

and partially inhibited by the addition of larger amounts of water.

II—The addition of water up to 4 per cent to an oil impregnated fabric caused a corresponding rise in permeability but of a smaller magnitude than in the case of the duck and gelatin treated cloth.

III—The probable explanation for this phenomenon is that at the point of maximum penetration a film of water covering the fabric interferes with surface adsorption of the mustard gas and possibly facilitates the passage of the gas along the surfaces by capillarity.

At higher water concentrations, as the interstices of the fabric are gradually filled with water, the passage of mustard gas would be first impeded and ultimately almost inhibited, since the speed of passage by solution in water and diffusion would necessarily be slow and associated with considerable hydrolysis.

IV—These results correspond with those observed regarding the influence exerted by a water film on the penetration of mustard gas into the skin.

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## ORIGINAL PAPERS

### THE PROXIMATE ANALYSIS OF COMMERCIAL CASEIN

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Although casein has had a fairly wide and growing application in many industries, it does not seem to have been customary to employ any comprehensive standard as the basis of specifications for its purchase. Since a large amount of casein was used in making waterproof glues for airplanes during the war, and since it was soon found that certain qualities of a commercial casein must be carefully considered in using it for glue making, it was found desirable to draw up specifications for casein for use in waterproof glues. This made necessary the adoption of methods of proximate analysis for casein.

Very little has been published in English concerning the proximate analysis of casein. A "borax solubility test"<sup>1</sup> and an "adhesiveness"<sup>2</sup> test have been described and appear to have found some use in buying casein for making paper size, but the tests are rather crude and their value does not seem to be thoroughly established. Reuter<sup>2</sup> proposes a qualitative test for iron, a borax solubility test, and the examination of a water extract for acidity, metals, sulfates, and chlorides. This system of analysis is of doubtful value. Höpfner and Burmeister<sup>3</sup> propose a system of analysis giving much more information. They determine moisture, fat, ash, nitrogen, and free acidity.

#### METHODS OF ANALYSIS

The methods described by Höpfner and Burmeister were adopted, with some modification, for the work at the Forest Products Laboratory of the U. S. Forest Service at Madison, Wisconsin. They have been employed in the examination of over 300 samples of casein and have served as the basis for Bureau of Aircraft Production specifications for the purchase of casein to be used in making water-resistant glue. The methods as employed at this laboratory are given below.

**SAMPLING FOR ANALYSIS**—If the sample is gathered from bins at the creamery, portions should be taken systematically from all parts of the bin. These should all be ground together, if that has not been done previously. After the powder has been thoroughly

mixed, the final sample is taken out to be sent to the laboratory. A 100-g. sample will be found to be sufficient for the determinations described. The sample received at the laboratory should be thoroughly mixed, 50 g. set aside for the determination of fineness, and the remainder reduced to 60-mesh size.

**COLOR**—If possible, the color of the casein should be observed at the creamery as it is taken from the driers. Grinding makes the casein appear much lighter in color. Commercial casein may be obtained which is almost pure white, and the color need never be more than a pale yellow or cream.

**ODOR**—In spite of the rather widespread belief that, as one casein jobber expressed it, "casein has an odor all its own," commercial casein may be obtained which is entirely free from odor, or, at most, has an odor resembling that of sweet milk. The rancid odor frequently associated with casein is not due to the casein, but to impurities or decomposition products of casein. The odor is readily determined as follows: About 10 g. of the casein are soaked in about 10 cc. of water and an equal volume of a rather thick "milk of lime" added with stirring. After the mixture has stood a few moments the odor is noted.

**FINENESS**—A 50-g. sample is placed in a 60-mesh sieve, the sieve is held in one hand and moved horizontally back and forth at the rate of about 120 strokes per min., being allowed to strike at the end of each stroke against the palm of the other hand which is held stationary. The portion passing through in 10 min. is weighed, and reported as per cent passing 60 mesh.

**MOISTURE**—This is most accurately determined by weighing out a 3-g. sample in a glass-stoppered weighing bottle, heating to constant weight in a vacuum oven at 70° to 80° C., cooling in a desiccator, and weighing. For most purposes it is more convenient and sufficiently accurate to use a porcelain evaporating dish and make the determination by heating in a Freas oven at 98° C., and atmospheric pressure for 5 hrs. How nearly constant weight is obtained in 5 hrs. is shown in Table I.

The maximum difference obtained at this laboratory between determinations made at atmospheric pressure and *in vacuo* is 0.47 per cent higher *in vacuo*. In most cases the difference was much less.

<sup>1</sup> A. O. Dahlberg, "The Manufacture of Casein from Buttermilk or Skim Milk," U. S. Dept. of Agriculture, *Bulletin* 661.

<sup>2</sup> *Papier-Zig.*, [2] 32 (1907), 3374.

<sup>3</sup> *Chem.-Zig.*, 36 (1912), 1053. See also Burr, *Milch. Zentr.*, 6 (1910), 385.

