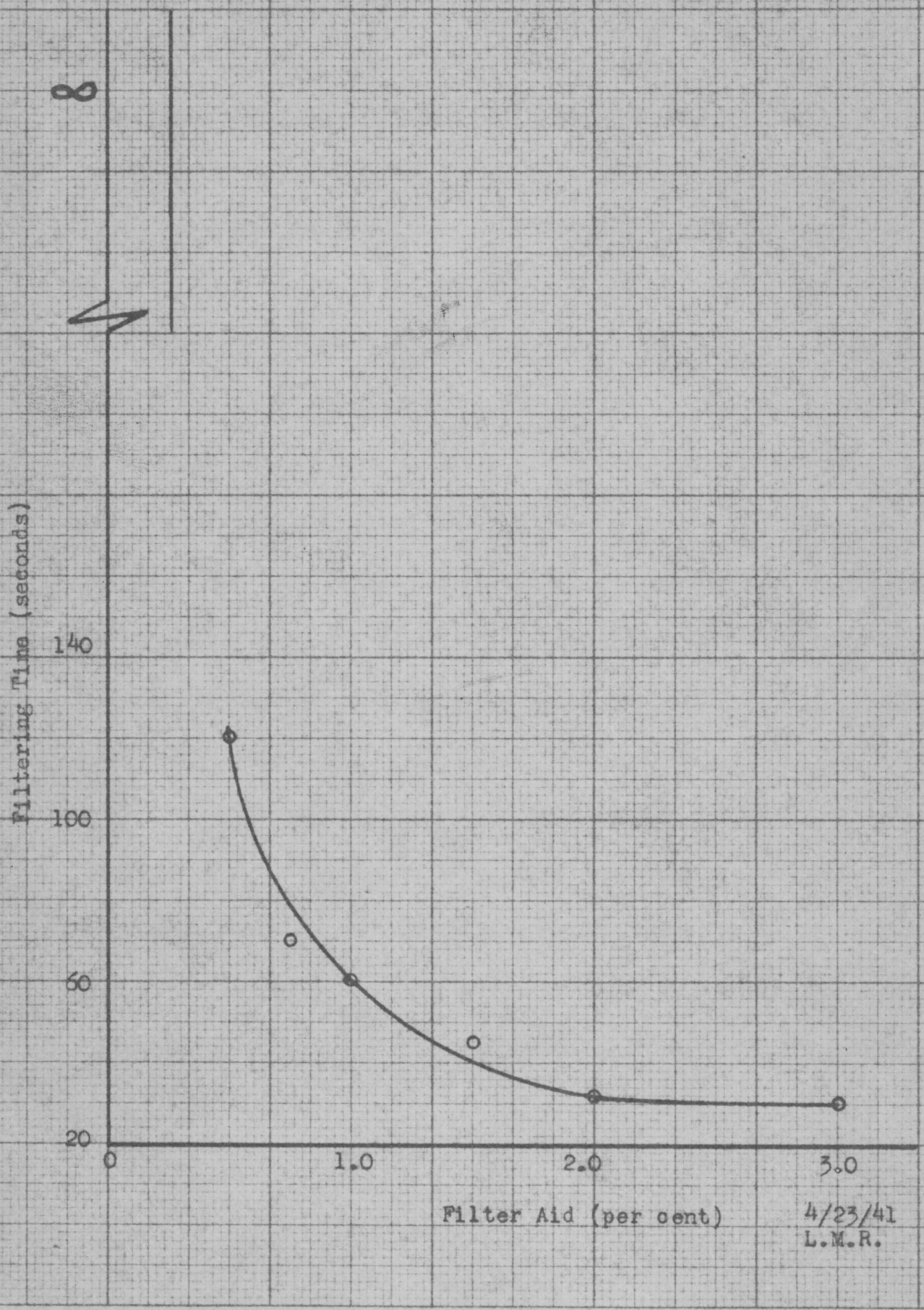
Effect of Per cent Filter Aid, Celite No. 545 on Filtering Time of 250 ml. of Casein Solution Containing 0.203 per cent Filterable Solids through a 7.5 cm. Buchner Funnel.



