UNIVERSITY OF IDAHO
AGRICULTURAL EXPERIMENT STATION
Department of Dairy Husbandry

Effect of Various Phases in the Manufacture of Casein By the Natural Sour Method on Its Physical and Chemical Properties

By

D. R. THEOPHILUS, H. C. HANSEN, R. S. SNYDER,
R. E. WOOD and R. L. OLMSTEAD

BULLETIN NO. 212 MAY, 1935

Published by the University of Idaho, Moscow, Idaho

A UNIFORM, high quality casein was produced by the following standard method of manufacture:

1. Skim milk with a fat content of approximately 0.01 per cent or less.

2. Titratable acidity of skim milk of 0.18 per cent or less.

3. Sufficient whey starter added to raise the acidity of the skim milk to 0.30 per cent. Temperature of the skim milk not below 70° F. and acidity of the starter not below 1.15 per cent or above 1.35 per cent.

4. Set at a temperature of 104° F. Allow to set until 0.64 per cent acidity has developed.

5. Coagulate by heating to 120° F., stir constantly.

6. Drain off the whey and break the curd into pieces about the size of an egg.

7. Wash the curd with cold water, use one-half as much water as original skim milk. Allow to stand in the cold water for 15 minutes, stir frequently.

8. Drain the wash water from the curd.

9. Press continuously at not less than 500 pounds pressure for 16 hours.

10. Dry at 120° F. for 8 to 10 hours. The drying tunnel should be of proper construction, equipped with accurate temperature controls and have sufficient air circulation.

Effect of Various Phases in the Manufacture of Casein by the Natural Sour Method on Its Physical and Chemical Properties*

D. R. THEOPHILUS[1], H. C. HANSEN[2], R. S. SNYDER[3], R. E. WOOD, and R. L. OLMSTEAD

INTRODUCTION

MANUFACTURE of casein by the natural sour method never has been standardized and no attempt has been made to correlate the methods of manufacture with the physical and chemical properties of the finished casein. Many buyers are using some physical or chemical tests in an attempt to measure the suitability of the casein for their specific purposes. Little uniformity exists in the tests used and opinions differ as to their respective value. The need for standards in grading and evaluating casein used for different purposes has been emphasized by several investigators (1, 4, 5, 6, 7, 8, 10, 12). Many writers (1, 2, 3, 5, 6, 7, 8, 10, 11) have suggested the possibility of some relationship between the methods of manufacture and the physical and chemical properties of casein.

The purpose of the work discussed in this bulletin was threefold: first, to determine the effect of various phases in the manufacture of casein by the natural sour method on its physical and chemical properties; second, to correlate, if possible, the phases of manufacture with the physical and chemical properties; and third, to suggest improved methods of manufacture.

A standard method for manufacturing casein by the natural sour method is presented in this bulletin. Casein made by this method was used as a standerd for comparison with caseins resulting from deviations within the various phases of manufacture. The phases studied were: original acidity of skim milk, original fat content of skim milk, setting temperature, acidity at time of coagulation, cooking temperature, time curd left in whey, number of washings, temperature of wash water, moisture in green curd, and drying temperature. Results were measured by selected physical and chemical tests.

EXPERIMENTAL PROCEDURE.

Whole milk from the University of Idaho Jersey and Holstein herds was used. The fat content of the skim milk was approxi-

---

*The data in this bulletin are taken from the theses presented to the Graduate Faculty, University of Idaho, by R. E. Wood and R. L. Olmstead in partial fulfillment of the requirements for the degrees of master of science.

[1]Associate Dairy Husbandman, [2]Assistant Dairy Husbandman, [3]Associate Agricultural Chemist.

mately 0.01 per cent and the titratable acidity about 0.17 per cent

Standard Method of Manufacture Selected

1. All curd was made in four 50-gallon cheese vats. Skim milk was weighed into the vats and warmed to 70° F.

2. Sufficient whey starter was added to raise the acidity of the skim milk to 0.30 per cent. The whey starter was developed and stored in a wooden barrel. Enough fresh whey was added to the barrel each day to maintain an acidity of from 1.15 to 1.35 per cent. Preliminary work demonstrated that a starter with an acidity of less than 1.15 per cent or above 1.35 per cent greatly increased the time necessary for coagulation.

3. Each batch was warmed to 104° F. and allowed to set until 0.64 per cent acidity had developed. Complete precipitation of the milk occurred at 0.64 per cent acidity.

4. The curd was coagulated by heating the milk to 120° F., stirring constantly. In heating the milk, steam was introduced directly.

5. All the whey was drained off and the curd broken into pieces about the size of an egg to facilitate draining and washing.

6. The curd was washed with cold water (55° F.), using one half as much water as original skim milk. It was allowed to stand in the cold water for 15 minutes, being stirred frequently, and then drained.

7. The curd was placed in a five-pound cheese mold and pressed in a continuous cheese press for 16 hours at a pressure of 1000 pounds per square inch.

8. After being ground in a food chopper the curd was spread one-fourth inch deep on drying trays.

9. The curd was dried for 16 hours in a thermostatically controlled drying tunnel at 120° F. The velocity of air was 95 cubic feet per minute through an intake 6 inches in diameter. Preliminary work (6) showed these drying conditions to be equivalent to 8 to 10 hours drying under commercial conditions.