TABLE I—EFFECT OF TIME ON MOISTURE DETERMINATION  
Temperature, 98° C.; Atmospheric Pressure

Time of Heating Hours	Average Moisture Content, Per cent	
	Casein 1	Casein 2
1	8.89	5.78
2	9.49	6.34
3	9.92	6.46
4	10.07	6.63
5	10.18	6.67
22	10.41	6.74
44	10.46	6.71

FAT—The residue from the moisture determination is transferred to an extraction thimble and extracted for 16 hrs. with anhydrous, re-distilled ethyl ether in a Cauldwell or Soxhlet apparatus. The ether is evaporated from the extract, and the residue, corrected for the moisture content of the casein, is called fat.

In order to determine the time required for complete extraction, samples of a casein of unusually high fat content were extracted for varying lengths of time in a Cauldwell apparatus with the results shown in Table II.

TABLE II—EFFECT OF TIME ON FAT EXTRACTION

Time Extracted Hrs.	Fat (Moisture-free basis) Per cent		Remarks
3	10.00		Sample "as received"
6	10.45		Sample "as received"
8	10.50		Sample "as received"
16	10.54		Sample "as received"
16	10.58		Residue from moisture determination

For practical purposes extraction is complete in 8 hrs., but it is customary and quite convenient to let the extraction run over night. It is important that the sample for the fat determination be finely ground.

ASH—A 3-g. sample is weighed out in a vitreosil dish and carefully charred over the low flame of a Bunsen burner. When completely carbonized, it is placed in an electric muffle furnace and heated at a dull red heat (not over 600° C.) until the ash is white, or at least light gray, and the weight is constant. A small amount of ammonium nitrate may be added to facilitate the combustion of the last traces of carbon. Care should be taken to avoid fusion of the ash if possible. Results are reported on a moisture-free basis.

The determination of the ash content of casein presents certain difficulties because of the presence of organic sulfur and phosphorus, alkali chlorides and phosphates, and alkaline-earth salts. With very low ash caseins, the phosphorus makes the use of platinum dishes dangerous; porcelain dishes cannot be used because the phosphoric acid resulting from the combustion of the organic phosphorus enters into combination with the porcelain, giving high results. Vitreosil dishes, however, have been found satisfactory at this laboratory.<sup>1</sup>

A temperature in excess of a dull red heat (about 600° C.) should not be used because of danger of loss of alkali chlorides. With low ash caseins the phosphates present in the casein or formed during combustion sometimes give trouble by fusing over particles of unburned carbon, making it very difficult to obtain a white ash. In these cases warning is given during the preliminary charring of the sample by a very marked tendency of the casein to swell and overflow the dish. If the heating at this time is carried out at the lowest possible temperature so as to keep this

swelling down to a minimum, and if too high a temperature is not used in the muffle furnace, it will be found that a light-colored ash may be obtained, especially by using ammonium nitrate near the end of the ignition. With most technical caseins, however, there is enough calcium oxide present to combine with practically all the phosphoric acid formed, thus readily giving a white, skeleton ash.

TABLE III—COMPARISON OF ASH DETERMINATIONS IN PORCELAIN AND VITREOSIL

CASEIN No.	Per cent Ash, Straight Ignition		Per cent Ash, Calcium Acetate Added In Vitreosil
	In Porcelain	In Vitreosil or Platinum	
217	1.13	0.38	2.19
218	1.24	0.38	2.29
221	2.21	2.00	2.33
222	2.59	2.52	2.61

In Table III it will be seen that the values obtained in porcelain dishes are high, especially for caseins very low in ash. Caseins having much over 2 per cent ash, however, give values nearly the same in porcelain as in vitreosil, indicating that there is now enough lime present to "fix" all the organic phosphorus. To check this point, these caseins were ashed in the presence of a large excess of lime, added as calcium acetate, so as to retain as much of the organic phosphorus and sulfur as possible. The results are given in the last column of Table III. Porcelain dishes cannot be used for caseins containing less than 2 to 2.5 per cent ash, and vitreosil dishes are preferable at all times. Since nearly all commercial casein contains at least 2 per cent ash, very nearly all the organic phosphorus and sulfur are "fixed" in the ash.

The ash content of commercial caseins is, therefore, not a direct measure of the mineral impurities present, since the organic phosphorus and sulfur of the casein itself have been retained; but since in nearly all cases practically all this phosphorus has been "fixed," the ash is roughly proportional to the mineral impurities present. In this sense, then, the determination is empirical. Of course, a correction could be made by deducting from the ash value an amount corresponding to the organic phosphorus, but this would be extremely difficult to estimate correctly, and for the purpose of judging casein the uncorrected values are satisfactory.