A plot of the data in Table XV, filtering time vs. per cent filter aid, is made in Fig. 7.

Fig. 7

Effect of Per cent Filter Aid, Celite No. 545, on Filtering Time of 250 ml. of Casein Solution Containing 0.17 per cent Filterable Solids through a 7.5 cm. Buchner Funnel.



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IV DISCUSSION

Soybean Meal. Meal A used in the preliminary work was obtained from beans of the Virginia variety, the oil of which had been extracted by flaking, heating to 122° F. and removing the oil by pressing to 2,080 lbs. per square inch. Only 9 per cent oil was removed, and by analysis 9.66 per cent remained in the meal. The meal as it comes from this oil removing process is a coarse material consisting of the smaller flakes adhering together to form larger cakes. Because of the caked nature of the meal, grinding was necessary. The attrition mill ground the meal very satisfactorily. The penetration of the extracting solution into the larger particles of meal containing a high percentage of oil is likely to be slow. Horvath (11) states that meal with the oil removed by pressing gives a poorer casein than does the solvent extracted meal with a lower oil content.

Difficulty in making screen analysis on Sample A arose as the finely ground meal would cake on the screens. This may be due to the electrostic charge on the particles or it may be due to the excessive amount of oil present causing it to cake. A coin placed on each of the screens helped to break the cake formed.

Meal B was obtained from the Ford Motor Company as it comes from the solvent extraction process. This meal was in a flaked form similar to oatmeal. The thickness of the flakes varied from 0.0010 to 0.0012 of an inch in thickness. Specifications of this meal were as follows; white, flaked, 44 per cent protein. Analysis for oil content gave 1.31 per cent oil.

Solution Concentration. Though solution concentrations for casein extraction were given in the literature[13], there is need for additional solubility data for casein in the extracting solution. The preliminary work, using Sample A, was concerned only with the concentration of casein and total solids of the casein solution and their relation to NaOH concentration. Because of the difficulty in filtering the entire slurry, only small samples were filtered and analyses made. The break in the curve, Fig. 2, at about 0.2 per cent NaOH, show this concentration to dissolve more casein per unit of NaOH than at other concentrations. Concentrations of NaOH greater than 0.3 per cent affect the per cent casein in solution very little.

Since Na_2SO_3 produces a higher quality casein than does NaOH, a more thorough investigation of the relation of Na_2SO_3 concentration to per cent yield of casein was made.

Again the break in the curve, Fig. 3, appears at a concentration of 0.2 per cent Na_2SO_3 indicating an optimum concentration. It is to be noted that extraction with distilled water alone gave a 25.4 per cent casein yield with a meal to water ratio of 1 to 10. Horvath states that this is due to the acid potassium phosphate present in the beans(10). A casein solution prepared from distilled water and meal, Sample C, 4-16 mesh, with a water to meal ratio of 10 to 1 has a pH of 6.5. The casein yield increased rapidly with small increase in Na_2SO_3 concentration up to 0.1 per cent, above which the increase was more gradual. Whey analyses show that the solubility of soluble sugars, legumelin and proteose is not affected by Na_2SO_3 concentration when in this low range below 0.5 per cent. For this reason, in calculating the per cent casein in the solution the average per cent solids of the whey was subtracted from the per cent total solids of the casein solution.

Rate of Solution. The initial work on rate of solution was done on the finely ground meal, Sample A. Two runs, Table VI, on Sample A indicate that the rate of solution is very rapid when the meal is finely ground and that an extended time may even cause precipitation of the casein without addition of an acid. From the larger particles of meal, Sample B, 40-60 mesh, the rate of solution is much slower, with only a 19 per

cent yield in 30 minutes. The reason for this probably being that the particles of meal were hard and with considerable oil content which prevented penetration of the extracting solvent.