METHODS OF ANALYSIS

Physical Tests

Color. Color was determined by comparison, using a color scale with the following gradations: 1 point, brown; 2 points, light brown; 3 points, amber; 4 points, light amber; 5 points, cream; 6 points, light cream; 7 points, straw; 8 points, light straw; 9 points, very light straw; 10 points, white. Samples for the standard color scale were made up of casein that would pass through a 20-mesh but not a 40-mesh screen. Preliminary results showed that the size and uniformity of the casein particles influenced color score. Samples were sealed in test tubes and kept in a dark, cool place when not in use.

Odor. Odor was determined by comparison, using an arbitrary odor scale with the following gradations: 5 points, musty, moldy, and cheesy; 6 points, rancid; 7 points, slightly rancid; 8 points, sour; 9 points, slightly sour; 10 points, clean. Before judging the casein for odor the lids were removed from the jars and the dead air allowed to escape.

Solubility. Instead of using the standard solubility test recommended by Sutermeister (5), a revised miniature test was developed. Casein was ground to pass a 20-mesh but not a 40-mesh screen to have all particles practically the same size. The same proportions of borax, water, and casein were used as in the standard method, but the amounts were varied.

Ten cubic centimeters of a 2.5 per cent sodium borate ($Na_2B_4O_7.10H_2O$) solution were placed in ¾x6-inch tubes; 1.66 grams of casein and a few drops of a one per cent alcoholic solution of corallin were added. The test tubes were then placed in warm water, 60° to 65° C., and stirred constantly with a glass rod until the casein was completely dissolved. When stirring was stopped any casein remaining undissolved immediately settled out. By continuing the stirring until no casein settled out the relative solubility of the different samples could be measured in minutes. This test had the following advantages over the Sutermeister test: first, the red color of the solution made it easier to distinguish rapidly any undissolved particles of casein; and second, the color together with the smaller quantity of solution in a test tube made it possible to establish a more definite end-point of solubility.

Viscosity. An orifice viscosimeter was assembled and used as follows: a 50 c.c. pipette with a 2 m.m. orifice was placed inside a water heated jacket in which a temperature of 60° C. was maintained by a gas burner. A 25 c.c. graduate was placed under the outlet of the pipette to receive the efflux from the orifice. The solution used was the standard Sutermeister solution (5) diluted with 75 c.c. of water. Dilution was found necessary to assure a steady flow of solution through the orifice. The time in seconds required for 25 c.c. of solution to flow through the orifice was used as the comparative viscosity.

Chemical Tests

Chemical tests for moisture, ash, fat, nitrogen, pH value, total acidity, free acidity, and conductivity were made according to the modified methods recommended by Snyder and Hansen (4). Conductivity as measured by resistance in ohms was used as an indication of the presence of impurities. All samples were placed in airtight glass jars and stored in a dark room until analyzed.

PRESENTATION OF DATA

Original Acidity of Skim Milk. The original acidity of skim milk was varied from 0.16 to 0.25 per cent. An acidity of 0.25 per cent was considered the maximum limit at which milk could be separated efficiently with the average cream separator. Trimble and Bell (7, 8) recommend that only clean sweet skim milk, free from abnormal fermentations, be used for casein manufacture.

Results of this investigation, *(Section B of Table I)*, indicate that the original acidity of the skim milk when varied from 0.16 to 0.25 per cent had little or no influence on any property of the finished casein. Apparently any milk free from abnormal fermentations will produce a good quality casein if it can be efficiently separated.

Original Fat Content of Skim Milk. When the original fat content of the skim milk was varied from 0.01 to 0.50 per cent, the physical properties of the finished casein were influenced much more than were the chemical properties (*Section C, Table I*). As the fat content of the skim milk was increased from 0.01 to 0.10 per cent, then to 0.25, and finally to 0.50 per cent, there was a decrease in the color score of 1.5 points, 2.0 points, and 2.23 points, respectively. The decrease in odor score was even more marked, for with the same increases in the fat content of the skim milk there were decreases in the odor score of 1.0 point, 3.25 points, and 3.75 points, respectively. When the fat content of the skim milk was 0.25 and 0.50 per cent, the finished casein in every instance had a distinctly rancid odor.

Rate of solubility was apparently influenced by the fat content of the skim milk. As the fat content increased the rate of solubility increased from 11.31 to 9.18 minutes.

When the fat content was increased from 0.01 to 0.50 per cent the viscosity decreased from 11.88 to 10.36 seconds, and the yield of casein per hundred pounds of skim milk was increased from 2.82 to 3.13 pounds, an increase of 11.0 per cent.

Moisture, ash, pH value, total acidity, free acidity, and resistance in ohms were not influenced to any marked extent by the fat content of the skim milk. However, an increase in fat content of the skim milk significiently influenced both the fat and nitrogen content of the casein. The increase in the fat content of the casein was 159 per cent, 382 per cent, and 1028 per cent when the fat content of the skim milk was increased from 0.01 per cent to 0.10, 0.25, and 0.50 per cent, respectively. This great increase in fat content of casein accounts, at least in part, for the increase in rate of solubility and decrease in viscosity, as well as increase in yield. Although the nitrogen content of the finished casein was not decreased appreciably when 0.10 and 0.25 per cent fat were present in the skim milk, nevertheless, when the fat content was increased to 0.50 per cent the nitrogen content was decreased 6 per cent.