In general, two methods of overcoming difficulties with fusible ashes have been proposed: (1) Leaching the charred material with water or acetic acid, completing the ashing of the residue, evaporating the leachings in the same dish, and finally heating to constant weight at dull redness,<sup>1</sup> and (2) adding basic materials such as calcium acetate,<sup>2</sup> or barium hydroxide,<sup>3</sup> before ashing. The first of these is time-consuming for routine work. The second is objectionable because the heating must be prolonged to obtain constant weight or else the temperature raised to a point at which there is danger of loss of chlorides. For caseins containing less than 2 to 2.5 per cent ash, the second method gives higher results (see Table III) than straight ignition in vitreosil.

NITROGEN—A  $\frac{1}{2}$ -g. sample is weighed out into an 800-cc. Kjeldahl flask, 20 cc. of concentrated sulfuric

<sup>1</sup> U. S. Department of Agriculture, Bureau of Chemistry, *Bulletin* 107, 38, 238.

<sup>2</sup> *Ibid.*, p. 21.

<sup>3</sup> *Ann. Chem. Pharm.*, 73, 339; 74, 336.

<sup>1</sup> See also Höpfner and Burmeister.

acid, 10 g. of crystallized sodium sulfate, and a small crystal of copper sulfate are added, and the contents digested until a clear solution is obtained, and then for 30 min. longer. 300 cc. of distilled water, 50 cc. of a 1 : 1 solution of sodium hydroxide, and about  $\frac{1}{4}$  g. of granulated zinc are added. About 250 cc. are then distilled over and caught in standard sulfuric or hydrochloric acid. (30 cc. of  $N/5$  acid will be sufficient.) The excess acid is back-titrated with standard sodium hydroxide solution, methyl red being used as indicator. Since the nitrogen determination is made as a measure of the impurities other than moisture, fat, or ash, results are reported on a moisture-, fat-, and ash-free basis.

The nitrogen content of pure casein has been quite satisfactorily determined to be close to 15.67<sup>1</sup> and it is customary in the determination of casein in milk,<sup>2</sup> for example, to coagulate with acetic acid, determine the nitrogen in the coagulum by the Kjeldahl method, and multiply by 6.38 (100/15.67) to obtain the casein. The same method can be used for determining roughly the per cent "casein" in the sample by multiplying the nitrogen (on the basis of the sample "as received") by this factor. By comparing this with the moisture-, fat-, and ash-free material in the sample, we obtain a measure of the organic materials other than casein and fat present. There has been some discussion of this "nitrogen factor" in the literature.<sup>3</sup> But since essentially the same information is given by observing how closely the nitrogen content computed on the moisture-, fat-, and ash-free basis approaches 15.67, and this method of calculation does not involve the use of any factor with its consequent multiplication of analytical errors, it is doubtless best to report only the nitrogen.

The use of the nitrogen content as a measure of the casein content is, of course, justifiable only if it is known that no other nitrogenous substances are present. Strictly speaking, this is not usually the case. The casein, unless very well washed, is contaminated with some whey protein which, as it has practically the same nitrogen content as casein, is thereby counted as casein. No other nitrogen-containing compounds are normally present.

**ACIDITY**—A 1-g. sample is placed in a flask and 25 cc. of  $N/10$  sodium hydroxide solution run in from a pipette. During this addition the flask is gently agitated. The flask is then stoppered and the agitation continued until the solution is complete. This should require only 5 or 10 min. The stopper is then removed and the portion of solution wetting it washed into the flask with a stream of water from a wash bottle, 100 cc. of distilled water (neutral to phenolphthalein) are added, and the solution back titrated at once with  $N/10$  acid, using 0.5 cc. of alcoholic phenolphthalein solution (1 g. per 100 cc.) as indicator. The acid is run in fairly rapidly with vigorous shaking of the flask so as to prevent precipitation of the casein locally. The number of cubic centimeters of  $N/10$  alkali used

up by 1 g. of moisture-, fat-, and ash-free casein is called the "acidity" of the sample.

The following precautions must be observed if this method is to give concordant results: (1) The flask should be kept stoppered except when making additions or titrating; (2) the amount of indicator specified must be used and it must be adjusted with alkali so that one drop added to distilled water does not change its reaction; (3) local coagulation of casein during titration must be avoided; (4) the total time during which the casein is allowed to stand in contact with alkali must not exceed 30 min. at room temperature.

If trouble is experienced in obtaining complete solution in 5 to 10 min., the following procedure will give the same results: After addition of the alkali, the tightly stoppered flask is kept in an ice box at a temperature of about 10° C., for 4 hrs. and shaken vigorously once an hour. The dilution and titration are then made as before, except that the acid and alkali solutions should be standardized at the temperature of the ice-box.

Pure casein under these conditions is stated to combine with about 9.0 cc. of  $N/10$  alkali per g.<sup>1</sup> Commercial casein may have a higher or lower acidity, depending upon the presence of free acids such as sulfuric or lactic on the one hand, or lime, etc., on the other.