The investigation using Sample C, 4-16 mesh, and Na_2SO_3 solution was more complete. Varying the time from 5 to 60 minutes gave a sufficiently wide range to give maximum extraction. The rate of solution is rapid during the first ten minutes, with the rate decreasing noticeably after ten minutes until extraction is completed in 25 to 30 minutes as shown in Fig. 3. Although the yield is noted to be only 33.2 per cent at the maximum, this is explained by the fact that considerable solution remained in the meal residue which could not be removed in the basket centrifuge.

It was found that with Samples A and B, that the particle size has considerable influence on the rate of solution of casein. However, with Sample C the situation is different. Table IX shows the results of the rate of solution from the different sizes of Sample C. As would be expected with the flaked character of the meal, there is no noticeable difference in the rate of solution from mesh size 4 to 60. It is likely that for meal smaller than 60 mesh the solution rate would be

greater as the thickness of flakes, 0.0010 to 0.0012 of an inch and the diameter of the particles passing through a 60 mesh screen are nearly equal.

The temperature effect on rate of solution was investigated from 85° F. to 125° F. Higher temperatures were not investigated because above 125° F. the casein is denatured and of inferior quality. Table IX indicates that temperature has little affect on the rate of solution as other variables, time, stirring rate, meal size and solution concentration remained constant.

The rate of solution is affected to a small extent by agitation. The run in which the mixture was slowly stirred with a tablespoon, Table X, the yield differed by only 4 per cent from the run with vigorous agitation. Since the per cent of filterable solids greatly increases with agitation, stirring sufficiently to barely keep the particles in motion is desired.

Separation of Casein Solution and Meal Residue. As early discovered in this work, the filtration of the slurry was the main problem to contend with. The samples for analyses in Table V were obtained by filtering through a Buchner funnel in a filter flask, with a vacuum of 27 inches of mercury obtained by a water aspirator. In order to obtain approximately 25 ml. of

solution it was necessary to change filter papers at least three times. The fine and mucilaginous material formed a very effective coating over the paper, immediately hindering the further passage of filtrate. Regardless of whether or not the vacuum was applied gradually or a maximum at the start, it was never possible to filter more than 10 ml. of filtrate through one 7.5 cm. filter paper before becoming completely plugged.

The super-centrifuge gave satisfactory separation when operated at 30,000 R.P.M. and fed through a tip 1/8 inch in diameter with a hydraulic head of 12 inches. However, the residue from 100 grams of meal would fill the bowl until a hole of approximately one inch remained in the center. If the bowl were allowed to fill more than this, separation was not complete. This method will produce results, but the use of a super-centrifuge for separating a material containing 5 to 6 per cent solids is cumbersome and expensive.

Because the super-centrifuge is not completely satisfactory, effort was directed toward a process of screening, settling and finally filtering the casein solution. The flake meal, Sample C, 4-16 mesh, produces a slurry which is easily

screened through a 16 mesh screen. However, this gives a casein solution with approximately 0.5 per cent filterable solids which is very difficult to filter. A maximum of 50 ml. can be filtered through one filter paper, in a 7.5 cm. Buchner funnel without complete stoppage. Some of the filterable solids will settle, though the mucilaginous and finer particles will not settle even on long standing. Fig. 5, a plot of per cent filterable solids vs. time of settling indicates that settling will not produce a casein solution with less than 0.15 per cent filterable solids. In a relatively short time, 20 minutes, the particles will settle to leave a solution with approximately 0.25 per cent filterable solids. Depending on the quality of casein required, the casein solution with 0.25 per cent filterable solids could be precipitated without further separation. The casein obtained from a 4 per cent casein solution containing 0.25 per cent filterable solids would be 94.2 per cent casein. The quality of such casein would be satisfactory for many purposes such as sizing material.