Results indicate that as the fat content of the original skim milk increases there is a detrimental influence exerted on the finished casein as indicated by some of its physical and chemical properties, particularly color, odor, nitrogen, and fat content. Although under commercial conditions the fat content of the skim milk is low and fairly constant, it must be remembered that small increases in the fat multiply their effect many times in the finished casein.

Setting Temperature. The time required to develop the desired acidity of 0.64 per cent at time of coagulation varied inversely with the increase in setting temperature from 80° to 110° F. At a setting temperature of 80° F. 13 hours were required to reach an acidity of 0.64 per cent; at 90° F., 8 hours; at 100° F., 4 hours; and at 110° F., 3 hours. With the longer time required for the setting temperatures of 80° and 90° F., the curd frequently developed gas and was crumbly, making it difficult to wash without loss in yield.

Differences in setting temperatures had no appreciable influence on color score, odor score, viscosity, moisture, ash, nitrogen, total acidity, or free acidity of the finished casein *(Section D, Table I)*. In the standard method, setting temperature 104° F., the rate of solubility was 11.60 minutes. Either raising or lowering the setting temperature caused a decrease in the rate of solubility, it being 12.50 minutes at 80° F. and 13.38 minutes at 110° F. As the setting temperature was increased there was a slight but uniform decrease in pH value, a decrease in resistance in ohms, and an increase in yield.

Setting temperatures of 100° to 110° F. are to be preferred because they lessen the time of manufacture and, with the exception of a slight decrease in rate of solubility, do not detrimentally influence the quality of casein.

Acidity at Time of Coagulation. Acidity of the skim milk at the time of coagulation is a very important factor in the manufacture of natural sour casein since it influences quite markedly the quality of the finished casein as measured by physical and chemical properties. Three acidities were used, 0.45, 0.60*, and 0.75 per cent. Titratable acidity was used in this work in preference to hydrogen-ion concentration because the average plant does not have the proper facilities for making pH determinations.

An acidity of 0.45 per cent had some very striking influences on solubility, ash, resistance in ohms, yield, color, and odor when compared with the standard method (*Section E, Table I*). When the acidity was lowered from 0.60 per cent to 0.45 per cent the rate of solubility decreased from 11.60 to 24.5 minutes; ash increased 56 per cent; resistance in ohms decreased 37 per cent; and yield decreased 58 per cent. Color score was decreased 1.11 points and odor score 1.0 point. The great decrease in rate of solubility and yield and the increase in ash may undoubtedly be attributed to the incomplete precipitation of the casein.

When the acidity was increased from 0.60 to 0.75 per cent, the color score decreased 2 points, viscosity decreased 17.6 per cent, and yield decreased 4.0 per cent. Rate of solubility was decreased from 11.60 to 15.44 minutes, pH value was increased 12.6 per cent, and resistance in ohms 13.0 per cent. When using an acidity of 0.75 per cent decreases in rate of solubility and yield apparently were due to the very hard granular character of the curd.

*An acidity of 0.60 per cent was used in the first series as a standard for comparison. Since it was noted that this acidity was not quite the iso-electric point, all other casein was coagulated at an acidity of 0.64 per cent.

These results indicate that at the time of coagulation a very careful adjustment of the acidity is desirable. Apparently 0.64 per cent of titratable acidity is very close to the iso-electric point and is the best acidity to use for coagulation. Any appreciable deviation, either below or above this acidity, gave much less satisfactory results as measured by the color, odor, solubility, and yield of the finished casein, because of either the incomplete coagulation or the hard, granular character of the curd. Low acidities increased the ash content and the resistance in ohms because of the incomplete precipitation of the casein.

Cooking Temperatures. As the cooking temperature was increased there was an increase in the rate of solubility and ash content and a decrease in pH value and total and free acidity (*Section F, Table I*). The changes in pH value and total acidity, while not particularly marked, nevertheless were regular and fairly uniform. The most outstanding effect of change in cooking temperature was the influence on solubility and yield when the temperature was lowered to 110° F. from the standard method of 120° F. At 110° F. the solubility was decreased from 11.60 to 16.06 minutes and the yield was decreased from 2.81 to 2.13 pounds. The decreased yield, which amounted to 24 per cent, may be accounted for by the incomplete coagulation of the curd. When the cooking temperature was raised above the temperature used in the standard method to 140° F. a very tough curd was formed and the ash was increased 27 per cent.

These results show that a cooking temperature of 120° F. was the best of those tried. A temperature of 110° F. should not be used because of the decrease in solubility and yield, while a temperature of 140° F. is undesirable because a tough curd is produced which makes it more difficult to wash out impurities, thereby resulting in a casein of higher ash content.

Time Curd Left in Whey. Leaving the curd in the whey 0, 10, 15, or 25 minutes affected none of the physical or chemical properties of the finished casein with the exception of solubility and resistance in ohms *(Section G, Table I)*. Rate of solubility was slightly decreased when the curd was left in the whey 15 or 25 minutes. Resistance in ohms was increased directly with the increase in length of time the curd was left in the whey. In commerical practice the whey is removed from the curd as soon as possible to save time in manufacturing. These results indicate that leaving the curd in the whey up to 25 minutes will have no detrimental effect in the finished casein as measured by its physical and chemical properties.

Effect of Number of Washings. In the commercial manufacture of casein the curd is usually washed once. A study was made of the effect of 0, 1, 2, 3, and 4 washings on the well-broken curd. Each washing was of 15 minutes duration, using a quantity of wash water equal to one-half the amount of the original skim milk.