Höpfner and Burmeister<sup>2</sup> determine acidity by the method of Lunge: 10 g. of the casein are shaken up with 100 cc. of water, filtered, and 50 cc. of the filtrate titrated with standard alkali solution. This method is supposed to give free acids directly, but fails because some casein goes into solution (even if the filtrate is perfectly clear) and uses up some alkali in the titration. To overcome this difficulty, one casein company suggests the following: 1 g. of casein is treated in a stoppered flask for 2 hrs. with 50 cc. of a 10 per cent solution of formaldehyde. It is then filtered, washed free from odor of formaldehyde, and the filtrate titrated with  $N/10$  sodium hydroxide. From this reading is subtracted the amount of sodium hydroxide solution required to neutralize 50 cc. of the formaldehyde solution.

TABLE IV—METHODS FOR ACIDITY

CASEIN No.	Acidity as Determined by—			Difference Between Complete Solution and $\text{CH}_2\text{O}$ Methods
	Complete Solution in NaOH— 4 Hrs. Standing Cc. $N/10$ Alk. per G.	Dissolved at Once Cc. $N/10$ Alk. per G.	Formaldehyde Method Cc. $N/10$ Alk. per G.	
65	11.65	11.70	3.68	7.97
224	11.35	11.40	3.69	7.66
9	11.15	11.20	3.67	7.48
228	10.45	10.30	2.66	7.79
37	10.05	10.10	2.99	7.06
120	7.93	8.03	0.90	7.03
144	7.40	7.35	—0.17	7.57
143	6.95	6.90	—0.27	7.22
161	6.25	6.35	—0.90	7.15
123	5.65	5.70	—0.41	6.06

A comparison of the results obtained by the methods of complete solution of the casein and the formaldehyde leaching method is given in Table IV. The formalde-

<sup>1</sup> Thorpe, "Dictionary of Applied Chemistry" (9.0 cc.); Robertson, *J. Phys. Chem.*, **15** (1911), 178 (9.10 cc.); Robertson, "Physical Chemistry of the Proteins," **1918**, p. 90 (8.0 cc.); Long, *J. Am. Chem. Soc.*, **28** (1906), 372 (8.90 cc.); Van Slyke and Bosworth, *J. Biol. Chem.*, **17** (1913), 211 (9.00 cc.); Hart, *J. Biol. Chem.*, **5** (1909), 445 (9.26 cc.).

<sup>2</sup> *Loc. cit.*

<sup>1</sup> "Dairy Chemistry" (Richmond).

<sup>2</sup> Leach, "Food Inspection and Analysis."

<sup>3</sup> *Z. offent. Chem.*, **15**, 53.

TABLE V—COMPARISON OF ANALYSES OF PURE CASEIN WITH DEALERS' SAMPLES

CASEIN No.	Source	Maker No.	Color	Odor	Moisture Per cent	Fat Moisture- Free Per cent	Ash Moisture- Free Per cent	Nitrogen		"Casein" Per cent	Moisture, Fat- Ash-Free Material Per cent	Acidity Cc.
								Cor- rected Per cent	Not Cor- rected Per cent			
Dealers' Samples												
201	Domestic	10	Slight yellow	None	6.51	2.17	4.45	14.85	12.96	82.7	87.30	10.3
204	Domestic	14	White	Sour	6.39	0.16	5.68	14.48	12.76	81.4	88.14	7.6
1	Domestic	16	Very yellow	Sour	10.09	0.41	2.61	14.34	12.51	80.2	87.19	10.9
2	Domestic	16	Yellow	Sour	9.88	1.03	2.94	15.08	13.05	83.4	86.51	9.0
3	Domestic	20	White	Sour	8.17	2.82	4.74	15.15	12.73	81.3	83.98	11.7
5	Domestic	15	Slight yellow	Slightly sour	7.94	0.59	4.79	14.83	12.92	82.4	87.12	7.3
6	Domestic	15	Slight yellow	Slightly sour	9.29	0.33	5.13	15.04	12.90	82.4	85.76	7.5
7	Domestic	9	Yellow	Slightly sour	8.69	0.39	4.19	14.86	12.95	82.7	87.12	7.4
8	Domestic	9	White	Sour	7.25	0.78	4.90	14.97	13.10	83.7	87.50	7.4
9	Domestic	..	Slight yellow	Slightly sour	5.85	2.56	4.48	13.40	11.73	74.9	87.32	12.8
10	Domestic	..	White	Slightly sour	5.30	3.86	3.55	14.84	13.02	83.1	87.69	10.1
11	Domestic	..	Yellow	Slightly sour	6.30	2.17	3.93	13.84	12.18	77.8	87.99	10.4
18	Domestic	17	White	Sour	5.59	2.90	3.64	14.62	12.89	82.2	88.16	8.7
19	Domestic	5	Slight yellow	Slightly sour	8.03	2.57	3.78	14.84	12.78	81.6	86.14	8.7
21	Domestic	1	Yellow	Slightly sour	6.46	0.80	3.82	14.57	13.00	83.0	89.22	11.9
22	Domestic	4	Yellow	Slightly sour	7.25	0.74	3.19	14.44	12.85	82.0	89.09	11.7
226	Domestic	20	White	None	9.72	0.75	4.35	14.54	12.46	79.6	85.67	10.8
227	Domestic	20	White	None	8.85	0.92	4.41	14.65	12.65	80.7	86.29	11.4
228	Domestic	20	White	None	9.50	0.90	4.43	14.80	12.68	80.8	85.67	13.3
				Maximum,	10.09	3.86	5.68	15.04	13.10	....	....	13.3
				Minimum,	5.30	0.33	2.61	13.40	11.73	....	....	7.3
				Average,	7.74	1.41	4.15	14.63	12.74	....	....	9.9
Pure Caseins												
171	Kahlbaum <sup>1</sup>	..	White	Sour	10.02	0.00	2.73	15.50	13.58	86.6	87.55	6.5
187	Kahlbaum <sup>1</sup>	..	White	Sour	7.42	0.22	0.95	15.58	14.26	91.0	91.50	8.46
216	Domestic <sup>2</sup>	..	White	None	5.70	0.19	0.24	15.20	14.27	90.9	93.87	8.2
217	Domestic <sup>2</sup>	..	White	Very sour	9.27	0.13	0.38	15.00	13.51	86.2	90.22	7.9
218	Domestic <sup>2</sup>	..	White	Sour	9.41	1.66	0.38	15.33	13.59	86.8	88.55	8.1
				Maximum,	10.02	1.66	2.73	15.58	14.27	....	....	8.46
				Minimum,	5.70	0.00	0.24	15.00	13.51	....	....	6.5
				Average,	8.38	0.44	0.94	15.32	13.84	....	....	7.8