The size of meal as well as the rate of stirring has considerable influence on the amount of filterable solids. Table XII indicates that the larger meal size, 4-16 mesh, produces

a screened casein solution which will settle more rapidly than the solution obtained from smaller meshed meals. This is explained by the fact that the fine and mucilagenous material is held within the larger particles to a greater extent than in the finer material. Solution obtained from meal of mesh 4-16 after 20 minutes settling contained 0.22 per cent filterable solids, while solution obtained in an identical manner from meal of mesh 40-60 and 20-40 contained 0.40 and 0.39 per cent filterable solids respectively. This difference of approximately 0.2 per cent is important as will be shown in relation to the filtering characteristics of the solution.

The rate of agitation affects not so much the rate of settling as it does the per cent of filterable solids which pass through a 16 mesh screen on screening the slurry. Table XIII shows the initial per cent of filterable solids to be higher when agitation was vigorous than when agitation was gentle. The amounts settled out in 20 minutes settling times were near the same for all degrees of agitation, but since the initial solids were greater with increased agitation it is definitely better to reduce agitation to a minimum.

Rate of Filtration. The casein solution produced by first screening and settling was still difficult to filter. The addition of filter aid made filtration possible, but excessive quantities of filter aid are necessary unless the per cent filterable solids are reduced to below 0.2 per cent. As recommended by John Manville and Co., Celite No. 545 was used. With a solution containing 0.303 per cent filterable solids, at least one per cent filter aid is required, and to get a maximum rate, 2.5 per cent filter aid is required as shown in Fig. 5. When the filterable solids are reduced to 0.17 per cent, filtration is possible with 0.5 per cent filter aid and an optimum amount of 1.25 per cent, Fig. 6. When in the range of one per cent filter aid, it is quite feasible that such a process could be used commercially. The great difference in the filtering characteristics of these solutions, yet differing in filterable solids by only 0.033 per cent, is a measure of how effective the mucilaginous and fine material is in stopping filtration. For this reason, any step in the procedure which can reduce the fine mucilaginous material is desired.

Recommendations for Future Work. Since the object of using the larger particles of meal is to retain the fine and mucilaginous material within the particles, there is a possibility of going one step further with this thought in mind. A fibrous material mixed with the dry meal may retain the fine and mucilaginous material within the body of the mixture so that the casein may be extracted by a leaching process. A material such as wheat or oat straw would not interfere with the meal residue being used as a livestock food, which would be a necessary requirement of the fibrous material chosen.

It is believed that the next step in this work should be to design and construct a pilot plant from which the various factors can be studied to a better advantage than it is possible to do in the small batch or bucket stage of development. The factors to be studied are the ratio of meal to solvent, and the number of extractions, with a more thorough investigation of time of extraction, stirring rate, settling time and per cent of filter aid required.

Limitation of the Investigation. The conclusions reached by this investigation are limited by the fact that only oneratio of meal to extraction solution was used.

Only two extraction solutions, NaOH and Na_2SO_3 were used.

This investigation was further limited to only one extraction from the meal.

V CONCLUSIONS

1. A slurry of finely ground soybean meal and an alkaline solution of either NaOH or Na_2SO_3 of concentrations varying from 0.025 per cent to 0.80 per cent cannot be satisfactorily separated by filtration through either rapid filter paper or 16 ounce filter cloth, as the fine and mucilaginous material immediately chokes the filter medium. The maximum amount of filtrate through one filter paper in a 7.5 cm. Buchner funnel is 10 ml.

2. Separation of a slurry with a meal to 0.3 per cent Na_2SO_3 solution ratio of 1 to 10, retaining 5 to 6 per cent solids, by means of a super-centrifuge is complete when operated at 30,000 R.P.M. and fed through a 1/8 inch diameter tip with a hydraulic head of 12 inches.

3. Between the temperatures of 85°F. and 125°F., temperature has very little effect on the rate of solution of casein in a 0.3 per cent Na_2SO_3 solution.

