

TECHNICAL NOTES

FOREST PRODUCTS LABORATORY

U. S. FOREST SERVICE

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THE ANALYSIS OF CASEIN

Commercial casein is an exceedingly variable product and in certain industrial applications, such as glue making, much trouble is caused by its lack of uniformity. With the growing importance of casein in industry a product of better and more uniform quality will be required and in order to control the methods of manufacture and furnish a basis for grading casein, methods of analysis must be adopted. Hitherto no serious attempt has been made to do this, with the exception of rather indefinite and questionable "solubility" and "adhesiveness" tests* though a more thorough procedure has been recommended.**

The following methods have been used at the Forest Products Laboratory in the examination of over 300 samples of casein, and have served as the basis of specifications for casein for the Air Service. They include determinations of the color and odor of the casein, and of its moisture, fat, ash, nitrogen and acid contents.

Color

The color is noted simply by observation of the samples as received. A good grade of casein is white or very pale cream in color.

Odor

The odor is determined by soaking a small handful of casein in about an equal volume of water. To this an equal volume of a rather thick paste of "milk of lime" is added with stirring, and the odor noted after a few minutes standing. The odor should be sweet or only faintly sour.

* U. S. Department of Agriculture, Bulletin No. 661, P. 30

** Hopfner and Burmeister Chem.-Zeit. 36, 1053, 1912.

Sampling for Analysis

The sample as received is thoroughly mixed and a portion of about 25 grams set apart for the moisture, fat, ash, nitrogen, and acidity determinations. The sample for fineness is then taken from the residue. If the casein does not pass the fineness test, the 25-gram sample is ground so that it will all pass through a 50-mesh sieve before making the other tests.

Fineness

A 50-gram sample is placed in a 50-mesh screen 6 inches in diameter. The screen is held in one hand, and moved horizontally back and forth at the rate of about 120 strokes per minute, being allowed to strike against the palm of the other hand held stationary. The shaking is continued not more than ten minutes and the portion passing through the screen is weighed. The degree of fineness required depends upon the use to which the casein is to be put.

Moisture

A 3-gram sample is weighed out into a porcelain evaporating dish about 2-3/4 inches in diameter and heated for five hours at 97-100°C. The dish is cooled in a desiccator and weighed, and the loss called moisture.

As an alternative method for determining the amount of moisture, the sample may be placed in a glass-stoppered weighing bottle and heated to constant weight in a vacuum oven at 75° C. The moisture content should not exceed 10 per cent.

Fat

The residue from the moisture determination is transferred to an extraction thimble and extracted for sixteen hours with anhydrous residue-free ethyl ether in a Caudwell or Soxhlet extraction apparatus. The ether is evaporated from the extract and the residue, corrected for the moisture content of the casein, is called fat. Casein should not contain more than 1 per cent fat.

Ash

A 3-gram sample is weighed out into a vitreosil evaporating 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 (about 600° C.) until the ash is white, or at least light gray, and the weight is constant. A small portion of ammonium nitrate may be added if necessary to obtain a light colored ash. Care should be taken to avoid fusion of the ash so far as possible. The dish is cooled in a desiccator and weighed. Ash is reported on the moisture-free basis. Natural-sour caseins should not contain more than 3 per cent ash, acid caseins not more than 4.5 per cent, and rennet caseins not more than 8.5 per cent.

Nitrogen

A 0.5-gram sample is weighed out into an 800 cc. Kjeldahl flask, 20 cc. of concentrated sulphuric acid, 10 grams of crystallized sodium sulphate, and a small crystal of copper sulphate are added, and the contents digested until a clear solution is obtained, and then for 30 minutes longer. Then 300 cc. of distilled water, 50 cc. of a 1:1 solution of sodium hydroxide and about a quarter of a gram of granulated zinc are added and about 250 cc. distilled over into standard sulphuric 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 or cochineal being used as indicator. Nitrogen is reported on the moisture-, fat-, and ash-free basis. It should not be less than 14.25 per cent.

Acidity

A 1-gram sample is treated with 10 cc. of carbon-dioxide-free water for 1 hour in a refrigerator at a temperature not to exceed 10° C. The water should be added in a fine stream in such a way as thoroughly to moisten all the casein and prevent lumps. Twenty-five cc. of tenth normal sodium hydroxide solution are then added and the flask kept at a temperature not to exceed 10° C.

for four hours. During this time it is vigorously but carefully shaken every hour by giving the flask a rapid rotary motion. At the end of this time 100 cc. of carbon dioxide-free water and 1 cc. of an alcoholic solution of phenolphthalein (containing 1 gram per 100 cc.) are added. The solution is then back-titrated with tenth normal acid, the acid being run in fairly rapidly with vigorous shaking of the flask so that no coagulation of casein can take place by local action, until the pink color is discharged. No attention is paid to the fact that this color reappears on standing 15 or 20 minutes. The acid and alkali solutions are standardized by titrating under the same conditions of temperature and amount of indicator as are used in the determinations. The phenolphthalein solution must be adjusted with alkali so that 1 cc. added to distilled water does not appreciably affect its reaction. The number of cc. of N/10 alkali used up by one gram of the moisture, fat and ash-free casein is called the acidity. Acidity should not exceed more than 10.5 cc. of N/10 sodium hydroxide per gram.

Summary

A good grade of casein will usually give the following results:

Color: White or very pale cream.

Odor: Sweet or only very faintly sour.

Fineness: As required by the use to which it is to be put.

Moisture: Not more than 10.0 per cent.

Fat: Not more than 1.0 per cent.

Ash: Not more than 3.0 per cent for natural sour, 4.5 per cent for acid, or 8.5 per cent for rennet caseins.

Nitrogen: Not less than 14.25 per cent.

Acidity: Not more than 10.5 cc. of N/10 sodium hydroxide per gram.