<sup>1</sup> Hammarsten method. <sup>2</sup> Van Slyke method.

TABLE VI—SUMMARY OF THE ANALYSES OF CASEINS MADE BY DIFFERENT METHODS

SOURCE OF CASEIN	Type of Casein	Number of Samples Averaged	Number of Makers Represented	Moisture			Fat (Moisture-Free)			Ash (Moisture-Free)			Nitrogen (Corrected)			Acidity		
				Maximum Per cent	Minimum Per cent	Average Per cent	Maximum Per cent	Minimum Per cent	Average Per cent	Maximum Per cent	Minimum Per cent	Average Per cent	Maximum Per cent	Minimum Per cent	Average Per cent	Maximum Cc.	Minimum Cc.	Average Cc.
				.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
A—Caseins Bought on Market																		
Domestic	Buttermilk	1	1	.....	.....	6.89	.....	.....	31.50 <sup>1</sup>	.....	.....	3.28	.....	.....	14.25	.....	.....	.....
	Grain curd	7	1	8.25	5.92	6.84	0.77	0.28	0.50	2.87	2.53	2.78	14.94	14.45	14.71	9.7	6.8	8.9
	Natural sour	12	5	9.78	6.21	7.70	1.44	0.21	0.88	5.38	2.08	3.46	14.89	13.62	14.38	12.5	5.1	9.7
	Sulfuric acid	23	7	10.18	6.23	7.97	2.48	0.15	1.41	5.34	2.62	3.92	15.32	13.22	14.42	13.9	5.7	10.4
	Acid, cooked curd	18	3	10.89	6.96	8.16	1.42	0.22	0.65	4.93	3.79	4.45	14.76	13.94	14.40	9.6	5.8	7.5
	Hydrochloric acid	14	6	8.60	4.86	7.37	1.15	0.00	0.56	6.10	3.14	4.52	14.98	13.83	14.39	10.7	6.7	8.7
	Rennet	3	3	10.32	5.54	8.29	0.83	0.39	0.63	8.39	7.18	7.97	15.10	13.82	14.41	11.3	5.5	7.9
India	Natural sour(?)	8	..	8.89	6.76	8.13	3.67	0.94	1.93	5.59	3.08	4.43	14.53	13.54	14.17	14.0	10.2	12.3
Argentine	Natural sour(?)	8	..	9.28	5.94	8.20	2.11	0.53	1.10	4.30	3.62	3.87	14.88	14.30	14.66	13.5	9.0	11.5
B—Caseins Made under Special Supervision																		
Domestic	Buttermilk	9	..	8.97	5.33	6.97	23.32	3.79	9.56	2.18	0.88	1.36	15.06 <sup>2</sup>	14.42 <sup>2</sup>	14.77 <sup>2</sup>	10.0 <sup>2</sup>	8.5 <sup>2</sup>	9.2 <sup>2</sup>
	Grain curd	11	1	11.10	8.50	9.48	0.31	0.10	0.33	3.76	1.08	1.65	15.25	14.37	14.84	11.7	6.1	9.9
	Natural sour (ejector)	18	1	9.44	6.70	7.87	0.94	0.00	0.27	2.62	1.33	2.15	15.40	14.54	14.84	10.7	7.4	8.7
	Sulfuric acid	34	1	9.63	4.15	7.81	1.65	0.05	0.35	5.36	3.02	4.05	15.00	13.50	14.46	10.8	6.2	7.6
	Sulfuric acid, cooked curd	8	1	9.98	7.68	8.89	0.46	0.00	0.12	5.58	3.92	4.25	15.58	14.70	15.04	6.5	4.4	5.9
	Hydrochloric acid, cooked curd	8	1	11.08	8.82	9.44	0.33	0.05	0.18	5.09	4.14	4.71	15.51	14.61	15.03	6.5	4.2	5.2
	Hydrochloric acid	6	1	8.56	6.32	7.10	0.24	0.08	0.16	6.05	5.51	5.74	14.77	14.02	14.32	8.4	5.2	6.7