Results show an improvement in the quality of casein with each additional washing (*Section H, Table I*). Although no commercial plant would attempt to produce casein without any washing, nevertheless, observation indicates that frequently the one washing they do use is not much better than no washing because of the prevalence of large masses of unbroken curd which make thorough washing impossible.

No washing resulted in a casein of poor quality, low in color score and solubility, high in ash and total and free acidity. The low resistance in ohms indicates the presence of impurities.

One washing distinctly improved the casein by increasing the color score, odor score, pH value, resistance in ohms, and the rate of solubility and by decreasing the ash content, and free and total acidity.

Two washings continued to improve the quality, but the effect was not so noticeable as with one washing except in the decrease in ash content from 3.15 to 2.41 per cent.

Three and four washings each continued to improve the quality, but the improvement was limited in comparison with the effect of one washing over no washing.

Successive washings reduced the impurities in casein as indicated by the decreased ash content, total acidity, and free acidity, and the increased pH value and resistance in ohms. Yield was decreased because of impurities washed out and the loss of small particles of curd in each wash water.

These results indicate that one *thorough* washing of *well-broken* curd is sufficient to produce a very good grade of commercial casein.

Effect of Temperature of Wash Water. Wash waters at temperatures of 55°, 65°, 85°, and 110° F. were used (*Section I, Table I*). As the temperature of wash water was increased above that of the standard method, 50° F., there was a marked increase in viscosity of the casein. Wash water at temperatures of 85° and 110° F. produced a very rubbery, tough curd, which when dried was flinty and difficult to grind. As the temperature of wash water was increased from 55° F. to 110° F. the fat content of the casein was reduced from 0.64 to 0.17 per cent and the viscosity was increased from 14.98 to 18.26 seconds. Wash water with a temperature at about 55° F. gave best results. Water with a temperature above 85° F. should not be used because a tough, rubbery curd results unless a casein high in viscosity is specially desired.

Effect of Moisture in Green Curd. Pressing the excess moisture out of the curd before it goes to the drier is of economic importance since it is cheaper to remove moisture and impurities by pressure than to drive off moisture by heat. The green curd was subjected to the following pressures: no pressure, 500 pounds per square inch, and 1000 pounds per square inch. The moisture content of each was 64.16, 53.61, and 49.10 per cent, respectively (*Section J, Table I*).

The reduction of moisture of the green curd from 64.16 to 49.10 per cent, due to increased pressure, improved the color score of the resulting casein 2.25 points and odor score 3.50 points. When green curd was held over night before pressing or drying, the resulting casein had a lower color and odor score, due primarily to growth of mold. No manufacturer would consider drying casein without pressing out the excess moisture unless the pressing facilities were limited.

These results indicate that excessive moisture in the green curd had no effect on any of the chemical or physical properties of the resulting casein with the exception of color and odor. It seems to make little difference, as far as the quality of the casein is concerned, whether the moisture is pressed out or driven off by heat. Since the former is more economical it is of interest to note that 500 pounds pressure was almost as effective as 1000 pounds in removing moisture.

Effect of Drying Temperature. Drying temperatures of 80°, 100°, 120°, and 140° F. were used (*Section K Table I*). Whittier and Gould (11) recommend drying temperatures of 128.8° to 131° F. In this investigation drying temperatures up to 140° F. had little or no effect on any property of casein except moisture, yield, and to a slight extent viscosity. It naturally follows that the lower the drying temperature the less moisture will be driven off, resulting in a higher moisture content of the casein, which in turn increases the yield. The slight increase in viscosity at 140° F. could be due to more casein and less moisture in the sample tested. The high yield at 80° F. is undesirable because the excessively high moisture content of the casein tends to cause spoilage. Drying at 140° F. drives out more moisture than necessary and lowers the yield. A drying temperature of about 120° F. would seem to be most satisfactory because it gave standard yields, accompanied by comparatively low moisture content and good color and odor scores.

DISCUSSION OF RESULTS

The standard method of manufacturing casein by the natural sour method, as used in this investigation, produced a uniform, high quality casein as measured by selected physical and chemical tests. Section A of Table I shows the average results (arithmetic means), standard deviations, and coefficients of variation of 60 lots of casein made by the standard method. Properties of color, odor, solubility, yield, and nitrogen content were controlled within very narrow margins, as indicated by the small coefficients of variation. Viscosity, ash, pH value, and conductivity were more difficult to control within narrow limits; while moisture and fat were found to be extremely variable. Determinations for total and free acidity were considered of little value because of difficulty in determining the exact end-point. Users of casein may expect a uniform, high quality casein from their producers if the directions for this standard method are followed.

Buyers of casein place considerable importance on color and odor as they consider them an index of the purity and keeping quality. The most desirable casein is alm t pure white and practically odorless. Apparently color and odor are adversely affected by the following conditions:

a. Insufficient pressure on the green curd,
b. Fat content of the skim milk of 0.10 per cent or above,
c. Any washing less than one *thorough washing,*
d. An appreciable increase or decrease in the acidity from 0.64 per cent at time of coagulation.

The influence of an increase of moisture in the green curd on color and odor agrees with the findings of Dahlberg (1) and the statements of Sutermeister (5). Drying temperatures up to 140° F. had no appreciable effect either on color or odor. The importance attached to color and odor by buyers of casein should be limited since results of this investigation show that, although they may be regarded as an indication of the usability of casein, they are not an index of all its physical and chemical properties. A light-colored casein free from any undesirable odors may be produced by following the standard method of manufacture given.