4. An extracting solution concentration of 0.2 per cent is near optimum concentration for both Na_2SO_3 and NaOH for a meal to solution ratio of 1 to 10 with the temperature range from 85° F. to 125° F.

5. Extraction from the flaked, solvent extracted meal, 4-16 mesh, is complete in from 25 to 30 minutes with stirring sufficient to keep the particles in motion and a meal to Na_2SO_3 solution ratio of 1 to 10.

6. Slurry obtained by mixing the flaked, solvent extracted meal, 4-16 mesh, with 0.3 Na_2SO_3 solution, 1 to 10 ratio, is readily screened through a 16 mesh screen.

7. A casein solution prepared from flaked, solvent extracted meal, 4-16 mesh and 0.3 Na_2SO_3 solution, 1 to 10 ratio, and screened contains approximately 0.5 per cent filterable solids.

8. Settling of a casein solution containing 0.5 per cent filterable solids for a period of 60 minutes will remove the filterable solids down to 0.15 per cent.

9. Filtration of the settled casein solution containing 0.17 per cent filterable solids is difficult without filter aid. The addition of 1.25 per cent filter aid to a 4 per cent casein solution containing 0.17 per cent filterable solids produces a slurry which will filter through a 7.5 cm. Buchner funnel with a pressure difference of 27 inches of mercury at a rate of 250 ml. in 45 seconds.

VI SUMMARY

Soybean meal is produced abundantly today as a by-product of the soybean oil industry. The present use of the soybean meal is mainly as a livestock food. However, the meal contains approximately 44 per cent of high grade casein, which has commercial possibilities as an industrial casein. This investigation studies the various problem encountered in the process of separating the casein from the soybean meal.

Casein is being produced from soybean meal at the present time, but to a limited extent and with difficulty. The method in general use is that of mixing the ground meal with a weak, alkaline solution, separating the slurry, and finally precipitating the casein from solution by the addition of an acid. The difficulty in this procedure lies mainly in the separation of meal residue and casein solution.

Attempts to filter the slurry through any type of filter medium resulted in rapid choking by a fine and mucilaginous material. Pressure sufficient to maintain a flow only caused the liquid to pass through freely without being filtered.

Since separation with a filter medium proved difficult, the super-centrifuge was tried. Separation was complete when operated at 36,000 R.P.M. and at a slow rate of feed. This method

is effective, but the use of a super-centrifuge for separating a material containing a high percentage of solids is very cumbersome.

Because of this, effort was directed toward developing a process which could be used commercially. The meal as it comes from the oil extraction process is in the form of flakes, similar to oat meal. The flakes are roughly 0.0010 to 0.0012 of an inch in thickness, with 99 per cent passing through a 4 mesh standard screen and 69 per cent remaining on a 20 mesh standard screen. Extractions were made from the meal of 4-16 mesh to determine the time required, and it was found that extraction was nearly complete in 25 minutes. The various factors affecting rate of solution; temperature, stirring rate, particle size were investigated. Temperature has very little effect on the rate of solution between the temperatures of 55° F. and 125° F. It is necessary to stir the slurry sufficient to keep the particles in motion, but additional agitation has little affect on the rate of solution. Particle size of the flaked meal has little affect on the rate of solution between meal from mesh 4 to mesh 60.

It was found that the slurry obtained from flaked meal, 4-16 mesh, could be easily screened through a 16 mesh screen, giving a casein solution containing approximately 0.5 per cent filterable

solids. Allowing the solution to settle will remove some of the filterable solids, with 60 minutes settling time leaving 0.15 per cent filterable solids.

The solution obtained by screening and settling is very difficult to filter. It was found that with a solution containing 0.17 per cent filterable solids, 0.50 per cent filter aid is necessary to make filtration possible, with 1.25 per cent increasing the filtration rate 2.5 times. For a casein solution containing 0.17 per cent filterable solids, 1.25 per cent filter aid is near the optimum amount required.

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