<sup>1</sup> Exceptionally high. Casein had a "greasy feeling" when rubbed between the fingers. <sup>2</sup> Results based on three samples only.

hyde method is not as convenient as the method of solution in sodium hydroxide, requires more time, and it is not quite clear what the results indicate. It will be seen that negative values are often obtained, probably indicating some combination (either physical or chemical) between the casein and the formaldehyde.

## SPECIFICATIONS

Government specifications<sup>1</sup> require that all casein used for making water-resistant glue for use in airplanes should pass the following tests:

Color: White or light cream  
 Odor: Nearly odorless, with not more than a trace of sourness  
 Moisture: Not more than 10.0 per cent  
 Fat: Not more than 1.0 per cent  
 Ash: Not more than 4.0 per cent  
 Nitrogen: Not less than 14.25 per cent  
 Acidity: Not more than 10.5 cc. N/10 alkali per g.

## RESULTS OF ANALYSES

In Table V are given the results of the analyses of samples of casein bought from dealers in casein and

<sup>1</sup> Department of the Navy, Bureau of Construction and Repair, *Aeronautical Specification 85*, January 15, 1919.

of five samples of pure casein made by the Hammarsten<sup>1</sup> and Van Slyke methods.<sup>2</sup> In Table VI-A is given a summary of the analyses of a much larger number of samples bought from makers of casein, classified according to the method of manufacture. In Table VI-B a summary will be found of the analyses of a large number of samples prepared in a commercial creamery by commercial methods, but under the supervision of an expert in dairy practice of the Dairy Division, Bureau of Animal Industry. In Table VIII the influence of certain factors in the manufacture of casein is shown. The same caseins furnish the data for Tables VI-B and VIII.

PURE CASEINS—It will be observed that the "pure" caseins in Table V are not entirely free from foreign substances. Most of them contain small amounts of fat (the high fat content of Casein 218 was due to insufficient washing with ether). The Hammarsten prep-

<sup>1</sup> Hammarsten, "A Text Book of Physiological Chemistry," translated by Mandel.

<sup>2</sup> *J. Biol. Chem.*, **35** (1918), 127.

arations are high in ash. It will be seen from the values for nitrogen or by comparing the "moisture-, fat-, ash-free material" with "caseins" that the samples contain appreciable amounts of organic impurities. The sour odor is due to this. Robertson<sup>1</sup> states that C. P. casein "nach Hammarsten" is contaminated by a small amount of an acid, water-soluble substance. The values for acidity are slightly low. The very low acidity value of Casein 171 is due probably to its high ash (CaO?) content.

**TYPICAL COMMERCIAL CASEINS**—The commercial caseins of Table V contain variable amounts of fat and ash. The fat in some cases is remarkably low.

In Table VI-A		TABLE VII	In Table VI-B	
	Per cent			Per cent
Grain curd <sup>1</sup> .....	2.66	Buttermilk.....	1.36	
Buttermilk (one sample).....	3.28	Grain curd <sup>1</sup> .....	1.65	
Natural sour.....	3.55	Natural sour (ejector) <sup>1</sup> .....	2.16	
Sulfuric acid.....	3.94	Sulfuric acid.....	4.05	
Acid, cooked curd.....	4.45	Sulfuric acid, cooked curd.....	4.25	
Hydrochloric acid.....	4.56	Hydrochloric acid, cooked curd.....	4.71	
Rennet.....	7.97	Hydrochloric acid.....	5.74	

<sup>1</sup> The grain curd method of precipitating casein with hydrochloric acid, and the ejector method of natural souring have been developed by the Dairy Division of the Bureau of Animal Industry, and will be published within a short time.

The ash content averages about 4 per cent. The low nitrogen values indicate the presence of (1) mineral matter not volatile at 98° C. but volatile at the temperature of ashing, (2) nitrogenous organic matter other than fat or casein containing less nitrogen than casein, (3) non-nitrogenous organic matter. From an examination of the constituents of milk<sup>2</sup> it will be seen that neither of the first two should be present in appreciable amounts. Any whey protein present, since it has about the same nitrogen content as casein, would not influence the nitrogen content. Other organic matter normally present is non-nitrogenous, chiefly, lactose or its fermentation products. Comparison of "moisture-, fat-, ash-free material" with "casein" shows that the organic impurities other than fat vary from about 3 to about 12 per cent, being usually about 5 per cent. The acidity varies over a wide range, being frequently less than that of the pure caseins (due to the higher CaO content?) but usually considerably higher.