Principal users of casein want it to be quickly and completely soluble; therefore, a high rate of solubility is desirable. Rate of solubility was decreased by having an acidity at time of coagulation either above or below 0.64 per cent and by increasing the setting and cooking temperatures above those of the standard method. Increase in rate of solubility resulted from increased washing, increased moisture content in green curd, and increased fat content of skim milk. An increase in fat is undesirable since it is uneconomical. Contrary to statements of Trimble and Bell (7), neither increased temperatures of wash water nor increased temperatures of drying, as used in this work, made the casein more insoluble. However, in agreement with the same investigators, higher temperatures of setting and cooking decreased the rate of solubility because of the formation of a tougher curd. Rate of solubility may be controlled by changes in technique, as above demonstrated. A high rate of solubility is desirable from the user's standpoint, but this test is a measure only of rate of solubility and not an index of quality.

Viscosity is considered an important property of casein by all users and is influenced by almost every phase in its manufacture. Increases in temperature at any point in the process of manufacture always increased the viscosity. Increasing the fat content of the skim milk decreased viscosity, probably because of the decreased amount of pure casein present. A high acidity at time of coagulation decreased viscosity, which is exactly opposite to Dahlberg's (1) results with grain curd casein. An increase in the acidity of the original skim milk and number of washings of the curd caused an increase in viscosity, which is in agreement with his work. Data presented support Dahlberg's contention that "the ash content in general indicates the viscosity." If a casein with a viscosity even higher than that resulting from the standard method is desired, it may be obtained by any one or a combination of the following changes: (a) increasing the temperature of the wash water; (b) increasing number of washings; (c) increasing the acidity of the original skim milk; (d) increasing the cooking temperature; (e) increasing the drying temperature; (f) increasing the moisture in the green curd. If a casein with a viscosity lower than that resulting from the standard method is desired, it may be obtained by increasing the acidity at time of coagulation to 0.75 per cent or by decreases in one or more of the above listed conditions below the standard method.

Yield of casein is determined by: first, completeness of precipitation from skim milk; second, loss of curd by draining and washing; third, loss of impurities by washing and pressing; and, fourth, loss of moisture in drying. Although more than one washing produces a higher quality casein, more than one washing is not desirable unless a premium can be secured which will more than compensate for the loss in yield caused by extra washing. Under a controlled period of time, high drying temperatures lower the yield because of the additional loss of moisture. Low drying temperatures result in a higher moisture content and corresponding higher yield, but the higher moisture may cause more spoilage in storage. Maximum yields commensurate with best quality can be secured with a setting temperature of 104° F., an acidity of 0.64 per cent at time of coagulation, a cooking temperature of 120° F., one thorough washing, and a drying temperature of 120° F.

According to Gould and Whittier (2) the ash content of casein is an indication of purity and adhesive strength, the less ash present the purer the casein and the greater the strength. Incomplete precipitation and poor washing increase ash content. Cooking at high temperatures causes a tough, rubbery curd which, since it is not easily washed, has a high ash content. Ash is materially reduced by successive, thorough washings. This agrees with the findings of Dahlberg (1) and Gould and Whittier (2). One thorough washing gives satisfactory results unless a premium may be secured for casein of lower ash content, since losses in yield occur with each successive washing.

Care should be exercised to maintain as low a fat content as possible in the skim milk for two reasons: first, even small increases in the fat content of the skim milk cause large increases in the fat content of the casein; and second, no manufacturer would knowingly sell fat at casein prices. Increased temperatures during any phase of manufacture, with the exception of drying temperatures, caused a decrease in the fat content. However, high temperatures of cooking or of wash water cause a tough curd, which lowers the quality of the casein. Under ordinary conditions the fat content of casein should not be a source of worry to users of casein, which has been indicated by Theophilus, Hansen, and Snyder (6) in their work on commercial casein. If abnormal quantities of fat are present, they are readily detected by a typical rancid odor.

Determination of nitrogen has been employed by many users as a measure of the purity of casein. Variations in nitrogen content are caused by increased or decreased amounts of impurities in casein. These variations, while small, are significant in that they indicate the value of nitrogen determinations as a measure of impurities. Results of this investigation agree with the work of Theophilus, Hansen, and Snyder (6) which showed that the nitrogen content of casein is rather uniform and varies little and with the conclusion of Snyder and Hansen (4) that nitrogen should be reported on a moisture free basis. Sixty samples of casein made by the standard method and used in this investigation had an average nitrogen content of 14.36 per cent when calculated on a moisture free basis and a standard deviation of 0.33. When, therefore, the nitrogen content, calculated on a moisture free basis, falls below 14.00 per cent, the quality of the casein should be questioned.

Users of casein are vitally interested in knowing the amount of alkali necessary to dissolve the casein and bring it to a desired reaction. Determinations of acidity are used as a relative measure of the alkali requirements of casein. The only phase of manufacture which appreciably affected the acid content of casein was the number of washings. Successive washings reduced the acidity. Extreme variations in results within the same group when determining free and total acidity made these determinations unreliable. The determination of pH value is the most reliable measure of acidity of any method used.