**FAT**—The fat content depends upon the raw material and its treatment before precipitation. Since the casein carries down the fat with it during precipitation, and since buttermilk usually contains more fat than skim milk, the fat content of the casein obtained therefrom is much higher than that of skim-milk casein. With skim-milk casein the fat content depends almost entirely upon the efficiency of the cream separator. With proper separation a fat content of less than 0.5 per cent can easily be insured.

**ASH**—The ash content is governed primarily by the acidity of the milk from which the curd is precipitated, and secondarily by the thoroughness with which the curd is washed. The formation of a large, chunky curd also seems to hinder removal of mineral matter by preventing the acid and later the wash water from coming thoroughly in contact with the casein inside the clots. Table VII condensed from Table VI shows the average ash contents observed in the different types of casein analyzed.

The tendency for the curd to form in large clots increases as we read down the lists of these precipitation methods. In Table VIII it will be seen that with the natural sour method, washing the curd after precipitation lowers the ash content, while it has little influence in the sulfuric acid method. This is due possibly to the fact that the different nature of the curd makes it less easy for the soluble mineral substances to be removed in the latter case.

Reference to Table VIII shows that the acidity of the milk from which the curd is precipitated has a decided influence on the ash content. The more acid used the lower is the ash content of the product. This is well shown in the cases of sulfuric-acid and sulfuric-acid cooked-curd caseins. With natural-sour caseins the ash content is lower than with corresponding amounts of mineral acids. This is doubtless due to the longer and more intimate contact of the curd with the acid. The development of the grain-curd method has shown that it is possible to get casein by mineral-acid precipitation with ash content even lower than in natural sour casein. In this method the aim is to add

TABLE VIII—INFLUENCE OF CERTAIN FACTORS IN MANUFACTURE ON ANALYSIS OF CASEIN

BASIS OF CLASSIFICATION	Natural Sour (Ejector)							Sulfuric Acid							Sulfuric-Acid Cooked Curd						
	No. Sam- ples	Acid <sup>1</sup> used for Pptn.	Times Curd Washed	Fat Per cent	Ash Per cent	Per cent N	Acidity Cc.	No. Sam- ples	Acid <sup>1</sup> Used for Pptn.	Times Curd Washed	Fat Per cent	Ash Per cent	Per cent N	Acidity Cc.	No. Sam- ples	Acid <sup>1</sup> Used for Pptn.	Fat Per cent	Ash Per cent	Per cent N	Acidity Cc.	
Washing of curd	13	0.92 Av. <sup>2</sup>	2-4	0.25	1.86	15.04	8.5	12	0.65 Av.	2-3	0.15	4.51	14.60	7.4	..	..	..	..	..	..	
	10	0.70 Av.	..	..	..	..	..	10	0.70 Av.	..	0.08	4.52	14.44	7.1	..	..	..	..	..	..	
	9	0.65 Av.	0	0.29	2.46	14.65	9.0	9	0.65 Av.	0	0.42	4.47	14.16	8.0	..	..	..	..	..	..	
	3	1.03	0 Av.	0.40	1.95	14.85	8.7	3	1.03	0 Av.	0.84	3.05	14.37	10.0	..	..	..	..	..	..	
Acid used for pre- cipitating casein from milk	5	0.89-0.91	2.2 Av.	0.23	1.97	14.94	8.8	7	0.73-0.84	1.3 Av.	0.20	3.81	14.67	7.5	..	..	..	..	..	..	
	5	0.72-0.85	2.2 Av.	0.15	2.10	15.05	8.6	9	0.65-0.71	1.0 Av.	0.23	4.48	14.43	6.9	4	0.65-0.76	0.10	3.85	15.23	5.9	
	..	..	..	..	..	..	..	15	0.57-0.63	1.3 Av.	0.13	4.86	14.37	7.6	4	0.55-0.63	0.14	4.68	14.84	6.0	

<sup>1</sup> Amount of acid used to precipitate the casein from the milk expressed as 100 times the grams of acid hydrogen per cc. of milk; for natural-sour casein determined by titrating the milk before running through the ejector; for sulfuric-acid casein, the milk in nearly every case had an initial acidity of 0.0022 g. hydrogen per 100 cc. (0.2 per cent lactic acid), hence this amount is added to the grams of acid hydrogen added as sulfuric acid to get the figures given in the table.

<sup>2</sup> Where the abbreviation "Av." occurs, the figure is the average value for the number of samples being considered.

#### CAUSES OF VARIATIONS IN CASEIN

**MOISTURE**—The moisture content depends entirely upon the details of the drying process and is practically independent of the methods of precipitating the curd.

<sup>1</sup> T. B. Robertson, "The Physical Chemistry of the Proteins," 1918, p. 39.