Conductivity has been suggested as a test for purity of casein by Snyder and Hansen (4), who state that "its extreme sensitiveness to variable factors in the preparation of casein makes its determination of value." Any phase of manufacture which lowers the ash content, such as increased washing, increase in acidity at time of coagulation, low setting temperature, or leaving the curd in the whey for any appreciable time, invariably increased the resistance in ohms. Since it has been established that ash content usually indicates viscosity, it is believed that conductivity may, in general, be used as a measure of the ash content and viscosity of casein.

Data presented demonstrate the feasibility of producing casein to meet specific needs if definite standards or specifications are made available. It appears that a good index of the quality of casein may be secured by determining color, odor, solubility, viscosity, nitrogen, pH value, and conductivity.

SUMMARY AND CONCLUSIONS

1. Results obtained from analyzing 220 lots of casein manufactured by the natural sour method under controlled conditions are reported.

2. The standard method of manufacturing used in this investigation resulted in a uniform, high quality casein.

3. Properties of color, odor, solubility, yield, and nitrogen content were controlled within narrow limits, but it was found more difficult to control as closely such properties as viscosity, ash, pH value, and conductivity. Moisture and fat were extremely variable. Of the physical and chemical properties studied, total and free acidity were of little value because of difficulty in determining the exact end point.

4. Each step in the manufacturing process was varied while the other steps were held constant in order to determine the effect of such variation on the physical and chemical properties of the resulting casein as measured by comparison with the casein made by the standard method.

5. Buyers prefer casein which is almost pure white and practically odorless. Thoroughness of washing was the most important factor of all the various changes made from the standard method. Either increasing or decreasing the acidity of the milk from 0.64 per cent at time of coagulation resulted in a lower color and odor score. A fat content of the skim milk of 0.10 per cent or above and insufficient pressure on the green curd adversely affected color and odor, but economy of manufacture would ordinarily result in control of these two factors.

6. Casein with a high rate of solubility is desired by buyers. Thoroughness of washing and an acidity of the skim milk either above or below 0.64 per cent at time of coagulation were again the most important of all factors studied. Both a setting temperature higher than 104° F. and a cooking temperature higher than 120° F. decreased the solubility due to the formation of a tough curd. Both a high fat content of the original skim milk and excessive moisture in the curd after pressing increased the rate of solubility of the finished casein but other considerations preclude such procedures except under special conditions.

7. All users of casein consider viscosity an important property. Viscosity was increased as the temperature at any step in the manufacturing process was increased; that is, cooking temperature of curd, temperature of wash water, and drying temperature. Increasing the original acidity of the skim milk resulted in a higher viscosity, but decreased the odor score and increased the acidity of the casein. Acidity of milk at time of coagulation should not be over 0.64 per cent. Increasing the number of washings and the

moisture in the green curd increased viscosity. The extent to which some of these changes are made will depend upon their compatability with other desirable characteristics of the casein.

8. Yield of casein is determined by: completeness of precipitation, loss of curd in draining and washing, loss of impurities by washing and pressing, and loss of moisture in drying. Lower yields are compensated for by casein of higher purity and better keeping qualities. Prices paid for different grades of casein will, therefore, affect manufacturing procedures.

9. Moisture content of the casein depended entirely on the drying process. This involved not only the drying temperature but also careful control of temperature and air circulation in the drying tunnel.

10. Low ash content of casein is usually considered as being indicative of greater purity and greater adhesive strength. Low ash content depends on the use of an acidity of 0.64 per cent at time of coagulation of the skim milk to obtain complete precipitation and thorough washing. The cooking temperature should not be over 120° F. as higher temperatures cause a tough, rubbery curd which is more difficult to wash.

11. High fat content of the finished casein is usually undesirable. The most important factor contributing to this condition was the fat content of the original skim milk. Higher cooking temperatures and more thorough washing with water of higher temperature tended to reduce the fat content but these procedures caused a tough curd which lowered the quality of casein.

12. Nitrogen content has been used as a measure of purity of casein. The number and thoroughness of washings, and the fat content of the original skim milk were the most important factors affecting nitrogen content. The extra fat in the skim milk would ordinarily be prevented because of the higher value of the fat. Higher nitrogen content was obtained by additional washing but resulted in lower yield. When the nitrogen content, calculated on the moisture free basis, falls below 14.00 per cent, the quality of the casein should be questioned.

13. The only important consideration found in producing casein of low acid content was thorough washing of the curd.

14. Conductivity, as measured by resistance in ohms, has been suggested as a test for purity of casein. Any phase of manufacture which lowered the ash content increased the resistance in ohms. Since ash content usually indicates viscosity, conductivity may be used as a measure of ash content and viscosity of casein.

15. Variations in the manufacturing process which had the greatest effect on the quality of casein were: thoroughness of washing, acidity at time of coagulation, setting temperature, and cooking temperature.

16. Quality of casein may vary according to the purpose for which it is used. Of the various physical and chemical tests studied

the following seemed to be the best indexes of quality: color, odor, solubility, viscosity, nitrogen, pH value, and conductivity.

17. Study of the results obtained by varying different steps in the manufacturing procedure indicated that casein of special characteristics can be made to meet definite specifications if desired.

BIBLIOGRAPHY

1. DAHLBERG, A. O. *Casein and Its Industrial Application.* E. Sutermeister, Chemical Catalog Company, New York, pp. 60-102. 1927.