<sup>2</sup> Leach, "Food Inspection and Analysis."

enough acid to give an acidity in the milk corresponding to the isoelectric point of the casein (which is also the point of minimum "solubility"), instead of limiting it to the least amount necessary to cause precipitation.

**NITROGEN**—The remarkably close agreement in the nitrogen contents of the domestic caseins in Table VI-A shows how little this value depends upon the

method of precipitating the curd. It is to be noted that the grain-curd caseins were all made by one creamery, which is employing the most modern methods. In Table VI-B the values for nitrogen are considerably higher with the grain-curd, ejector method of natural-sour, and cooked-curd caseins. This is probably due to the fact that in these cases the character of the curd is such as to permit of more thorough elimination of impurities during washing. In Table VIII it will be seen that washing the curd thoroughly after precipitation raises the nitrogen content, indicating a removal of such organic impurities as lactose. The acidity of the milk does not influence the nitrogen content.

**ACIDITY**—As is to be expected, there is a fairly close relation between the acidity and the ash content of the casein—the higher the ash, the lower the acidity, but this value is influenced also by other factors, such as the presence of some of the acid used for the precipitation. The acidity, therefore, is influenced first by the same factors as govern the ash content, namely, the acidity of the milk, and second, by the thoroughness of washing the curd. In this connection the change in acidity with ash content of the skim-milk caseins of Table VI-B is to be noted. The caseins of Table VI-A are not so well washed and hence this relation is more obscure. In Table VIII it will be seen that washing the curd thoroughly after precipitation tends to lower the acidity.

A comparison of A and B, Table VI, shows that the present commercial methods of making technical casein can be improved to a considerable extent. At present it does not seem to be the practice to wash the curd sufficiently after precipitation. The ejector method for natural-sour and the grain-curd method for acid casein seem to give the best results.

#### SUMMARY

1—A system for the proximate analysis of casein consisting of determinations of color, odor, moisture, fat, ash, nitrogen, and acidity is described.

2—The results of the analysis of some 200 samples of caseins of different types and methods of manufacture are reported.

3—The fat content of caseins depends upon the efficiency of the cream separation (for skim-milk casein) and is independent of the method of manufacture.

4—The ash content varies slightly with the thoroughness of the washing of the curd, but chiefly with the method of precipitation, the important factors being the amount of acid used and the length of time and intimacy of contact between curd and acid. The kind of acid seems to be immaterial, provided that different acids are used in equivalent amounts.

5—The nitrogen content depends chiefly on the thoroughness of the washing of the curd. It indicates the presence of 3 to 12 per cent of organic impurities (lactose, etc.).

6—The acidity in general increases as the ash content decreases, but is also largely influenced by the

amount of acid impurities present, which in turn varies with the thoroughness with which the curd is washed.

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#### A MODIFICATION OF THE PHENYLHYDRAZINE METHOD OF DETERMINING PENTOSANS

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Everyone who has made determinations of pentosans by the phloroglucinol method realizes that a shorter and less expensive method is very much needed. This is especially true at this time when it is almost, if not impossible, to buy phloroglucinol even at the high price asked for it. Our purpose in this short investigation was to try to overcome this difficulty by modifying the phenylhydrazine method so as to make it shorter and at the same time obtain results by this method which would agree with the provisional phloroglucinol method.

Recent investigations make it more and more evident that many compounds other than the pentoses and pentosans give furfural when they are distilled with hydrochloric or sulfuric acid, and hence neither the phloroglucinol method nor any other method of determining the furfural coming from such a distillation will enable one to say definitely that the original substance contained a certain per cent of pentosans.

We thought it should be possible to precipitate the furfural with phenylhydrazine and determine the excess of phenylhydrazine in the filtrate by the use of some compound which would oxidize the phenylhydrazine. Tests were made using solutions of phenylhydrazine sulfate with iodine, potassium dichromate, ferric sulfate, sodium hypobromite, and Fehling's solution. With the iodine almost theoretical results were obtained when a large excess of it was used. The amount of ferrous salt found was always less than the theoretical. No better results were obtained with the other oxidizing agents. These results are in harmony with what Chattaway<sup>1</sup> found in his work on the hydrazines. R. Adan<sup>2</sup> found that the reaction between phenylhydrazine, zinc, and copper sulfate always gave less than the theoretical amount of nitrogen. He stated that secondary products such as chlorophenylhydrazine and diazo compounds were formed when hydrochloric acid was present. E. Ebler<sup>3</sup> found that a quantitative yield of nitrogen was

<sup>1</sup> *J. Chem. Soc.*, **91** (1907), 1323; **95** (1909), 1065.

<sup>2</sup> *Bull. soc. chim. Belg.*, **21** (1907), 211; abstracted in *J. Chem. Soc.*, [II] **91** (1907), 657.

<sup>3</sup> *Z. anorg. Chem.*, **47** (1905), 371; abstracted in *J. Chem. Soc.*, [II], **90** (1906), 53.