2. GOULD, S. P., and WHITTIER, E. O. *Effect of Calcium and Phosphorus on Adhesive Strength of Paper-Coated Casein.* Journal of Ind. and Eng. Chem., Vol. 24, p. 791. 1932.

3. SHAW, R. H. *Methods of Casein Analysis.* Jour. of Ind. and Eng. Chem. Vol. 12, pp. 1162-1173. 1920.

4. SNYDER, R. S. and HANSEN, H. C. *A Study of Technic in Chemical Analyses of Casein.* Jour. of Ind. and Eng. Chem., Vol. 5, pp. 409-412. Analytical Edition, 1933.

5. SUTERMEISTER, E. *Casein and Its Industrial Application.* Chemical Catalog Company, New York. 1927.

6. THEOPHILUS, D. R., HANSEN, H. C., and SNYDER, R. S. *Chemical Study of Casein Made by Natural Sour Method in Eight Idaho Plants.* Concentrated Milk Industries, pp. 16-21. November, 1931.

7. TRIMBLE, C. S. and BELL, R. W. *Method of Making Grain Curd Casein.* Milk Plant Monthly, Vol. 19, p. 40. August, 1930.

8. TRIMBLE, C. S. and BELL, R. W. *Methods of Manufacturing Acid-Precipitated Casein from Skim Milk.* U.S.D.A., Cir. No. 279. July, 1933.

9. TRUAX, T. R. *The Gluing of Wood.* U.S.D.A. Bul. No. 1500. June, 1929.

10. United States Department of Agriculture, Yearbook of Agriculture, pp. 300-302. 1933.

11. WHITTIER, E. O., and GOULD, S. P. *Effect of Excessive Temperature and Time of Drying on Solubility of Casein,* Jour. Ind. and Eng. Chem. Vol. 24, p. 1081. Sept., 1932.

12. WHITTIER, E. O., GOULD, S. P., and BELL, R. W., SHAW, M. B., and BICKING, G. W. *Commercial Casein.* Jour. Ind. and Eng. Chem., Vol. 25, pp. 904-908. Industrial Edition, August, 1933.

13. ZOLLER, H. F. *Studies on Technical Casein.* Jour. Ind. and Eng. Chem., Vol. 12, pp. 1171-1173. December, 1920.

TABLE I.

Physical and Chemical Properties of Casein Resulting From Variations in the Manufacturing Process Compared With Casein Made By the Standard Method.

	No. of batches	Color	Odor	Solubility (minutes)	Viscosity (seconds)	Yield (per cent)	Moisture (per cent)	Ash (per cent)	Fat (per cent)	Nitrogen (per cent)	pH value	Total acidity (c. c.)	Free acidity (c. c.)	Resistance in ohms
A.—Casein Manufactured by Standard Method.														
Standard Method	60	7.98	9.7	11.60	11.64	2.81	5.02	2.80	0.41	14.36	4.43	13.81	7.83	974
Standard ± Deviation		0.35	0.46	0.42	0.77	0.10	1.061	0.36	0.17	0.3267	0.42	1.836	1.79	145
Coefficient of Variation		4.4	4.7	3.6	6.6	3.6	21.1	12.9	41.5	2.3	9.5	13.3	22.9	14.9
B.—Effect of Original Acidity of Skim Milk (See section A for comparison. Average original acidity of skim milk, 60 batches by standard method, 0.165 per cent.)														
Original acidity of milk (per cent) 0.16	6	8.00	10.00	11.63	11.30	2.88	5.27	2.77	0.40	14.39	4.45	14.07	7.02	980
0.19	6	8.05	10.00	11.63	11.75	2.98	5.52	2.92	0.42	14.43	4.48	14.39	8.10	895
0.22	6	8.15	9.75	11.66	12.30	2.85	5.68	2.97	0.43	14.53	4.62	14.28	7.60	871
0.25	6	8.15	9.00	11.66	12.57	2.88	4.94	2.99	0.45	14.38	4.58	14.68	8.20	845
C.—Effect of Fat Content of Original Skim Milk (See section A for comparison. Average fat content of skim milk, 60 batches by standard method, 0.012 per cent.)														
Fat content of skim milk (per cent) 0.01	6	8.00	9.75	11.31	11.88	2.82	4.99	4.33	0.61	14.19	4.21	12.11	9.11	930
0.10	6	6.50	8.75	10.12	11.67	2.92	5.09	2.93	1.58	14.21	4.22	11.91	8.98	974
0.25	6	6.00	6.50	9.68	11.25	3.02	4.59	3.09	2.94	14.10	4.28	11.80	8.52	1054
0.50	6	5.87	6.00	9.18	10.36	3.13	4.11	3.08	6.88	13.32	4.13	11.80	9.42	967

TABLE I (Cont.)

		No. of batches	Color	Odor	Solubility (minutes)	Viscosity (seconds)	Yield (per cent)	Moisture (per cent)	Ash (per cent)	Fat (per cent)	Nitrogen (per cent)	pH value	Total acidity (c. c.)	Free acidity (c. c.)	Resistance in ohms
D.—Effect of Setting Temperature (See section A for comparison. Average setting temperature, 60 batches by standard method, 104° F.)															
Setting temperature (degrees F.)	80	6	8.00	9.00	12.50	11.07	2.71	4.16	2.75	0.64	14.49	4.14	15.23	11.63	1279
	90	6	8.37	9.00	11.93	11.43	2.91	3.40	2.45	0.60	14.47	4.28	14.89	8.80	1276
	100	6	8.12	9.00	11.63	11.02	2.92	4.39	2.88	0.25	14.37	4.32	15.27	9.40	991
	110	6	8.00	9.25	13.38	11.60	3.00	4.15	2.95	0.33	14.31	4.38	15.64	9.28	901
E.—Effect of Acidity at Time of Coagulation (See section A for comparison. Average acidity, 60 batches by standard method, 0.64 per cent.)															
Acidity (per cent)	0.45	6	6.87	8.75	24.50	11.60	1.17	7.03	4.37	0.42	14.06	4.97	11.93	10.30	611
	0.60	6	8.00	10.00	11.62	11.60	3.12	6.13	3.12	0.51	14.16	4.58	12.50	10.30	792
	0.75	6	6.00	9.75	15.44	9.56	2.70	6.84	2.86	0.45	14.16	3.87	13.90	10.70	1100
F.—Effect of Cooking Temperatures (See section A for comparison. Average cooking temperature, 60 batches by standard method, 120° F.)															
Cooking temperature (degrees F.)	110	6	7.87	9.50	16.06	11.61	2.13	4.18	3.01	0.63	14.04	4.14	15.2	10.5	986
	120	6	8.00	10.00	11.87	12.00	2.92	4.50	2.98	0.62	14.05	4.21	15.0	9.0	1067
	140	6	8.00	9.00	11.62	12.66	2.90	4.55	3.57	0.58	14.38	4.39	14.3	7.6	961
G.—Effect of Time Curd Left in Whey (See section A for comparison. Time left in whey, 60 batches by standard method, zero.)															
Time in whey (minutes)		6	7.87	9.75	11.86	11.12	2.83	5.49	2.51	0.44	14.61	4.21	11.44	7.31	1048
	10	6	7.87	9.75	11.75	11.46	2.86	5.11	2.75	0.40	14.62	4.17	12.38	7.21	1084
	15	6	7.38	10.00	12.50	11.18	2.88	5.79	2.86	0.54	14.77	4.23	12.90	7.67	1156
	25	6	7.38	9.75	12.50	11.46	2.86	4.50	2.41	0.40	14.54	4.02	12.93	8.40	1234

TABLE I (Cont.)

		No. of batches	Color	Odor	Solubilty (minutes)	Viscosity (seconds)	Yield (per cent)	Moisture (per cent)	Ash (per cent)	Fat (per cent)	Nitrogen (per cent)	pH value	Total acidity (c. c.)	Free acidity (c. c.)	Resistance in ohms
H.—Effect of Number of Washings (See section A for comparison. Number of washings, 60 batches by standard method, one.)															
Number of Washings	0	6	7.00	9.25	19.87	11.22	2.93	5.06	3.47	0.39	14.30	4.17	16.02	10.93	837
	1	6	8.00	9.75	11.56	11.75	2.84	5.15	3.15	0.34	14.54	4.59	14.30	4.92	1453
	2	6	9.00	10.00	10.94	11.86	2.84	5.35	2.41	0.35	14.70	4.79	13.09	2.83	1663
	3	6	9.00	10.00	10.69	12.32	2.71	5.24	2.36	0.29	14.69	4.89	12.93	2.31	1894
	4	6	9.50	10.00	10.50	12.05	2.65	5.65	2.28	0.32	14.68	5.03	12.57	2.77	2211
I.—Effect of Temperature of Wash Water (See section A for comparison. Average temperature of wash water, 60 batches by standard method, 55° F.)															
Temperature of wash water (degrees F.)	55	6	8.00	10.00	11.96	14.98	2.92	5.21	3.08	0.64	14.35	4.91	13.10	6.50	916
	65	6	8.00	10.00	11.96	16.48	3.03	5.49	3.06	0.43	14.64	4.91	13.20	6.88	898
	85	6	8.00	10.00	11.50	16.39	3.06	4.60	3.07	0.21	14.53	4.93	13.08	7.91	953
	110	6	8.00	10.00	11.44	18.26	3.18	5.01	3.15	0.17	14.55	4.93	12.97	6.34	991
J.—Effect of Moisture in Green Curd (See section A for comparison. Average moisture in green curd, 60 batches by standard method, 49.23 per cent.)															
Moisture in green curd (per cent)	64.16	6	5.87	6.25	10.25	12.95	2.84	3.79	2.28	0.49	14.37	4.82	13.1	8.91	950
	53.61	6	8.00	9.00	13.37	10.99	2.81	4.79	2.08	0.44	14.36	4.27	14.4	12.00	922
	49.10	6	8.12	9.75	11.58	11.60	2.78	4.80	2.07	0.27	14.53	4.27	13.8	8.97	1048
K.—Effect of Drying Temperature (See section A for comparison. Average drying temperature, 60 batches by standard method, 120° F.)															
Drying temperature (degrees F.)	80	6	8.42	9.00	11.25	11.00	3.25	8.85	2.26	0.32	14.67	4.71	10.55	6.05	1230
	100	6	8.10	9.25	11.38	11.62	3.00	5.35	2.63	0.30	14.48	4.49	11.35	6.48	1039
	120	6	8.15	9.75	11.44	11.64	2.81	5.02	2.80	0.41	14.38	4.42	13.81	7.83	974
	140	6	8.10	9.25	11.59	12.28	2.56	3.23	2.39	0.36	14.47	4.44	11.90	6.00	1071